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Complementary and Alternative Medicine: Prebiotics and Probiotics

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PREFACE

The field of prebiotics and probiotics has experienced rapid growth in recent years, reflecting a profound shift toward understanding and harnessing the microbiome for improved health across species. *Complementary and Alternative Medicine: Prebiotics and Probiotics* provides a comprehensive exploration of the pivotal role these microbial allies play in promoting gut health, enhancing immunity, and supporting overall well-being. This book delves into the various applications of prebiotics and probiotics, examining their effects not only in humans but also in animals, aquaculture, and veterinary medicine, illustrating their wide-ranging impact. Beginning with an overview of the benefits of prebiotics and probiotics on animals, this book explores how these compounds contribute to livestock productivity and disease prevention. Chapters focused on poultry and ruminant health discuss how supplementation of these beneficial microorganisms supports growth, improves feed efficiency, and enhances resilience against pathogens. The use of probiotics in aquaculture, particularly in functional feeds, is also examined, highlighting their potential to support sustainable fish farming by promoting gut health and immunity in aquatic species. The book further investigates the unique immunomodulatory effects of popular probiotic strains such as *Lactobacillus* and *Bifidobacterium*. These strains are increasingly recognized for their roles in regulating immune responses, offering protection against infections, and potentially even mitigating inflammatory conditions. Detailed discussions cover the use of probiotics in managing specific health concerns, from supporting glucose regulation in type 2 diabetes to assisting in cardiovascular and cancer care, illustrating their potential as valuable tools in integrative health strategies. Prebiotics, which serve as fuel for beneficial gut bacteria, are explored for their contributions to digestive health and beyond. Their role in promoting gut health in children, pregnant women, and individuals with metabolic disorders is examined, underscoring the broad applications of these dietary fibers in maintaining balance within the gut microbiome. The book also addresses the interplay between prebiotics and probiotics, highlighting their combined, synergistic effects on gut health and immunity in humans and animals alike. Beyond traditional applications, this book addresses innovative uses of prebiotics and probiotics in fields as diverse as fisheries, aquaculture, and veterinary medicine. For instance, the potential of yeast-based therapeutics in aquaculture, the role of probiotics in preventing zoonotic diseases like brucellosis, and the use of encapsulated probiotics in functional foods reveal the depth of possibilities these natural compounds offer. Chapters discussing specific health conditions, such as necrotic enteritis in broilers and liver diseases, demonstrate targeted applications and practical guidelines for their use. Emerging research is also presented, including the promising use of bacteriophages in conjunction with probiotics to control *E. coli* in poultry and prevent coccidiosis in ruminants, thereby reducing reliance on antibiotics. These strategies represent the forefront of integrative approaches aimed at enhancing animal welfare and public health while addressing antibiotic resistance concerns. *Complementary and Alternative Medicine: Prebiotics and Probiotics* offers a wealth of knowledge for researchers, healthcare professionals, veterinarians, and those interested in the science behind these natural health promoters. This book not only highlights the therapeutic potential of prebiotics and probiotics but also envisions a future where gut health serves as a foundation for holistic well-being across species. By bridging the gap between traditional medicine and modern science, this book aims to inspire readers to explore the full potential of these natural allies in advancing health and resilience in a sustainable manner.

Editor

Contents

Chapter 01

An Overview of Beneficiary Effects of Prebiotics and Probiotics on Animals

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ABSTRACT

The use of prebiotics and probiotics in the animal feeds provides several beneficial effects ranging from an improved digestive health to an overall strong immune system. Prebiotics are non-degradable fibers that act as substrate for beneficial gut microbiota. Upon consumption, prebiotics improve the beneficial bacteria growth and activity resulting into better nutrients-utilization and absorption, necessary for animals undergoing dietary transitions. These substances maintain the healthy gut microbiota leading to an improved immunity, reduced gastrointestinal infections, and better pathogen resistance. Furthermore, they reduce the stress, help in adapting the environment, and promoting overall health. Whereas, probiotics are live microorganism providing health benefits to the animal when given in adequate dose. They colonize in the gastrointestinal tract and compete with pathogenic bacteria for receptors and nutrients necessary for their growth, thus do not allow the harmful bacteria to grow leading to reduced gut related infections. Probiotics also enhance the nutrients absorption efficiency, nutrient availability, and the digestion process. Simultaneous feeding of prebiotics and probiotics to animals shows synergistic effects such as improved growth rates, efficient nutrient absorption, better reproductive performance. Furthermore, a healthy gut lead to reduction in antibiotics usage and its resistance, thus helps in attaining the sustainable farming practices.

KEYWORDS
Prebiotics, Probiotics, Gut microbiota, Animals

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INTRODUCTION

The term probiotic is derived from the Greek words pro and bios which means for life (Metchnikoff, 1907). It is defined as live microbes when administered in a suitable amount provide health benefits to the host (Akram et al., 2023; Joint, 2002). They introduce organisms from the external world in microflora. These are given as feed additives in the form of capsules, gel, powder, pellets, and paste in animal feed, especially for poultry and pigs (Aslam et al., 2023; Gill, 2003). Probiotic target rumen in which feed is digested (Williams and Newbold, 1996). The most commonly used probiotic is *Bifidobacterium.* Probiotics provide health benefit as it maintains balance within intestinal microbial ecosystem (Salminen et al., 1996), produces bacteriocins which are antimicrobial substances (Mazmanian et al., 2008), work against enzymes that contribute in development of cancer so probiotic blocks these enzymes (Rafique et al., 2024; Gill, 2003). Prebiotics are defined as non-digestible food components like carbohydrates, lipids, and proteins that selectively promote the growth of one or more microbes such as *Bifidobacterium* or *lactobacilli* in the intestine and modulate gut microbiota (Gibson and Roberfroid, 1995: Śliżewska et al., 2013). One commonly used prebiotic is inulin. For polysaccharides and oligosaccharides to be included as prebiotic, it should be able to survive stomach acid and digestion by our bodies' enzymes, can be broken down by bacteria in the intestines, helps good bacteria in the gut grow and work better for our health (Mehnaz et al., 2023; Gibson et al., 2004).

Background

With passage of time, the number of people will reach in billions. Constant growth of human population is closely linked with an elevated level of food of plant and animals' origin. In the past, people used different types of feed additives to improve the quality of meat, eggs, milk, and fish. The health and nutrition of consumer totally was depended upon the animal production, and its intestinal pathogens, such as yersinia, campylobacter, listeria and salmonella acted as the main causes of food deterioration and caused zoonosis. People started to use additional medicine or feed supplements that changed or altered the microflora and enhanced the productivity and animal growth. Long-time use of those substances caused the development of drug-resistant microorganism, resulting into exertion of negative effects on health of people, and also negative consequence on environment (Truszczyński and Pejsak, 2006; Biernasiak et al., 2010). At the end, the use of growth promoters was banned by European Union on 1st January 2006.

The first concept was suggested by Metchnikoff in 1907, which checked that bacteria have favorable effect on intestinal microflora (Metchnikoff, 1907). The term probiotic was invented by Ferdinand Vergin, who compared harmful and beneficial effect of antibiotics or other antimicrobial agent on intestinal microbiota (Vergin, 1954). It was not an easy task to judge the protection level of probiotic strains crucial for optimization of their use (Anadón et al., 2014). Features of probiotics steer to ameliorated the health of animals, increase yield (Isolauri et al., 2004) and elevated immunity of animal (Patel et al., 2015). Probiotic commodities may hold one or more selected microbial strains. Microorganisms are used as feed supplements in the EU are mostly bacteria. These bacteria are beneficial for animals. Chiefly Gram-positive bacteria belonging to the following geni: Bacillus, Enterococcus, Lactobacillus, Pediococcus, Streptococcus. Some fungi and yeast strains of Saccharomyces cerevisiae and Kluyveromyces species are probiotics. Bacteria linking to the genera Lactobacillus and Enterococcus are components of the natural microbiota of the animal alimentary tract, and in amounts of 107–108 and 105–106 CFU/g, respectively. Some bacteria create problem like these e.g. Enterococcus genus bacteria may collaborate in dissemination of antibiotic resistance, and Bacillus cereus strain are efficient to produce endotoxins and emetic toxins (Rafique at al., 2024; Anadón et al., 2006).

In addition to probiotics, prebiotics were also used as natural feed additives. In 1921, Rettger and Cheplin reported that after devouration of carbohydrates the human intestinal microbiota was enriched with lactic bacteria (Rettger and Cheplin, 1921). The mammalian immune or defense system enfolds byzantine network of innate and adaptive component of tissue, and plays indispensable role in host defense against various harmful agents. The orientation of ecological, commensal microorganism of mammals interdependently evolved towards mutualism and hemostatis (Dethlefsen et al., 2007). This is necessary for perfect relationship of host to protect the commensals from plundering of host resources whereas immune resistance from unmalicious to external stimuli (Macpherson et al., 2005). Microbiome immune interaction harmonious in variety of non-contagious, non-transmissible gastrointestinal disease including inflammatory bowel disease (Zhang, 2017), celiac disease (Valitutti et al., 2019) additionally, extra intestinal disorder syndrome ranging rheumatic arthritis (Raheem et al., 2023; Maeda and Takeda, 2019), it also cause metabolic syndrome (Belizario et al., 2018), neurodegenerative disorder (Main and Minter, 2017) and then move towards malignancy (Gopalakrishnan et al., 2018). In early life, mammalian host play fundamental role in maturation of host immune system (Gensollen et al, 2016). Infants are more susceptible to environmental foray to microbiota then long-lasting adverse effect on immunity (Russell, 2012).

Common prebiotic substances including; non-absorbable carbohydrates (oligosaccharides and polysaccharides), peptides, proteins, and lipids. Legumes, fruit and cereals are natural sources of prebiotics. Although, many prebiotics products are formulated by using industrial chemical and various enzymatic method (Śliżewska, 2013). Some commonly used prebiotics; oligofructose, trans-galacto-oligosaccharides (TOS), gluco-oligosaccharides, glico-oligosccharides, lactulose, lactitol, malto-oligosaccharides, Xylo-oligosaccharides, Stachyose and raffinose (Monsan, 1995; Orban et al.,1997; Patterson et al.,1997; Collins and Gibson, 1999; Patterson et al.,1997). These above compounds make a nutritional substrate for useful intestinal bacteria (Yasin et al., 2023; Grajek and Olejnik, 2005). Increase the account of Bifidobacterium and reduce intestinal colonization by pathogenic bacteria ae the main prebiotics factor on health of chicken (Jung et al., 2008; Biggs, 2008).

Importance of Gut Health in Animals and Impact on Overall Wellbeing

Gut health can be defined as maintaining the gastrointestinal well-being of animals without disease so that animals can perform their normal body functions and bear stressors (Kogut and Arsenault, 2016). Animal overall health is closely related to the health of the intestine. It is essential for the functioning of production animals as ruminants (Bailey, 2013) and also responsible for excretion and hydrated equilibrium (Guarino et al., 2020). Gut health can result in less use of antibiotics and keeps them away from diseases (Seal et al., 2013)

If there is problem in gut, it will affect the immune system, absorption of feed, nutrient digestion and product output will be low leading to economic losses and susceptibility to illness. Several factors affect gut health as age (Sender et al., 2016), quality of feed, surroundings, and animal handling (Bailey, 2013) Changes in microbiota can lead to Inflammatory Bowel Disease and heart diseases (Seo et al., 2020).

The gut's defense system consists of 70-80% of total body immune cells and has a vital role in the development of the host (Furness et al., 1999) Microbiome of the gut also helps in coping with stress, formation of the blood-brain barrier, microglial differentiation, inflammatory signaling pathways, animal behavior management through the fermentation process (Sharon et al., 2016; Nicholson et al., 2012). In adult normal flora, the human colon is home to an estimated 3.9 ×

1013 bacteria (Sender et al., 2016). Based on their impact on the human body, intestinal flora can be classified into three categories Probiotics, which confer health benefits, neutral bacteria, and pathogenic bacteria, which can cause harm or illness (Jones and Foxx-Orenstein, 2007). The human intestine lacks an enzyme that breaks bonds of prebiotics so they remain in the GIT tract, there they undergo degradation by intestinal flora and selective fermentation, leading to the production of secondary metabolites such as acetate, propionate, and short-chain fatty acids which create an acidic environment in the colon for growth of beneficial gut bacteria. These are absorbed by intestinal epithelium or transported to the liver through the portal vein and pose beneficial effects on host physiology such as pathogen resistance, enhancement of intestinal barrier epithelium, and reduction of blood lipid levels (Slavin, 2013; Cockburn and Koropatkin, 2016; Guarino et al., 2020).

Many studies reported positive effects of prebiotics on the growth of poultry (Abd El-Hack et al., 2020). Additionally, yeast-derived mannan oligosaccharides (MOS) have been observed to directly mitigate gastrointestinal pathogens like Escherichia coli and Salmonella. This is achieved through their interaction with the flagella of these pathogens, consequently impeding their colonization by disrupting their adherence to gut epithelial cells (Fomentini et al., 2016). Salmonella infection can be reduced by Short Chain Fatty Acids (Van Immerseel et al., 2006). Propionate, a type of acid, can stop Salmonella from invading the cells lining the gut, while butyrate, can turn down the activity of Salmonella genes responsible for invading those cells (Van Immerseel et al., 2006). Lactose is a disaccharide sugar present in milk that has glucose and galactose; it may be considered a prebiotic. The addition of lactose to broiler diets leads to taller and longer folds in the cecum; it also reduces the thickness of the intestinal lining and lowers the pH level in the gut (Tellez et al., 1993). The growth of broilers can be boosted by using a diet containing soybean and corn with lactose; it also enhances egg production in laying hens (Fomentini et al., 2016). Prebiotics can also play a role in preventing animals from heart or enteric diseases. It can also be used as an antibiotic alternative and it does not cause resistance.

Benefits of Probiotics for Animals by the Introduction of Beneficial Live Microorganism into the Gut

Probiotics provide benefits to the animals in a number of ways as shown in Figure1. Their beneficial effects depend on their mode of actions, dose administered, and strain used (Patel and Shukla, 2015; Tellez et al., 1993). Upon ingestion of the accurate amount, they introduce beneficial live bacteria into the microbial population of the gastrointestinal system (Guarino et al., 2020). They support in the maintenance of microorganism population in the digestive tracts, thus improving the proportion of beneficial bacteria (Fomentini et al., 2016). These bacteria do not allow pathogenic organisms to obtain nutrition, grow, localize and cause disease in the gut (Isolauri et al., 2004).

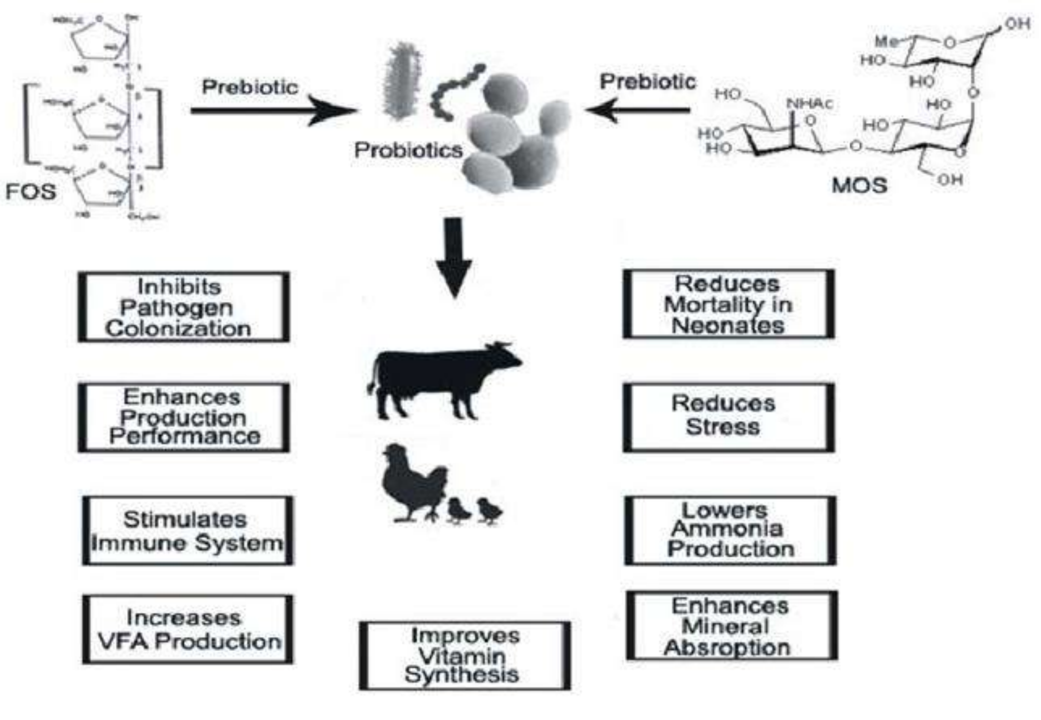

Fig. 1: Benefits of using probiotics in the animals

Probiotics Strains used in Animal Nutrition and their Effects

There are several probiotics strains used in the animal feed plans to increase their production, reproduction and performance efficiency, as described in table1. These include: i) *Lactobacillus acidophilus*: the main benefits of this strain include the production of lactic acid which supports the gut health and maximizes the nutrient absorption by decreasing the pH of gastrointestinal tract (GIT) and inhibiting the growth of pathogenic bacteria (Russell et al., 2012; Patterson et al., 1997). ii) *Bifidobacterium animalis*: it exerts its beneficial effect by enhancing the gut-barrier-function, strengthening the immune system, and improving the overall GIT health (Gill, 2003). iii) *Saccharomyces boulardii*: This strain is structurally a yeast, but its characteristic to support the GIT health through maintenance of microbial population and improvement of the gut barrier faction encourages its usage as a probiotic (Salminen et al.,1996). iv) *Enterococcus faecium*: it benefits the animals by improving the digestion process efficiency, defense system, and overall gut health (Guarino et al., 2020).

Table 1: Probiotics strains used in animal nutrition

Lactobacillus species	Bifidobacterium species	Others
L. acidophilus	*B. bifidum*	*Bacillus cereus*
L. casei (rhamnosus)	*B. longum*	*Escherichia coli*
L. reuteri	*B. breve*	*Saccharomyces cerevisiae*
L. bulgaricus	*B. infantis*	*Enterococcus faecalis*
L. plantarum	*B. lactis*	*Streptococcus thermophilus*
L. johnsonii	*B. adolescentis*	
L. lactis		

Research Studies Indicating the Benefits of using Probiotics

a) Improved digestive function: Research studies revealed that probiotics supplementation leads to an improved the feed digestion efficiency by as they promote the beneficial microbiota growth, maximize the nutrients absorption, and reduce the growth of harmful bacteria (Main and Minter, 2017). For example, its usage in poultry resulted into better feed conversion ratios, and in ruminants it reduced the digestive issues (Śliżewska et al., 2020). b) Strong immune system: Regular use of probiotics in the animals exerts beneficial effects them through modulation of immune responses (Depoorter and Vandenplas, 2021), optimizing the immunity cells, making them more resistant to morbidities and improving their health (Gao et al., 2023; Maeda and Takeda, 2019). Studies showed significant reduction in disease incidence rates among animal groups fed on probiotics. c) Improved Performance: Benefits such as improved production of milk, meat, eggs, wool, and hair, and better growth rates are the key indications for use of probiotics supplements in many animal species (Śliżewska et al., 2013). Studies have also been performed in equines which showed an improvement in the performance and stamina of the racing horses resulted by probiotics usage (Slavin et al., 2013). One markable study in broiler chicken fed on probiotic-supplemented diet suggested an improvement in the GIT structure (Mazziotta et al., 2023; Cunningham et al., 2021), immune responsiveness, and reduction in pathogen colonization (Santacroce et al., 2019), resulting a better health and performance (Abd El-Hack et al., 2020; Lu et al., 2003). Findings of the above studies illustrate that probiotics use in different animal species exerts significant positive effects on the animals such as an improved digestion (Trush et al., 2020), strong immune system, and optimum performance (Lu et al., 2021; Drago, 2019). However, several factors influence the efficacy of probiotic strains which include dosage form, formulation available, and physiological status of the animal, demanding the selection of Targeted Probiotic Strategy (TPS) in animal nutrition (Martín and Langella, 2019; Williams and Newbold et al., 1996).

Combined Effects of Prebiotics and Probiotics

Simultaneous diet supplementation of prebiotics and probiotics can produce a marked improvement in the health status, growth and immunity of the animals (Piqué et al., 2019; Salminen et al., 1996). In this approach the prebiotic substances act as food substrate for probiotics, which support them in getting colonized and exert their beneficial effects in the gut (Bedada et al., 2020; Anadón et al., 2014).

Prebiotics includes non-digestibel compounds such as oligosaccharides, fructooligosaccharides (FOS), and inulin (Fenster et al., 2019; Patel et al., 2015). These sugars selectively target and enhance the growth and functioning of beneficial microbiota (probiotics) in the animal digestive system. As these prebiotic sugars serve as fermentation substrate for probiotics, they result into an increase in the production of short-chain fatty acids (SCFAs) (butyrate, acetate, and propionate) which play a critical role in maintaining the health and function of GIT (Rettger and Cheplin, 1921).

Furthermore, combined use of prebiotics with probiotics in the animal diets creates such environment which favors the survival of probiotic bacteria, and support their colonization and activity in the animal digestive system (Yang et al., 2021; Dethlefsen et al., 2007). Probiotics fulfill their nutrients and energy requirements from prebiotics substance, which is essential for their survival and activity in the gut (Sotoudegan et al., 2019; Zhang et al., 2017). This synergistic relationship supports the probiotics in the effective modulation of gut microbial population, increasing the digestion efficiency, and strengthening the immune system (Quigley, 2019; Valitutti et al., 2019).

Studies Supporting the Synergistic use of Probiotics in Different Animal Feeds

a) Poultry Production: A study conducted on broiler chicken suggested that combined supplementation of prebiotics (FOS or inulin) probiotics (*Lactobacillus spp*. or *Bacillus spp*.) lead to improvement in the growth rate, FCR, gut morphological feature, and an overall better production and health, in contrast with control group (Krysiak et al., 2019). These beneficial effects were obtained due to the increased nutrient absorption, reduced colonization of pathogenic bacterial population, and generation of the effective immune responses (Gopalakrishnan et al., 2018). b) Equine Nutrition: Research in equines has also supported the benefits of the simultaneous use of prebiotics and probiotics in the diet (Abd El-Hack et al., 2020). Results of a trial consisting on the combined use of prebiotics (mannan oligosaccharides, MOS) with probiotics (*Enterococcus faecium*) demonstrated an improved GIT structural integrity, reduced cases of post-weaning diarrhea, and a better growth performance in foals (Yeşilyurt et al., 2021). This synergistic action supported in maintaining gut microbial population, reducing GIT inflammation, and ensuring optimum nutrient utilization (Yong et al., 2020). c) Ruminant Health: Research suggested that the combined use of prebiotics (galactooligosaccharides, GOS) with probiotics (*Lactobacillus spp. or Bifidobacterium spp*.) in ruminants (bovine, caprine and ovine specie) helps in maintaining the rumen pH, promoting the population of beneficial microbiota, and reducing the GIT-related disorders (Anee et al., 2021). Thus, it has positively affected the rumen microbial fermentation, nutrient utilization, performance and growth (Ruiz Sella et al., 2021).

Mechanisms of Action

Every probiotic and prebiotic substance possess a specific mechanism of action (Figure 2). Obtaining the desired results from their usage depends on their interaction and way they exert their effects on the body which include: a) Gut Microbiota Modulation: Using the prebiotics and probiotics in combination increases the beneficial bacteria population and reduces the growth of pathogenic specie which supports in balancing the GIT Microbiota (Bhogoju and Nahashon, 2022; Patterson et al., 1997). This modulation leads to healthier gut function, efficient nutrient absorption, stronger immunity system in the animals (Al-Shawi et al., 2020). b) SCFA Production: Probiotics oriented fermentation of the prebiotics leads to SCFAs production in the gut (Alayande et al., 2020). These SCFAs, particularly butyrate, contribute to the overall GIT health and performance by providing energy to the gut epithelial cells, enhancing gut barrier function, and exerting the anti-inflammatory action (Anadón et al., 2019). c) Immune Regulation: Probiotics and prebiotics interact symbiotically and exert a positive impact on the animal immune system (Plaza-Diaz, 2019) and over all disease-resistance by balancing the defensive system and reducing the inflammations, making the animal resistant to diseases (Arsène et al., 2021).

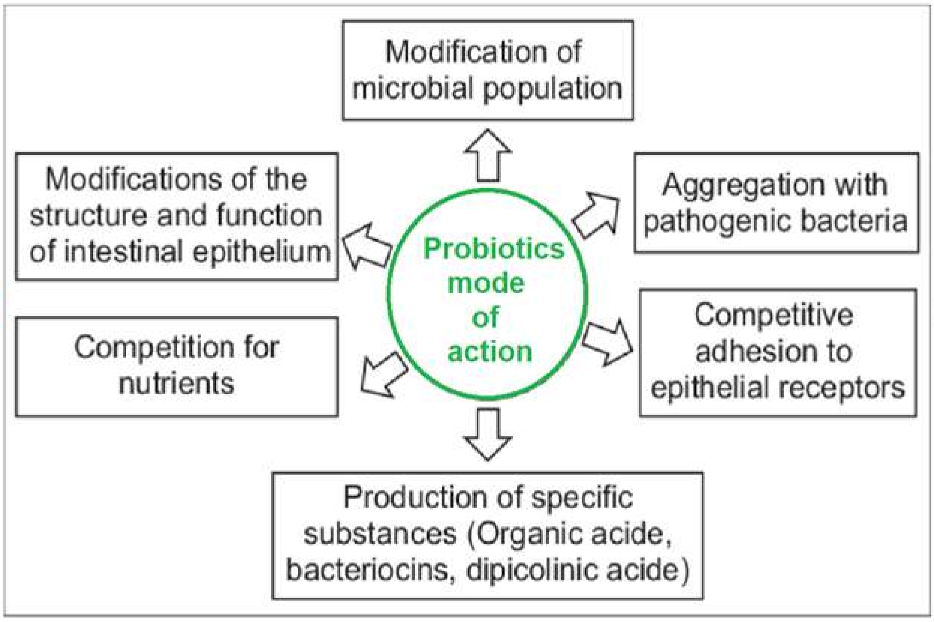

Fig. 2: Mechanism of action

Emerging Trends in Prebiotic and Probiotic Research for Animal Nutrition

In the past years, animal nutritionists have paid significant attention to the probiotics and prebiotics substance due to their prospective characteristics to improve the GIT function, nutrient utilization efficiency, productive and reproductive performance, and immunity of several animal species (Limbu et al., 2024). With research evolution in the field of animal nutrition, several trends have emerged which initiated the prebiotics and probiotics development and application in the animal feeds (Jha et al., 2020). These trends include:

a) Advanced Formulation Techniques: Development of the probiotics and probiotics products dosage by following the new methods helps in the optimum utilization of the active substances of the products (Judkins et al., 2020). These techniques are: i) Microencapsulation: Unique encapsulation techniques are being developed to ensure the stability and efficient delivery of the prebiotics and probiotics substances in the animal feed (Cameron and McAllister et al., 2019). Microencapsulation maximizes the efficacy of these sensitive ingredients by protecting them from the unfavorable environmental fluctuations (changes in pH and temperature) in the Gut, ensuring their targeted release in the GIT (Pandey et al., 2019). ii. Synbiotics: They are combined form of prebiotics and probiotics for their simultaneous use in the animal feed (Stavropoulou and Bezirtzoglou, 2020). They support in improving the GIT health and gut microbial population balance by synergistically providing the substrate (prebiotics) (Żółkiewicz et al., 2020) and beneficial microbiota (probiotics) for the fermentation process (Yaqoob et al., 2022).

b) Precision Nutrition: This includes the development of nutritional strategies by focus specific objectives. i) Species-Specific Formulations: It is an emerging trend to customize the prebiotic and probiotic formulations according to specific animal species to meet their individual physiological and nutritional requirements (Franco-Robles et al., 2020). To improve the animal health and performance, it is essential to develop the targeted nutritional strategies (TNS) by gaining the deep understandings regarding the composition and responsiveness of the gut microbiota of a particular specie (Melara et al., 2022). ii) Dosage Optimization: It is important to determine the optimum-dosage levels of prebiotics and probiotics for maximum utilization of their benefits without any adverse effects (Enan et al., 2023). Ongoing studies are now focusing on dose-response relationships and the impact of varying dietary concentrations on gut microbiota composition and metabolic processes (Azad et al., 2020). c) Functional Ingredient Exploration: Obtaining the deep insights regarding characteristics and development of prebiotics and probiotics for their better usage in improving the animal wellbeing (Reid et al., 2019) i) Novel Prebiotics: Nutrition scientists are now in search of new prebiotics sources (resistant starches, arabinoxylans, and xylooligosaccharides) to increase the number of substrates available that can be used to support the growth of beneficial microbiota in the GIT (Shehata et al., 2022). ii) Next-Generation Probiotics: Advanced microbiological techniques are now being developed for microbial identification and selection which supports in discovering and characterizing the novel probiotic strains with specific functional properties such as enhanced acid and bile tolerance, competitive exclusion of pathogens, and production of bioactive compounds (Tomasik and Tomasik, 2020).

Challenges in Prebiotic and Probiotic Application

a) Formulation Stability: Prebiotics and probiotics are sensitive substances and face stability issues when they are processes, stored, and passed though the GIT (Panitsidis et al., 2023). Their stability can be ensured by using advanced formulation technique such as microencapsulation, but this requires an optimized and economical production on large scale (del Valle et al., 2023). b) Dosage and Delivery: Efficacy of the prebiotics and probiotics supplements is influenced by the several factors such as determination of optimum-dose levels, delivery methods (feed grade, water grade, or direct administration) (Suez et al., 2019; Wieërs et al., 2020), compatibility with animal different species and lactation stage, feed composition, feed processing techniques, and environmental condition (Fei et al., 2023; Bottari et al., 2021). c) Species-Specific Responses: Effective supplementation of prebiotics and probiotics requires an in-depth understanding of the animal-microbes interaction (El-Saadony et al., 2021), cross-species effects (Veiga et al., 2020), and the development of tailored-formulation approaches due to variations in the GIT microbiota composition, digestive physiology, and immune system responses among different animal species (Abd El-Hack et al., 2023).

Future Research Directions

a) Long-Term Effects: It is important to investigate the long-run sustainable benefits of prebiotics and probiotics use on the GIT health, immunity, and production performance (Shoukry et al., 2023; Zommiti et al., 2020). b) Multi-Omics Approaches: The use of advanced Omics technologies such as metagenomics, metatranscriptomics, metabolomics, and host transcriptomics play critical role in obtaining the in-depth analysis of the relationship between probiotic and prebiotic supplementation and the GIT microbial dynamics, functional pathways, and host-microbe interactions (Dagnaw Fenta et al., 2023).

c) Gut-Brain Axis: Investigating the effect of prebiotics and probiotics usage on the gut-brain axis and neuroimmune interactions in animals can reveal the potential indications such as relieving stress, improving behavior, enhancing the post-environmental challenges recovery (Munni et al., 2023). d) Environmental Sustainability: It includes how the use of prebiotics and probiotics aligns with the sustainable livestock production goals, and what kind of environmental effects are they producing, such as on manure composition, greenhouse gas emissions, and nutrient recycling in agricultural systems (Hernandez-Patlan et al., 2023; Yousefi et al., 2019). e) Precision Nutrition Strategies: For optimization of the prebiotic and probiotic supplementation efficacy and cost-effectiveness, it is essential to develop the Precise Nutrition Strategies (PNS) which focus on the individual animal specifications and characteristics, GIT health biomarkers, and microbial profiles (Elghandour et al., 2024). If the above-mentioned challenges are successfully addressed, it is possible to unlock the full potential of prebiotics and probiotics in nutrition science which will improve the animal health and production, leading to a sustainable livestock industry (Belhassen, 2023).

Conclusion

Regular inclusion of the prebiotics and probiotics in the animal feeding-regime results into many beneficial effects ranging from an efficient digestive system to overall strong immunity. Prebiotics are non-degradable fiber acting as substrate for healthy gut-bacterial population. Consuming prebiotics causes an improved growth and activity of beneficial bacteria leading to an efficient utilization of absorption of nutrients. Moreover, it improves the immunity, reduces the chances of GIT related illness, and helps the animal combat the heath stress by adapting the environment. Whereas, probiotics are live microorganisms. Commonly used strains of probiotics include *Lactobacillus acidophilus*, *Bifidobacterium animalis*, *Saccharomyces boulardii*, and *Enterococcus faecium*. They improve the animal health by colonizing in the GIT and competing with the pathogenic bacteria for receptors and nutrients essential for their growth. Thus, leads to reduced gut-diseases by inhibiting the growth of pathogenic bacteria. Probiotics also lead to an improved nutrients-absorption efficiency, nutrients-availability, and the digestibility. Combined feeding of probiotics and prebiotics to the animal synergistically improves the body growth rate, nutrient absorption, reproductive performance, gut health, and sustainability in the farms economics. Probiotics exert their beneficial effects by different mechanisms such as modifying the microbial population, modifying the structure and function of gut epithelium, aggregating with pathogenic bacteria, competing for nutrients, producing specific substances (organic acid, bacteriocins, dipicolinic acid). In the past years, considerable work has been done on the beneficial effects of probiotics and probiotics, and studies are focusing on emerging aspects such as microencapsulation, precision nutrition, functional ingredient exploration, and finding new generation of probiotics with better efficiency. Successful production and usage of probiotics and prebiotics face several challenges such as formulation stability, dosage formation and delivery methods, and dealing with specie-specific responses. Large numbers of animal scientists, nutritionist, and microbiologists are focusing to explore the undiscovered areas of these in the domain of these substances.

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Chapter 02

Role of Prebiotics and Probiotics in Biomedical Sciences

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ABSTRACT

Prebiotics and probiotics imparted various health benefits. In the past few decades, they have become an area of great interest. These have been demonstrated to adapt to the preexisting intestinal microflora. Prebiotics include GOS, Inulin, XOS, FOS, and the most frequently used probiotic strains are *Lactobacillus* and *Bifidobacterium* which collectively expands the existence of probiotics. When both of these work together, they are referred as synbiotics. This chapter emphasize on the impact of pre and probiotics in different biomedical sciences particularly, oral health, immune system, cardiovascular system, hepatic encephalopathy and colorectal cancer. It further focuses on how they improve symptoms of such disorders and particularly which pre and probiotics and their dosage assist in recovery.

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INTRODUCTION

Human beings provide a natural environment for a large, dynamic and complex microbial ecosystem. In early life, the colonization of bacteria in the gut starts, which impersonates that of its mother (Dreyer and Liebl, 2018). Higher diversity and complexity of gut microbiota demonstrated in healthy adults. A population density gradient forms by these microbes, ranging from about 10^2 per milliliter in the stomach to about 10^{11}-10^{12} per milliliter in colon (Guarner and Malagelada, 2003). A large number of these microbes are present in large intestine. The conformation of microbiota may be affected by diverse factors comprising diet, the process of aging, genome, use of antibiotics, lifestyle of the host and also some endogenous factor like mucin secretion (Backhed et al., 2005).

Many metabolic and in addition protective functions can be accomplished by gut microbiota. Amendment in their composition and microbiome diversity is associated with different chronic diseases like obesity, cardiovascular disorders, type 2 diabetes, oxidative stress-related diseases, immune-mediated diseases and nonalcoholic fatty liver disease (Selvanantham et al., 2016). Constructive/beneficial intestinal microbiota can ferment indigestible dietary ingredients, known as prebiotics and obtain their survival energy by degrading nondigestible substances (Gibson et al., 2004). So, prebiotics can selectively affect these microorganisms. Alternatively, the gut microbiota influences metabolism and various functions of intestine. However, they can induce immunomodulatory molecules through which pathogens can be suppressed in healthy individuals, with help of antagonistic effects by lactic acid, generated by lactobacillus and Bifidobacterium genera (Shokri et al., 2018).

The development of bacterial resistance alongside antibiotics would provide inadequate effectiveness. Therefore, in recent years, relationship of probiotics with human health has become an area of great interest. Probiotics demonstrate distinctive ability to promote human health (Le Barz et al., 2015). Moreover, probiotics are being related clinically as coadjuvants for the exclusive properties like antiobesity, anti angiogenic, antidiabetic and antiinflammatory activities (Palumbo et al., 2016). Mostly, majority of the probiotics are commercially available as drugs or foodstuffs that contain live microbes. Hence, safety calculation of probiotics is of utmost significance before use (Ishibashi, 2001). It provides a long list of health benefits and considered as bio-therapeutic agent.

Origin

In 1995, Glenn Gibson and Marcel Roberfroid first time presented the concept of ''prebiotics'' (Gibson and Roberfroid, 1995). It was defined as an ingredient of non-digestible food, which selectively stimulates the activity or development of the single or restricted number of bacteria present in the colon, and hence develops enhancement in host health". By this definition, only limited compounds of carbohydrates, for instance short as well as long chains of lactulose, β-fructans and GOS can be categorized as prebiotics. 6th Meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP) in 2008 described "dietary prebiotics" as a particularly fermented ingredient that cause particular alterations in the activity or composition of gut microbiota, and therefore benefits host health (Gibson et al., 2010). FAO/WHO defines prebiotics as non-digestible components of food that related with distinction of the microbiota deliberate health benefits on the host (Pandey et al., 2015).

The word Probiotics is derived from a Greek term that means "for life" and earlier this term was defined as non-pathogenic living organisms and their consequences on host health. Vergin introduced this term Probiotics, while studying the injurious effects of microbial substances and antibiotics, on gut microbiota. He perceived that "probiotika" was suitable for the gut microbiota (Pandey et al., 2015). Different scientists gave different definitions of probiotics as described in Table 1.

Table 1: Definitions of Probiotics

Sr. no.	Definition	Reference
1.	Microorganisms produced substances that influence the development of further microorganisms	Lilly and Stillwell, 1965
2.	Microorganisms or substances that balance intestinal microbes.	Parker, 1974
3.	A live microbial feed additive that improve balance of intestinal microbe which proves beneficial to host animal.	Fuller, 1989
4.	An applicable mixed culture or monoculture microorganism, when given to humans or animals, benefits to host by developing properties of primeval microflora.	Havenaar and Huis, 1992
5.	Living microorganisms exerts benefits over intrinsic basic nutrition by ingesting in definite numbers.	Schaafsma, 1996
6.	Such foods which contain live bacteria, are favorable to health	Salminen et al., 1998
7.	A microbial adjunct that modulate systemic and mucosal immunity to benefit host physiology, also improves microbial and nutritional balance.	Naidu et al., 1999
8.	A product comprising, microorganisms in adequate amount, which remould microflora and improve host health.	Schrezemeir and De Vrese et al., 2001
9.	Live microorganisms, administered in sufficient number lead to benefit host health.	Hunter et al., 1999

Criteria

Prebiotics

Criteria for prebiotics are as follows:

1) Neither absorbed in the upper part of the gastrointestinal tract nor hydrolyzed by mammalian enzymes.
2) Able to modify colonic microflora towards healthier composition.
3) Cause systemic and luminal effects that prove beneficial to the health of host.
4) It should have resistance against to the acidic pH of the stomach (Gibson et al., 2010).
5) These compounds can stimulate the activity or development of colonic bacteria which leads to the improvement of host health (Sareen et al., 2012).

Probiotics

Criteria intended for microbes to be incorporated in the groups of probiotics are:

1) A strain, which is proficient of employing beneficial influence on host health, e.g. resistance to disease or improved their growth (Manigandan et al., 2012).
2) Nontoxic and nonpathogenicity.
3) Capability to adhere to the epithelial cells of intestine
4) Competence to survive on interaction with bile and at low pH while passing through gastrointestinal track.
5) Capacity to survive in food products and probability meant for manufacture of pharmacopoeia lyophilized preparations.
6) Ability to fast proliferation, with temporary or permanent colonization.
7) Generic specificity

Types of Probiotics

The majority of the probiotics are bacterial (Table 2), particularly with the species of *Lactobacillus* and *Bifidobacterium*, most commonly used bacteria (Mishra and Acharya, 2021).

Table 2: Different bacterial speices that act as probiotics.

Bacteria	Species
Lactobacillus	*L. Crispatus, Lactobacillus acidophilus, L. Gallinarum, L. casei, L. delbrueckii ssp. Bulgaricus, L. Fermentum Reuteri, L. plantarum, L. rhamnosus, L. reuteri, L.Paracasei, L. Paracasei, , L. Johnsonii, L. Breves*
Bifidobacterium	*B. Breve, B. Bifidum, B. Infantis, B. Longum Thermophilus, B. Animalis Adoloscentis*
Other moulds and yeast	*A. cerevisiae A. oryzue, C. pintolopesii, A. niger,*

Types of Prebiotics

There are various types of prebiotics. Most of them are carbohydrate groups (oligosaccharide carbohydrates (OSCs) but not all (Figure 1). Some of the carbohydrate prebiotics are as follows:

Fructans

This group consists of oligofructose or inulin and fructo-oligosaccharide. Previous studies reported that fructans can selectively develop lactic acid bacteria. Whereas current investigation showed that which bacteria can ferment them depends upon the chain length of fructans (Scot et al., 2014).

Galacto-Oligosaccharides

It is the product of lactose extension and is further divided into two sub-groups:

1) The Galacto-Oligosaccharides at C3, C4 or C6 with abundant galactose.
2) Production of GOS by enzymatic trans-glycosylation from lactose (Gibson et al., 2010).

There are some other types of GOS that depends upon sucrose extension, known as raffinose family oligosaccharides (RFO). However, its influence on intestinal microflora has not been interpreted yet (Whelan, 2013).

Starch and Glucose-Derived Oligosaccharides

Resistant starch is a category of starch i.e. resistance to digestion in upper gut. A high level of butyrate is produced by RS, which stimulates robustness so included in prebiotics (Sánchez-Zapata et al., 2011). *Bifidobacterium adolescentis* and *Ruminococcus bromii* can degrade RS. There is a glucose-derived oligosaccharide, known as polydextrose. It has not been proven but some indications suggest that it can influence Bifidobacteria (Costabile et al., 2012).

Other Oligosaccharides

Pectin is a polysaccharide from which certain oligosaccharides are originated. These types of oligosaccharide are known as pectic oligosaccharides (POS). These are established on the extension of rhamnose (rhamnogalacturonan I) or galacturonic acid (homogalacturonan) (Yoo et al., 2012).

Non-Carbohydrate Oligosaccharides

To meet the conditions of definition of prebiotics, carbohydrates are involved but there are certain non-carbohydrate compounds, acclaimed to be categorized as prebiotics e.g. cocoa-derived flavanols. Experiments determined that flavanols can stimulate lactic acid bacteria (Tzounis et al., 2011).

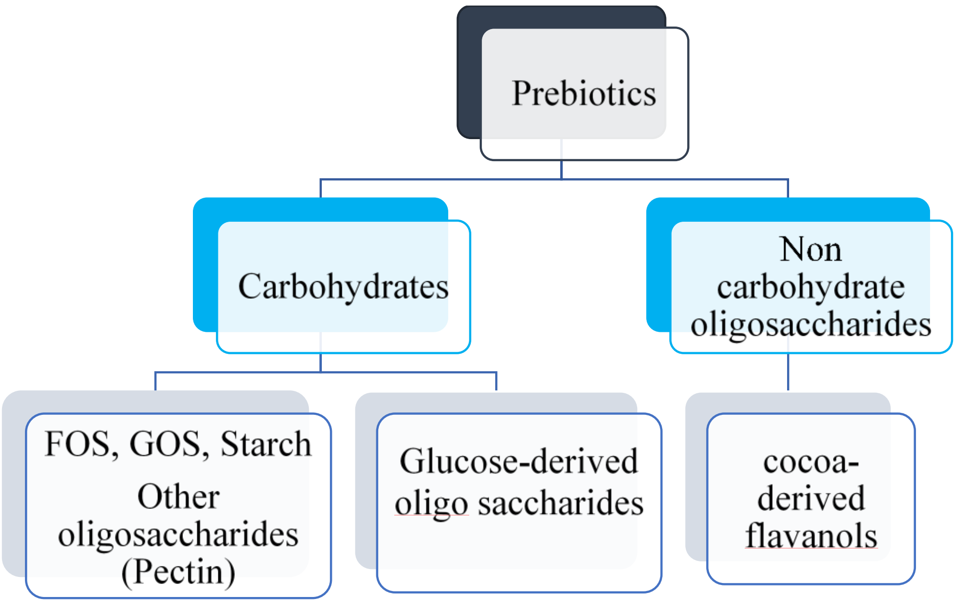

Fig. 1: Prebiotics and its types.

Mechanism of Action

Probiotics act by adhering to intestinal walls and creating an ecological niche (Coconnier and Lievin, 1997). They dynamically influence the development of other bacteria as under particular conditions they reduce pH levels and also produce H_2O_2 and bacteriocins (pediocin and nisin) (Saavedra, 1995). They competitively use nutrients for their development for instance carbohydrates (*Clostridium difficille*). Immune mechanism can be stimulated by probiotics. Such as *Streptococcus thermophiles* and *Bifidobacterium bifidum* were directed to children suffering from rotaviral diarrhea, causing immediate seroconversion of IgM and IgA antibodies assisted by the development of cells manufacturing IgM (Saavedra et al., 1998). Studies have shown the magnification of manufacturing of IgA antibodies in GIT prompted by probiotics (Buts and Bernasconi, 1990).

The beneficial effects of probiotics are not only restricted to GIT, they can also digest lactose, reduce lactose non-tolerance as its level diminishes in fermented dairy foodstuffs (Kim and Gilliland, 1983). They develop short chains of fatty acids, synthesize K and B group vitamins and polyamines (spermidine putrescine and spermine) (Buts et al., 1994). Moreover, folic acid, riboflavin, niacin level were elevated up to 20 times and B group vitamin levels were raised by bacteria in yogurt and lactic acid bacteria respectively (Deeth and Tamime, 1981).

Relationship between Prebiotics and Probiotics

Prebiotics and probiotics mutually prove beneficial. Mostly prebiotics can selectively affect the activity or growth of probiotics, which are strain and dose-dependent. During fermentation, while the path through the gut or during storage period, prebiotics function as a particular advance substrate for the strain of probiotics. Combinations of these two induce live microbial dietary supplements and generate a friendly atmosphere for their existence in gut flora. This atmosphere improves the healthy balance of microbiota (Reddy et al., 2011). The grouping of prebiotics and probiotics may have synergistic and additive influence in stimulating enhanced health conditions (Saraf et al., 2010).

Role of Prebiotics and Probiotics in Biomedical Sciences

Prebiotics and probiotics have vast applications in biomedical sciences. Its roles particularly in oral health, cardiovascular system, immune system, hepatic encephalopathy, irritable bowel syndrome and colorectal cancer are as follows and presented in figure 2.

Probiotics and Oral Health

Probiotics have a massive contribution to oral health, reducing symptoms of various dental problems. Its impact on various problems is as follows:

Probiotics and Dental Caries

Dental caries are one of the oldest dental problems. The impact of probiotics has been deliberated by using multiple test strains in numerous experiments. *L. casei* (Busscher et al., 1999) and *Lactobacillus rhamnosus GG* (Ahola et al., 2002) have the potential to inhibit the growth of *streptococci*. Yogurt comprising *L. reuteri* consumed for 2 weeks showed a definite decline in *S. mutans* count (Caglar et al., 2006). During yogurt intake period temporary decline in *S. mutans* was detected and ceased after a few days of consumption. It signifies that to accomplish an effect, continuous administration of probiotics is essential. Givenemerging evidence about the impact of probiotics on pathogens, though in clinical dentistry probiotic execution with successive less invasive involvement; might challenge the operative approach (Anderson and Shi, 2006). Yet definitely more detailed studies are needed beforehand this objective can be accomplished.

Probiotics and Periodontal Disease

Orally administered probiotics also benefit chronic periodontitis. Antagonistic interactions can control periodontal pathogens. Krasse et al. (2006) described *L. reuteri* administration can reduce gingivitis and gum bleeding. *Prevotella intermedia* and *Porphyromonas gingivalis* growth obstruct by inhabitant lactobacilli flora; 65% and 85% correspondingly (Koll-Klais et al., 2006). Probiotic strains at an optimum concentration of 10^8 CFU/ml incorporated in periodontal dressings were exhibited to reduce most recurrently isolated periodontal pathogens count: *Actinomyces sp, Bacteroides sp.* and *S. intermedius* (Volozhin et al., 2004). Utilizing periodontal dressing that contained *L. casei* and collagen showed a diminution period of 10 to 12 months afterward periodontal treatment.

Probiotics and Yeasts

Amongst the most common infectious agents is *Candida albicans* in the oral cavity. Impaired immunity conditions at older age increase its incidence. A rapid deterioration in *C. albicans* in mice was observed by Elahi et al. (2005) after the administration of the probiotic strain while testing colonization pattern of *L. fermentum* and *L. acidophilus.* Probiotics continuous consumption is necessary to reduce fungi in oral cavity, and maintains a prolonged effective period after termination of application. Stimulation of humoral and cellular aspects of mucosal protection by different lactobacilli depends upon c-interferon levels and salivary nitrous oxide. An association between the comprehensive abolition of *C. albicans* and the highest interleukin-4 secretion was observed by (Elahi et al., 2005). This result was based on animal studies, for humans further testing is required.

Probiotics and Halitosis

The oral malodor, halitosis can be controlled by consistent probiotics use. Kang et al. (2006) reported inhibition of the manufacture of volatile sulfide components (VSC) by *Fusobacterium nucleatum,* subsequently administration of *Weissella cibaria* (Kang et al., 2006). This may be attributable to the production of H_2O_2 by *W. cibaria* which hinders the proliferation of *F. nucleatum*. *Streptococcus salivarius* has also established an inhibitory influence on VSC by challenging species that cause elevation in VSC levels intended aimed at colonization sites (Burton et al., 2006). Effects of probiotics on various oral problems are mentioned in table 3.

Table 3: Effects of Probiotics on Oral health including different oral problems.

Oral health	Probiotics	Results	Reference
Dental caries	Yogurt comprising *L. reuteri*	decline in *S. mutans* number	Caglar et al., 2006
	L. reuteri	reduced gingivitis and gum bleeding	Krasse et al., 2006
	L. casei	Reduced pathogen period	Volozhin et al., 2004
Periodontitis	lactobacilli flora	Inhibit growth of *Prevotella intermedia* and *Porphyromonas gingivalis*	Koll-Klais et al., 2006
Candida albicans	*Lactobacillus rhamnosus LC705*	decline in candida infections	Hatakka et al., 2007
	Weissella cibaria	Inhibit production of volatile sulfide	Kang et al., 2006
Halitosis	*Streptococcus salivarius*	components (VSC) by *Fusobacterium nucleatum*	Burton et al., 2006)

Prebiotics and Cardiovascular System

In 2013, cardiovascular diseases (CVD) caused 30% of the death in US according to states. Direct positive prebiotic effects in this concern have not been manifested as yet but the indirect role of prebiotics on CVD is summarized in this sector (Mozaffarian et al., 2015). In a double-blind, placebo-controlled randomized clinical trial healthy individuals were exposed for 3 weeks with 10 g per day inulin. This regimen did not any significant consequence proceeding cholesterol level but reduced blood triacylglycerol and liver lipogenesis (Letexier et al., 2003).

Russo et al. (2008) elucidated the previously mentioned results by ingestion of inulin supplemented pasta configuration of 3% durum wheat vital gluten, 11% inulin and 86% semolina. Vogt et al. (2006) demonstrated administration of oral lactulose and *L. rhamnose* for 4 weeks with 25g/day in healthy individuals causing decline in amalgamation and level of TAG however no effect on cholesterol. A study on overweight individuals by up to 3 risk aspects of metabolic syndrome has demonstrated that administration of Bimuno®Galacto-oligosaccharides for 12 weeks reduced circulating cholesterol, TAG, and total HDL cholesterol ratio (Vulevic et al., 2013).

Antagonistically, prebiotics produced some SCFAs, which may have a deleterious effect on lipid profile. For instance, acetate can alter into acetyl-CoA, and acts as a substrate to produce fatty acids (Beyen et al., 1982). Even previous study reported that prebiotics is declared to be advantageous for various obesity-associated diseases, distinctly non-alcoholic fatty liver problems (Lambert et al., 2015).

Similarly, reduced serum cholesterol levels were observed in Maasai people by consuming fermented milk (Watson and Preedy, 2011). Hypercholesterolemic patients (32) in a trial exhibited significant deterioration in triglyceride, total serum, LDL, and elevation in HDL (14.5%) by ingesting low-fat yogurt comprising B. longumBL1 (Homayouni et al., 2012).

Prebiotics and the Immune System

Immunity functions can be improved by consuming prebiotics that elevate the protective microorganism (probiotic) population. Prebiotics have capability to induce the manifestation of immunity molecules, particularly cytokines.

Captivatingly, the development of an immune system of fetal can be affected as maternal prebiotics metabolites can cross the placenta (Thorburn et al., 2014). The features of distinguished prebiotic reaction on the immune system are deliberated below:

1) **FOS:** Numerous researches have exhibited that consuming FOS improves antibody counter to the influenza virus (Lomax et al., 2012). This group of prebiotics also decreases diarrhea-related fever in children. Excluding these, it can reduce the interval of disease, practice of antibiotics and occurrence of feverish seizures in offspring (Saavedra et al., 1999).

2) **GOS:** Several investigations indicated that consumption of GOS cause decline in IL-1β, however elevated C-reactive protein, IL-10 and IL-8 in adults. Likewise improves functioning of NK cells (Vulevic et al., 2015). Additionally, following GOS ingestion in infants; decreases the probabilities of eczema and atopic dermatitis (Kukkonen et al., 2007).

3) **AOS (acidic oligosaccharides):** In Low risk newborns probability of atopic dermatitis is lessen by application of AOS (Gruber et al., 2010).

4) **Inulin and oligofructose mixture**: Antibodies response with regard to viral vaccines: measles and influenza by this mixture (Firmansyah et al., 2001).

Hepatic Encephalopathy

Hepatic encephalopathy occurs when liver does not function appropriately. The foremost cause for hepatic encephalopathy is the increase in blood ammonia level. It causes various neurological and psychiatric complications, besides cognition impairment, may leads to coma and then death. In 1966, it was displayed that lactulose may possibly diminish the ammonia level in gut and progress in life quality of individuals affected with hepatic encephalopathy (Mudd et al., 2016). It exerts beneficial effects on through different ways:

1) Lactic acid is the artifact of lactulose fermentation that has the ability to reduce pH of colonic lumen by discharging H^+. Ammonia converts into ammonium by reacting with protons. This transformation make concentration gradient, it escalates the reuptake of ammonia into gastrointestinal tract from blood (Elkington et al., 1969).

2) Bacteria instead of converting amino acids into ammonia energy, consumes the energy of lactulose fermentation in existence of lactulose in the gut.

3) Lactulose inhibit glutaminase that can convert ammonia into glutamine (Mudd et al., 2016).

Lactitol also proves beneficial while treating hepatic encephalopathy and its side effects are much lower than lactulose (e.g. nausea and flatulence) (Weber, 1996).

Role in Irritable Bowel Syndrome

IBS is characterized by different bowel habits and severe chronic pain. Crohn's disease is a type of IBD, include any part from GIT from mouth to anus. In this disease, population of *Faecalibacterium prausnitzii* and *Bifidobacteria* a alongside *Bacteroides* to *Firmicutes* were reduced (Johnson et al., 2013). A randomized, double blind, placebo-controlled experiment exhibited that FOS dose (20 g/day) had no beneficial effect on IBS patient (Olesen and Gudmand, 2000). However, 6 weeks consumption of FOS (5 g per day) showed enhancement in IBS symptoms (Paineau et al., 2008).

Probiotics moderate intestinal microflora and immune response (Derwa et al., 2017). The balance of anti-inflammatory and pro inflammatory cytokine levels may altered by enteric bacteria that are vulnerable for intestinal disorders (Shi et al., 2016). In order to examine the effects of probiotics numerous animal trials showed notable anticolitis effects by regulating anti-inflammatory cytokines and down regulation of TNF-alpha and COX-2 (Chen et al., 2012). Though probiotic lessened inflammation; reduce severity of disease, whereas did not treat the basis.

Colorectal Cancer

Colorectal cancer is a multifactorial disease it ranged from genetic mutation to development of adenomatous polyps; further points towards invasive and metastatic cancer (Candela et al., 2011). It has been revealed that prebiotics (fermented goods) may have beneficial effects to oppose its development, along with its expansion by inducing apoptosis, for instance butyrate. Moreover, clinical trial established that colorectal cancer risk can be reduced by symbiotic therapy (Bifidobacterium, Lactis and *Lactobacillus rhamnosus* plus inulin) by generating necrosis of colonic cells, by reducing the multiplication rate, which causes improvement in function of epithelial barrier (Rafter et al., 2007).

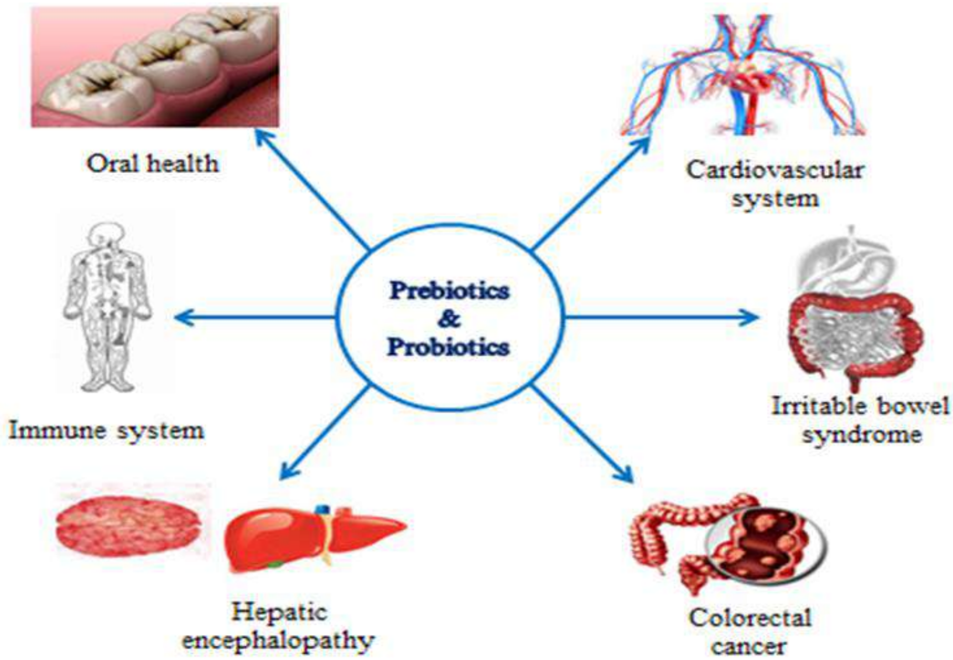

Fig. 2: Prebiotics and Probiotics in Biomedical sciences

Conclusion

In recent years astounding progress has been made in conduction of placebo- controlled, double-blind, randomized clinic studies and in endorsement and development of *in vitro* and *in vivo* research. It expanded the impact of intestinal microflora in biomedical sciences. Altering the gut microbiota, and utilizing prebiotics and probiotics signifies an essential therapeutic strategy in order to prevent and treat human diseases. Regardless of strong scientific evidence about prebiotics and probiotics further thorough research with randomised clinical trials is obligatory to validate the well-being and efficacy.

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Chapter 03

Immunomodulatory Effect of Lactobacillus and Bifidobacterium as Probiotics

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ABSTRACT

This chapter explains the immunomodulation by Lactobacillus and Bifidobacterium strains as probiotics within the human gut microbiome. The probiotics have a significant influence on the microbiota balance by promoting the regular microbial populations, strengthening the immune responses, and regulating the inflammatory processes. Their actions are based on cytokine synthesis modulation, gut barrier reinforcement and interaction with immune cells. While strains of Lactobacillus produce short chain fatty acids and vitamins that can stimulate immune function, those of Bifidobacterium also play a vital role in this process. Furthermore, they engage with dendritic cells and T cells producing regulatory T cells and regulating immunity in the long run. The chapter underscores the fact that there are probiotics for the prevention of allergies, inflammatory bowel disease and infections. Along with this, it covers emergent topics including precision probiotics and microbiome engineering which will result in personalized healthcare for each individual. To sum up, probiotics specifically Lactobacillus and Bifidobacterium are critical for gut health and the maintenance of overall bodily functions.

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INTRODUCTION

Probiotics are the commensal bacteria which because of being slimy and the exclusive microenvironment in the gut, they restrict the spread of the pathogenic bacteria therefore, making the healthy intestinal microbiota flora dominant in the gut (Raheem et al., 2021). The gut microbiome that comprises an unfathomably high number of various microorganisms is recognized as the largest determinant of many physiological processes like the digestion of foods and the extraction of nutrients, especially by affecting the type of communication between the digestive tract and the immune system. Having the disbalance of the gut microbiota, which already confirmed to be one of the most essential health guidelines, has highlighted the principal position of probiotics in the maintenance of good health (Cappellucci et al., 2024).

Importance of Gut Microbiome

Being in possession of just a perfect organism of the stomach and an updated bowel microbiota is the employment of a base which leads to the being of much better beings of us and the years of ever long life expectancy (Yeşilyurt et al., 2021). Plenty of research that is to illuminate the microbiota's linkage with multiple health aspects, for instance, with the immune system and psychiatry and the metabolism have been accomplished already, the microbiota is now called co-participators. Keep in mind that the only way to prevent the body to develop diseases like obesity, diabetes, and etc (Pei et al., 2024). Upon the influence of the abnormal microbiome is finding a solution for the problem. The microbiota lost during period of imbalance (for example in case of the woman during her pregnancy) can be recovered by probiotics experiment that leads to our health wellness restoration (Aghamohammad et al., 2023).

Benefits of Probiotics

The consumption of the probiotics can be done in two ways: through the use of supplements or consuming foods which are fermented. This has a stimulation effect for a better resultant gut microbiome which includes a higher density firm gut flora, rich in better digestion of nutrients, production of antibodies, and regulation of other functions as well (Kaźmierczak-Siedlecka et al., 2021). An underlying magnifying effect of probiotic addition harbors in prophylaxis against chronic diseases with limited inflammation.

Factors Affecting Gut Microbiota

There are many factors which pose direct effect on overall stomach microorganism population. These components include type of diet, our lifestyle, medication history and stress (Lv et al., 2021). Also, the best part is that these probiotic foods or supplements are very easily available in any food store at very cheap prices, unlike other methods that supposed to bacteria in your gut. Taking probiotics in the form of foods or supplements is one of the most convenient and practical methods you can add in your daily diet.

Exploring Probiotics: The Good Bacteria

Probiotics work via several means to provide their good benefits. They generate antibiotics that stop dangerous bacteria from growing, encourage the synthesis of immuno-stimulating chemicals, and fortify the intestinal barrier to keep germs out (Nemati et al., 2024).

Mechanisms of Action

Probiotics modulate cytokine synthesis, improve mucosal immunity, and control inflammatory reactions among other methods by which they interact with the host immune system. These interactions help explain probiotics' immunomodulatory effects (Aghamohammad et al., 2023).

Gut Barrier Function

Encouraging the integrity of the intestinal barrier—a physical and immunological barrier against infections—is one of probiotics' main functions. Probiotics stop dangerous bacteria from translocating from the colon into the circulation by fortifying the gut barrier (Choi et al., 2022).

Immune-Stimulating Compounds

It has been shown that certain probiotic strains increase the synthesis of cytokines, immunoglobulins, and other immunological mediators, therefore strengthening the body's defense systems (Nemati et al., 2024). This immune-boosting action works especially well when one is under stress or unwell.

How Probiotics Work in the Body

Both systemically across the body and locally in the stomach, probiotics may affect immune responses (Park et al., 2024). Probiotics contribute to immunological homeostasis, the equilibrium of the immune system, via interacting with immune cells and signaling pathways.

Local Effects in the Gut

Probiotics become part of the "gut team" and keep it supplying a proper functioning of gut-associated lymphoid tissue (GALT) which is related to a good health (L. Zhou et al., 2024). The protagonist from these two stories are a lovable pair of agents, who work mostly on the background, thus making the antigens from the food tolerated in the right ways. Probiotics, in particular, give the GALT mighty power of functionality, preventing it from being inflamed but, rather keep it calm for better performance of the gut's immune system.

Interaction with Immune Cells

There are two innate layers that are characterized by immune cells which can comprehend immune system and also they contain data about probiotics (Sadrifar et al., 2023). It is the cells that are being attacked the most which in turn leave the probiotics in unfavorable conditions. Such chat has both ways: the personal and the collective features (P. Zhou et al., 2024). The next way involved covers the utilization of either immune cell metabolites or this second alternative is through the immunocyte surface, which results in linking to specific receptors (Al-Najjar et al., 2024).

One of the important tasks dendritic cells (DCs) performs as antigen carriers is to initiate and regulate immune responses. Strains of Lactobacillus and Bifidobacterium can stimulate production of pro-inflammatory cytokines and induce T cell responses through their interaction with DCs.

Different tasks such as activation and regulation of immune responses are considered as the main operational duties of dendritic cells (DC) which are the professional antigen presenting cells (APC). Insulation of dendritic cells by LA and Bifidobacterium together is responsible for substances which will be the root of inflammation and finally will activate T cells (Elekhnawy and Negm, 2022).

Probiotics may likewise impact T cells, the cells considered as the chiefs of cell mediated immunity. Amongst other things, regulatory T cells, a cell species that effect immunological balance and resistance to autoimmune disorders may be differentiated by some Lactobacillus and Bifidobacterium strains (Synodinou et al., 2022).

Production of Immunomodulatory Compounds

Along with that, probiotics are renowned to produce a series of substances whose job is to regulate the immune system. Short-chain fatty acid (SCFA) is one of the most studied substances that is usually the product of the metabolism of fibers in the gut by active bacteria. Scientifically, SCFA, particularly butyrate, anti-inflammatory effects are observed even at the molecular level and they control the immune function (Khattab et al., 2023).

Probiotics are found to produce vitamins, absolutely needed for the activities of immune cells and lately, discovered to be the producers of B vitamins and vitamin K. In addition to this, they generate antibiotics which can be used to address infections, including bacteriocin: this comprises of the matter which is capable of fighting with the reproduction and spreading of the bad bacteria in the stomach.

Cross-Talk with Gut Microbiota

Probiotics foster an eco-system at the level of gut microbiota that are connected with each other and in some ways a very good cooperators towards the immune system. normal immunological responses and host’s immunological homeostasis are maintained, at least in part, by the interaction which the probiotics, commensal bacteria, and host cells engage in (Ma et al., 2023).

Sources of Probiotics in Food

A person will be able to obtain probiotics from a lot of dietary sources. But the ferments, like yoghurt, are the richest natural sources of these beneficial bacteria. Probiotic food consumption might be good for keeping the rich, varied population of the intestinal ecosystem (Aghamohammad et al., 2023).

Fermented Dairy Products

Well-liked probiotic sources include yoghurt, kefir and certain varieties of yogurt, that contain the live cultures Lactobacillus and Bifidobacterium. These dairy end-products are fermented which raises their liveliness and microbial content (Mazziotta et al., 2023).

Fermented Vegetables

Besides, these lactic acid bacteria which are responsible for the fermentation of foodstuff like sauerkraut, kimchi and naturally pickled vegetables also relieves people of any symptoms of vitamin C deficiency thus providing individuals with a strong antioxidant. Consequently, more bacteria in the stomach will be helpful, if you consume these fermented plants (Choi et al., 2022).

Non-Dairy Fermented Foods

People on a vegan diet lactose intolerant people and others do not need to miss out on the essential nutrients they would get from fermented dairy because they can have kombucha miso and tempeh as their substitutes. It cultivates a high diversity of probiotic gut bacteria (Yang et al., 2024).

Table 1 shows the status of some most notable probiotic foods and its strains: These foods, like yogurt, kefir, and sauerkraut, have included bacterial species like Lactobacillus and Bifidobacterium that are also called probiotics which support gut health.

Table 1: Common Probiotic Food Sources and Their Strains

Probiotic Food Source	Probiotic Strains
Yogurt	Lactobacillus, Bifidobacterium
Kefir	Lactobacillus, Bifidobacterium
Sauerkraut	Lactobacillus
Kimchi	Lactobacillus
Miso	Lactobacillus

Types of Probiotics: Lactobacillus and Bifidobacterium

The two most popular 'friends' within probiotic bacteria genera, Lactobacillus and Bifidobacterium, are highly revered for their multiple health promoting benefits. Usually found in fermented products and of a wide abundance in nutritional additives, these probiotic strains offer a number of health advantages for the immune system and the gut (Tarique et al., 2022).

Lactobacillus

Lactobacilli is widely present in the vaginal and gastrointestinal tract lactate acid producing bacteria. Lactobacilli constitute the largest species of this beneficial group. Lactic acids are organic acids that are produced due to by bacteria possessing the capability of fermenting carbs. Probiotic milk products and supplements contain Lactobacillus among other types of bacillar bacterial lineages (Tarique et al., 2022).

Bifidobacterium

There are anaerobic bacteria belonging to Bifidobacterium species which live in the colon and contribute to digestion of food fibers. They produce short-chain fatty acids and go to battle with the bad bacteria for the resources, therefore they are indispensable for the maintenance of the gut health. Some strains of Bifidobacterium exist in breast milk and yoghurt (Braga et al., 2024).

Combined Benefits

Often, the functioning of Lactobacillus and Bifidobacterium will prove synergistic to result in a bettering of the efficacy of probiotic therapy. These baking the targeted gut areas and regain the balance of immune system helps the probiotic creatures supply the comprehensive immune system and digestive health support (Kim et al., 2022).

The Role of Lactobacillus in Probiotic Benefits

Among the most well researched probiotics, Lactobacillus strains have been linked to a host of health advantages. These helpful bacteria enhance intestinal barrier function, have immunomodulatory effects, and improve general health (Dong et al., 2022).

Immunomodulation by Lactobacillus

Species of Lactobacillus interact with immune cells in the lymphoid tissue linked to the gut to affect the cytokine and immunoglobulin synthesis. These immunomodulatory effects improve immune monitoring of infections and help control inflammatory reactions (Tarique et al., 2022b).

Gut Barrier Support

Lactobacillus strains increase the production of mucus and the expression of tight junction proteins, therefore enhancing the integrity of the gut epithelium. Probiotics from Lactobacillus lower intestinal permeability and stop the transit of toxic chemicals by fortifying the gut barrier (Aghamohammad et al., 2022).

Anti-Inflammatory Effects

Anti-inflammatory substances produced by certain Lactobacillus species, notably butyrate and derivatives of indole, assist reduce inflammatory reactions in the gastrointestinal system. Lactobacillus probiotics help to maintain immunological homeostasis by balancing pro- and anti-inflammatory messages (Heldner et al., 2023).

Allergy Prevention

When the immune system reacts too strongly to innocuous things like dust or pollen, allergies result. Research has shown that early exposure to probiotics—especially Lactobacillus strains—can help build immunological tolerance and hence avoid allergies. Anti-inflammatory cytokines are produced and regulatory T cells are believed to be induced as mediators of this impact (Rousseaux et al., 2023).

Bifidobacterium: Another Key Probiotic Species

Strains of bifidobacterium are well known for their advantages to immune system and gut health, which makes them useful components of probiotic preparations. Short-chain fatty acids are produced, dietary fibers are fermented, and immunological responses are modulated by these probiotics (Begum et al., 2021).

Fermentation of Dietary Fibers

Species enzymes of bifidobacteria split up the complex carbohydrates which includes resistant starch and dietary fiber. They refer to the group of the microorganisms which produce the short-chain fatty acids by way of the fermentation and therefore can provide power for colonocyte and simultaneously anti-inflammatorily influence them (Ferreira et al., 2021).

Short-Chain Fatty Acid Production

Bifidobacterium fermentation forms three key short-chain fatty acids, these being acetate, propionate and butyrate. These metabolites can be classified into pH controlling substances, food substances for helpful bacteria in the colon, or immunomodulators according to their functions (Rocha-Ramírez et al., 2021).

Immune Modulation by Bifidobacterium

In gut-associated lymphoid-tissue, bifidobacterium strains that come in contact with dendritic cells and T cells eventually load them to mature and activate, thus affecting immune cell development and activation. Symbiotic bacteria that belong to bifidobacteria family afford auxiliary T cell response in the regulation of excessive inflammation, and maintains the homeostasis of immune system (Cristofori et al., 2021).

Immunomodulatory Properties and Mechanisms

Probiotics, especially species of Lactobacillus and Bifidobacterium, interact with the host immune system to produce their immunomodulatory effects. By intricate cross-talking with cytokines, immune cells, and signaling pathways, these helpful bacteria mould immune responses and encourage tolerance (Synodinou et al., 2022).

Cytokine Regulation

The control of cytokine emission from immune cells in the gut is one of the functions executed by probiotics. Probiotics manage the immune response and suppress excessive inflammation by balancing the pro- and anti-inflammatory cytokines (Elekhnawy and Negm, 2022).

Mucosal Immunity

Probiotics such as Lactobacillus and Bifidobacterium are found to boost secretory IgA antibodies and antimicrobial peptides, thus mucosal immunity will be enhanced. These immuno-elements preserve the immune barriers against allergens and pathogens (Kostolomova et al., 2022).

Inflammatory Pathway Modulation

Probiotics exhibit their regulating potential via activating the specific macroreceptors such as nucleotide-binding oligomerization domain-like receptors and toll-like receptors. As a result, they influence the inside circulate inflammatory signaling pathways of immune cells. Probiotics will continue inhibiting the activation of MAPK signaling as well as NF-kB – the gut pathways that cause inflammation. Therefore, the suppression of pro-inflammatory pathways will immerse in the body hence, weakening inflammation (Liu et al., 2022).

The following table presents probiotics' role in how the system works. It encompasses immune regulation of cytokines, immunological protection of mucosa and to modulate inflammatory pathways.

Table 2: Mechanisms of Probiotic Action

Mechanism	Description
Cytokine Regulation	Balancing pro-inflammatory and anti-inflammatory cytokines in the gut
Mucosal Immunity	Enhancing the production of secretory IgA antibodies and antimicrobial peptides
Inflammatory Pathway Modulation	Modulating toll-like receptor and NF-kB signaling pathways in immune cells

Lactobacillus and Immune Regulation

The Lactobacillus species of pro-biotics, in terms of immunomodulatory aspects, have been the most extensively studied ones for their effect on the human immune system. These friendly bacteria get involved into some processes which change cytokine production or even immune response and they do it by collaborating with such immune cells as dendritic cells and macrophages (Tarique et al., 2022).

Macrophage Activation

Macrophages may be induced to generate cytokines including tumor necrosis factor-alpha (TNF-alpha) and interleukin-10 (IL-10) by lactobacillus species. Maximizing immune surveillance, this activation prepares macrophages for phagocytosis and antigen presentation (Lv et al., 2021).

Dendritic Cell Maturation

Enhancing T cell activation, dendritic cells exposed to chemicals produced from Lactobacillus mature and upregulate co-stimulatory molecules. Lactobacillus and dendritic cells interact to provide immunological memory and adaptive immune responses (Sadrifar et al., 2023).

Tolerance Induction

Probiotics from the Lactobacillus family have been shown to activate regulatory T cells, which are essential for preserving immunological tolerance and averting autoimmunity. Lactobacillus species aid to reduce abnormal immune responses by encouraging a tolerogenic milieu in the gut (Pei et al., 2024).

Bifidobacterium and Immune Responses

The capacity of probiotics from the bifidobacterium to control immunological responses and encourage intestinal immune tolerance is well recognized. Through interactions with mucosal surfaces and immune cells, these helpful bacteria affect the inflammatory and cytokine pathways (Al-Najjar et al., 2024).

Cytokine Modulation

Strains of Bifidobacterium can control how much interleukins and interferons immune cells produce. Probiotics of the Bifidobacterium family assist to reduce over reactionary immune responses by adjusting the cytokine profile towards anti-inflammatory messages (Khattab et al., 2023).

Intestinal Epithelial Integrity

As they increase the production of mucins and tight junction proteins, species of bifidobacterium help to maintain the integrity of the intestinal epithelium. Inflammation is reduced by this barrier-strengthening action, which stops poisons and bacteria from translocating across the gut lining (Ma et al., 2023).

Anti-Inflammatory Metabolites

In the stomach, short-chain fatty acids like propionate and acetate produced by fermentation of Bifidobacterium have anti-inflammatory effects. A healthy inflammatory response and immune cell activity are maintained in part by these metabolites (Aghamohammad et al., 2023).

Synergistic Effects of Lactobacillus and Bifidobacterium

Although strains of Lactobacillus and Bifidobacterium have each been linked to immunomodulatory effects, research has also indicated that their combined usage may have synergistic benefits. This is so because, while having distinct modes of action, these two probiotic strains may enhance one another's benefits.

For instance, it is well known that Lactobacillus strains may generate SCFAs, and that Bifidobacterium strains can increase NK cell activity. These strains may affect gut health and immunological responses more significantly when combined.

Combining these two probiotic species allows formulations to target various gut areas, control a range of immune responses, and improve gut health generally (Mazziotta et al., 2023).

Complementary Mechanisms

Strains of Bifidobacterium and Lactobacillus have complimentary effects on the immune system and gut flora. By the means of which Bifidobacterium strains promotes fermentation and immune regulatory processes, and Lactobacillus species encourages immunity at the mucosal level and gut barrier function, therefore, stabilizing microbial interactions (Tarique et al., 2022).

Enhanced Immune Regulation

Among lactic acid and bifidus groups of probiotics a combination intake (taken together) has been shown to enhance the immune system control and anti-inflammatory response. As a whole, these probiotic strains communicate with immune cells, provide signals for the synthesis of enzymes and cytokines which in turn maintain immunological homeostasis (Kim et al., 2022).

Comprehensive Gut Health Support

Probiotic formulations with both Bifidobacterium and Lactobacillus provide complete support for immune system, gastrointestinal health, and general health. Utilizing the synergistic benefits of these helpful bacteria, people may maximise their immune responses and microbial balance (Dong et al., 2022).

Clinical Applications and Evidence

The clinical settings of infectious diseases, inflammatory bowel disease (IBD), and allergies are among various clinical contexts in which the immunomodulation of Lactobacillus and Bifidobacterium has been extensively investigated (Tarique et al., 2022).

Allergies

Several research provides evidence of the fact that early probiotic intake, especially of Lactobacillus species, lowers the odds of childhood allergy development of the little ones. For instance, eczema and allergic sensitization were less likely to strike newborns who received L. rhamnosus during their first six months of life, according to a research that was published in the Journal of Allergy and Clinical Immunology (Heldner et al., 2023).

Inflammatory Bowel Disease (IBD)

Among the chronic inflammatory diseases of the gastrointestinal tract that comprise IBD are ulcerative colitis and Crohn's disease. According to studies, probiotics especially Bifidobacterium strains can lessen inflammation and restore the gut microbiota's balance, therefore easing IBD symptoms.

Probiotics Bifidum and B. longum, among others significantly increased clinical remission rates in ulcerative colitis patients, according to a meta-analysis published in the World Journal of Gastroenterology (Rousseaux et al., 2023).

Infectious Diseases

Additionally investigated is the ability of probiotics to both cure and prevent viral disorders. According to a Cochrane Database of Systematic Reviews systematic study, probiotics specifically, strains of Lactobacillus and Bifidobacterium can lower the frequency and length of respiratory tract infections in children (Begum et al., 2021).

Prebiotics and the Probiotic-Host Crosstalk

Supporting probiotic survival and function are prebiotics, non-digestible fibers that specifically encourage the development of beneficial bacteria in the gut. Prebiotics improve probiotic strain colonization and function by giving them a substrate for fermentation (Ferreira et al., 2021).

Prebiotic Effects on Gut Microbiota

Prebiotics such resistant starch, oligofructose, and inulin go to the colon undigested and feed probiotic microorganisms. Short-chain fatty acids and other metabolites that support the gut flora and host health are produced by probiotics via fermentation of prebiotics (Rocha-Ramírez et al., 2021).

Symbiotic Approaches

In symbiotic preparations, the combined benefits of probiotics and prebiotics may be increased. Prebiotics feed probiotics, which increases their survival and activity in the gut, while probiotics convert prebiotics into bioactive molecules (Cristofori et al., 2021).

Host-Microbiota Interactions

Excluding prebiotics, probiotics and as well as the interaction of host-gut microbiome help to strengthen the immune system and engender intestinal wellness plus put general wellbeing in good shape. At the same time, they not only constitute a beautiful web of interaction but they also do together as one. Prebiotics can take into account that the probiotic species, which the consumers already know, i.e. Lactobacillus and Bifidobacterium, be at their best and, by their turn, lead the activity of the immunity and format the microenvironment (Synodinou et al., 2022).

Safety Considerations for Probiotic Consumption

Although prebiotics have numerous health benefits, please take care about the effect and 'safety side' along with the habitual health measures while using it. Thus, getting accustomed to the negative sides and preventive measures of due probiotics foods intake or taking of supplements of probiotics is the need of the hour which will make that suitable usage of probiotics foods or supplements as preventive or curative and not detrimental (Elekhnawy and Negm, 2022).

Quality Control and Product Selection

Decide once again on the fermented foods and probiotics from respectable representatives that are equipped with the quality assurance process. In order to trust the efficiency and safety of the product, select the products of determined clinical strains Bifidobacterium and Lactobacillus exclusively (Kostolomova et al., 2022).

Dosage and Administration

Also, in case you choose supplementary probiotics, make sure to follow the directions printed on the product's label. The stomach problems like pain or gaseous feeling in the beginning, can be minimized by increasing little by little and start with lower dose this should be done is the best way (Liu et al., 2022).

Individual Sensitivities and Medical Conditions

Individuals who already have some medical problem need to must seek medical advice from their physician prior to engaging into probiotics therapy. Prescribing the exact species of probiotics is indispensable, since some of them may worsen the effect of the prescription drugs or aggravate the existing diseases that a patient has (Mazziotta et al., 2023).

Table 3 below shows several safety considerations including get a safe probiotic product on market, stick to the experiments and consider visit your healthcare provider if you have medical conditions.

Table 3: Mechanisms of Probiotic Action

Safety Consideration	Description
Quality Control	Choosing reputable probiotic products with specific strains and clinical evidence
Dosage Guidelines	Following recommended dosages to prevent adverse effects and optimize benefits
Consultation with Healthcare Provider	Seeking professional advice for individuals with medical conditions or sensitivities

The Emerging Future of Probiotics in Healthcare

Probiotics in medicine have a bright future with creative uses and therapeutic treatments as long as study on the complex processes of probiotic-host interactions continues. Probiotics are changing the way that people approach health and illness management with everything from individualized microbiome-based therapies to focused immune regulation (Dong et al., 2022).

Precision Probiotics

Precision probiotics catered to specific microbial profiles are being made possible by developments in microbiome sequencing and analysis. Using certain bacterial strains that are associated with health outcomes, customized probiotic formulations may correct particular gut imbalances and enhance immune system performance (Heldner et al., 2023).

Therapeutic Applications

The application of probiotics as supplements for an increasing number of ailments is no longer news, including the treatment of allergies, metabolic problems, and inflammatory bowel diseases. Aims of guided probiotics are to relieve dysbiosis symptoms, return gut balance, and reprogram immunological reactions (Begum et al., 2021).

Microbiome Engineering

Synthesis biology and other the new technologies which are emerging, have made it to be possible to produce probiotics having the improved functions and therapeutic potential. Among the engineered strains, those that home in precisely on harmful bacteria, communicate with host cells more effectively, deliver acting agents or prescribe different cures should be researched since they have great potential (Ferreira et al., 2021).

Summarizing, due to the fact that the derivation of two species, Lactobacillus and Bifidobacter, plays the major role in the establishment of correct immune system function, intestinal health prevention, and general health promotion, probiotics are all that we need in order to achieve the goals. Such bacteria, which help to create balanced ecosystems, pass essential trophic functions, rely on interaction with the open immunity system and regulate cytokines production which results in more advanced immunologic and inflammatory conditions. By their constant use and researches in the field of probiotic science practitioners are able to make the therapy as individual as possible; a number of creative approaches to probiotic treatment are possible in the near future in healthcare. Embracing the prospect of probiotics as a pillar of health of all intestines might help people strengthen an inside wall, enhance resistance to infectious agents, and also to reserve the "healthy state" of their gut (Rocha-Ramírez et al., 2021).

Conclusion: Probiotics, a Vital Part of Gut Health

Firstly, probiotics improve the immune system and microbiome of the gut. In fact, the lactobacillus and bifidobacteria are the most helpful bacteria for the immune system, gut health, and general health. Immunomodulators are the probiotics which core job is to regulate the immune system, cure inflammations, and prevent from infections (Nemati et al., 2024).

The understanding of the relationship of probiotics to the gut microbiota and the host immune system enables the people to make the right choices that will promote overall well-being. Day-to-day regimen can be improved for digestion, resilience of immune system, and balancing existing microbial environment by consuming foods, supplements and symbiotic formulation (L. Zhou et al., 2024).

It is possible that the drugs will be now designed especially for each person individually instead of making a one for all medicine progress. In other words, the use of probiotics is made clever. Besides that, we plan to do genetic engineering of the probiotic microbes that will be used to treat the immune system and other diseases. Also, we will create artificial microbiome and renovate the microorganisms with changed functionality (Park et al., 2024).

Probiotics are called like the god-gifts to our digestive system from where they give us the pen and show us the way to write the destiny of our health. Simultaneously, with the same substances that are significant for digestion, they boost metabolism, immunity support, and energy retention. Now see that after practicing for a couple of times you will be familiar with this road that will make you healthier and get you a nice figure (Al-Najjar et al., 2024).

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Chapter 04

Use of Probiotics and Prebiotics in Livestock Productivity

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ABSTRACT

The digestive health of livestock is integral to their overall well-being and productivity in agricultural operations. A healthy digestive system ensures efficient absorption of nutrients from feed, directly impacting growth rates and performance, especially in meat-producing animals. Additionally, a well-functioning digestive system acts as a barrier against pathogens, supporting the animal's immune system and reproductive performance. Probiotics and prebiotics play significant roles in promoting digestive health by enhancing the population of beneficial bacteria in the gut and creating a favorable environment for their growth. Probiotics, such as *Lactobacillus* and *Bifidobacterium* species, contribute to improved digestion and nutrient absorption while inhibiting the growth of harmful pathogens. Prebiotics, including oligosaccharides and fiber, selectively promote the growth of beneficial microorganisms in the gut, leading to a healthier and more balanced gut microbiota. Ultimately, maintaining optimal digestive health in livestock not only benefits animal welfare but also translates into economic advantages for producers through higher production yields and improved quality of animal products like meat, milk, and eggs. Future research is likely to focus on tailoring livestock nutrition based on individual animals' microbiome profiles. Understanding the unique microbial communities in different species and optimizing diets to support specific beneficial bacteria can enhance overall animal health and performance.

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INTRODUCTION

Digestive health or gut health plays a crucial role in livestock production as it directly influences the overall well-being, growth, and productivity of animals. Several key aspects highlight the importance of digestive health in livestock production. A healthy digestive system is essential for efficient absorption and utilization of nutrients from feed. Proper digestion ensures that the nutrients, including proteins, carbohydrates, fats, vitamins, and minerals, are broken down and absorbed in a form that can be used by the animal's body for growth, maintenance, and reproduction (Ohara et al., 2020).

Livestock with optimal digestive health tend to exhibit better growth rates and improved performance. This is particularly crucial in meat-producing animals where rapid and efficient growth directly impacts the economic viability of the operation. A well-functioning digestive system acts as a barrier against pathogens and helps prevent the entry of harmful microorganisms into the bloodstream. Probiotic bacteria in the gut can also compete with and inhibit the growth of pathogenic bacteria, contributing to disease resistance (Van Zyl et al., 2020)

The gut plays a significant role in supporting the animal's immune system. A healthy digestive system contributes to the overall immune response, helping animals resist infections and diseases. This is particularly important in intensive livestock farming systems where animals may be more susceptible to diseases due to close confinement. Digestive health influences the reproductive performance of animals. Nutrient availability and hormonal balance, both of which are influenced by the digestive system, play key roles in the success of reproductive processes, including estrus, conception, and fetal development (Amin, 2014).

Ultimately, maintaining optimal digestive health in livestock results in economic benefits for producers. Healthy animals are more efficient in converting feed into valuable products such as meat, milk, or wool, contributing to higher production yields and profitability. The digestive process influences the quality of animal products such as meat, milk, and eggs. Proper digestion contributes to the development of desirable characteristics in these products, including taste, texture, and nutritional composition. Probiotics are live microorganisms, mainly bacteria and yeast that confer health benefits to the host when administered in adequate amounts. In the context of animal nutrition, probiotics are used to promote a healthy microbial balance in the digestive tract (Ezema, 2013).

They exert positive effects on the host by enhancing the population of beneficial bacteria, which can improve digestion, nutrient absorption, and overall gut health. Probiotics can also help in preventing the colonization of harmful pathogens by competing for resources and producing substances that inhibit their growth. Common probiotic strains used in animal nutrition include various species of *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces* (Simon et al., 2021).

Prebiotics, on the other hand, are non-digestible substances that selectively promote the growth and activity of beneficial microorganisms already present in the host's gut. These substances are typically carbohydrates, such as oligosaccharides and fiber, that resist digestion in the upper gastrointestinal tract and reach the colon where they serve as a substrate for beneficial bacteria. By providing a favorable environment for the growth of beneficial microorganisms, prebiotics contribute to a healthier and more balanced gut microbiota. Common prebiotics in animal nutrition include fructooligosaccharides (FOS), inulin, galactooligosaccharides (GOS), and various types of dietary fibers (Wilson and Whelan, 2017).

Table 1: Differences between probiotics and prebiotics

Characteristic	Probiotics	Prebiotics
Definition	Live microorganisms providing health Benefits when administered in adequate amounts.	Non-digestible substances promoting the growth and activity of beneficial microorganisms in the Gut.
Nature	Living organisms (bacteria, yeast).	Non-living, typically complex carbohydrates.
Function	Directly contribute to the microbial population, offering health benefits Through their presence and activities.	Indirectly benefit the host by promoting the growth and activity of existing beneficial Microorganisms in the gut.
Administration	Administered as supplements or added to Feed.	Added to the diet as ingredients or naturally Present in certain feed materials.
Examples	*Lactobacillus acidophilus*, *Bifidobacterium animalis*, *Saccharomyces cerevisiae*.	Inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), MOS, dietary fibers.

Gut Microbiota in Livestock

The gut microbiota of livestock plays a crucial role in their overall health, growth, and performance. Recent research has focused on understanding the composition and function of these microbial communities within the gastrointestinal tract of various livestock species. The gut microbiota in livestock, including cattle, sheep, and poultry, has been found to influence nutrient utilization, immune system development, and resistance to pathogens. This symbiotic relationship between thehost and its microbiota has far-reaching implications for the production and sustainability of livestock farming (Rawal et al., 2024).

Studies have revealed that the composition of gut microbiota in livestock is influenced by various factors, including diet, genetics, and environmental conditions. For instance, changes in the diet composition can lead to alterations in the microbial community structure, impacting the animal's ability to digest and absorb nutrients efficiently. Understanding these interactions can help optimize animal nutrition, leading to improved feed efficiency and growth rates. Moreover, the gut microbiota is involved in the fermentation of complex carbohydrates, producing short-chain fatty acids that serve as an energy source for the host and contribute to overall metabolic health (Blaak et al, 2020).

Furthermore, the gut microbiota in livestock has been linked to disease resistance and overall animal welfare. A balanced and diverse microbial community can enhance the host's immune response, providing protection against various pathogens. Researchers are exploring strategies to manipulate the gut microbiota through probiotics, prebiotics, and other nutritional interventions to promote a healthier microbial balance in livestock. This emerging field of research holds promise for advancing sustainable and efficient livestock production practices (Kraimi et al., 2019).

Gut Microbiota Composition and Function

The composition and function of gut microbiota in various livestock species have been extensively studied, revealing critical insights into their impact on host health and performance. Research indicates that the microbial community in the gastrointestinal tract plays a pivotal role in nutrient metabolism, immune system modulation, and disease resistance. For instance, studies in cattle, as highlighted by Ley et al. (2008), demonstrate the co-evolution of mammals and their gut microbes, emphasizing the intricate relationship between host genetics and microbiota.

Additionally, investigations into pigs by McCormack et al. (2017) underscore the potential link between gut microbiota and feed efficiency, offering avenues for optimizing nutrition strategies.Similarly, research on chickens, as explored by Stanley et al. (2014), emphasizes the importance of the gut microbiota in maintaining overall health and productivity. These findings collectively underscore the significance of understanding and manipulating the gut microbiota to enhance the well-being and productivity of diverse livestock species.

Importance of a Balanced Microbial Community

The importance of a balanced microbial community for efficient digestion and nutrient absorption in animals is well-documented in scientific literature. Research has consistently shown that the gut microbiota plays a crucial role in breaking down complex dietary components, aiding in the digestion of fibers, proteins, and carbohydrates. This symbiotic relationship enhances the overall efficiency of nutrient utilization by the host. Studies, such as those conducted by Sonnenburg and Bäckhed (2016) highlights the microbial influence on the extraction of energy from indigestible polysaccharides, contributing to the host's metabolic processes. Additionally, a balanced microbial community contributes to the production of short-chain fatty acids, as elucidated by den Besten et al. (2013), which not only serve as an energy source but also exert positive effects on host metabolic health. Understanding and promoting a balanced microbial community in the gut are crucial for optimizing nutrient absorption, fostering animal health, and enhancing overall efficiency in livestock production.

Suitable Sources of Probiotics for Livestock

Probiotics, live microorganisms beneficial to the host when administered in adequate amounts, are sourced from various strains of bacteria, yeast, and fungi. Research has identified *Lactobacillus* and *Bifidobacterium* species as prominent bacterial probiotics suitable for livestock. For instance, a study by Wang et al. (2017) examined the effects of a *Bifidobacterium* strain on the growth performance and immune response of weaned piglets, revealing positive impacts on gut health and immune function. Additionally, *Saccharomyces cerevisiae*, a yeast probiotic, has been extensively studied for its ability to enhance fiber digestion and nutrient absorption in ruminants. Understanding the diversity of probiotic sources is crucial for tailoring nutritional strategies to specific livestock species and optimizing their health and productivity.

Mode of Action of Probiotics

The mode by which probiotics exert their beneficial effects in livestock nutrition are multifaceted. Research has demonstrated that probiotics contribute to gastrointestinal health by competing for adhesion sites with pathogenic bacteria, as explored by Yang et al. (2015) in their study on broiler chickens. Furthermore, the production of antimicrobial substances, such as organic acids and bacteriocins, has been identified as a key mechanism through which probiotics inhibit the growth of harmful pathogens. Additionally, probiotics play a role in modulating the immune response, influencing the balance and functionality of the host's immune system. A comprehensive understanding of these mechanisms is crucial for developing targeted probiotic interventions to improve livestock health and performance.

Applications in Disease Prevention

Probiotics have demonstrated substantial potential in disease prevention across various livestock species, offering a sustainable and effective approach to reduce the incidence and severity ofinfections. Research by Callaway et al. (2008) highlights the use of probiotics as a strategy to mitigate the impact of bacterial pathogens in cattle. In this study, the administration of a specific *Bacillus*- based probiotic reduced the shedding of *Escherichia coli* O157:H7 in cattle feces, demonstrating the ability of probiotics to modulate the gut environment and limit the dissemination of pathogenic bacteria.Poultry farming, particularly broiler production, has faced challenges related to infectious diseases, such as necrotic enteritis. A study by Wu et al. (2014) investigated the preventive effects of probiotics on necrotic enteritis in broilers. The research demonstrated that the supplementation of broiler feed with a combination of *Lactobacillus*-based probiotics reduced the severity of necrotic lesions in the intestines, highlighting the potential of probiotics as a preventive measure against poultry diseases. In swine production, where gastrointestinal diseases pose significant economic threats, probiotics have shown promise in disease prevention. A case study conducted by Yang et al. (2019) examined the use of a *Bacillus*-based probiotic in weaned piglets to control post-weaning diarrhea. The results indicated a reduction in the incidence and severity of diarrhea, along with improvements in growth performance, suggesting that probiotics can play a role in preventing gastrointestinal disorders in swine. Aquaculture, an industry susceptible to bacterial and viral diseases, has also witnessed the application of probiotics for disease prevention. A study by Nayak (2010) investigated the use of probiotics in shrimp aquaculture, demonstrating their ability to enhance the immune response and protect against pathogenic infections. The findings underscore the potential of probiotics as a sustainable and environmentally friendly alternative to antibiotics in aquaculture disease.

These case studies exemplify the diverse applications of probiotics in preventing diseases across livestock sectors. While specific mechanisms vary, the overall theme is the ability of probiotics to modulate the gut microbiota, enhance immune responses, and create an environment less conducive to the proliferation of pathogenic organisms. Continued research in this field holds the promise of refining probiotic interventions for disease prevention in livestock production, contributing to the overall health and sustainability of farming practices.

Probiotic Strains Effective Against Specific Pathogens

Research into probiotic strains effective against specific pathogens is pivotal in developing targeted strategies for disease prevention in livestock. A notable study by La Ragione et al. (2004) investigated the efficacy of *Enterococcus faecium* SF68 in preventing enteric infections in piglets caused by enterotoxigenic *Escherichia coli* (ETEC). The research revealed that *E. faecium* SF68 administration significantly reduced the incidence and severity of ETEC-induced diarrhea in piglets, suggesting the strain's potential as a specific probiotic intervention against enteric pathogens in swine.In poultry production, *Salmonella* and *Campylobacter* are common pathogens that pose significant challenges. A study by Kizerwetter-Świda et al. (2019) explored the inhibitory effects of *Lactobacillus reuteri* strains against *Salmonella enteritidis* in chickens. The research demonstrated that specific *L. reuteri* strains effectively reduced *Salmonella* colonization in the ceca of broiler chickens, showcasing the strain-specific effectiveness of probiotics in controlling specific poultry pathogens.

Additionally, the application of probiotics to combat bacterial infections in aquaculture has been a focus of research. A study by Balcazar et al. (2007) investigated the potential of *Bacillus subtilis* as a probiotic against the fish pathogen *Aeromonas hydrophila*. The findings indicated that *B. subtilis* supplementation in the diet of Nile tilapia significantly reduced the mortality rate associated with *A. hydrophila* infection, highlighting the strain-specific probiotic efficacy in aquaculture.

Furthermore, ongoing research in the field continues to identify novel probiotic strains effective against specific pathogens. The strain-specificity in probiotic action underscores the importance of precision in selecting and applying probiotics in livestock systems. As our understanding of microbial interactions advances, tailoring probiotic interventions to target specific pathogens becomes increasingly feasible, offering a valuable tool in the arsenal against infectious diseases in diverse livestock sectors (Papadimitriou et al., 2015).

Improving Feed Efficiency

The studies investigating the efficacy of probiotic strains against specific pathogens in livestock provide valuable insights into targeted disease prevention strategies. La Ragione et al. (2004) conducted a noteworthy investigation using *Enterococcus faecium* SF68 in piglets to combat enterotoxigenic *Escherichia coli* (ETEC) infections. The study demonstrated a significant reduction in both the incidence and severity of ETEC-induced diarrhea, indicating the potential of *E. faecium* SF68 as a specific probiotic intervention for enteric pathogens in swine. This study underscores the importance of identifying probiotic strains with targeted efficacy against particular pathogens to enhance the precision and effectiveness of disease prevention strategies in pig farming.

In the realm of poultry production, Kizerwetter-Świda et al. (2019) explored the inhibitory effects of *Lactobacillus reuteri* strains against *Salmonella enteritidis* in broiler chickens. The findings highlighted the strain-specific effectiveness of *L. reuteri* in reducing *Salmonella* colonization in the ceca of broiler chickens. This study contributes to the understanding of probiotic strain selection as a crucial factor in controlling specific poultry pathogens. The application of *L. reuteri* strains showcases their potential as targeted interventions for enhancing food safety and minimizing the prevalence of *Salmonella* infections in poultry.

Balcazar et al. (2007) delved into the use of *Bacillus subtilis* as a probiotic against the fish pathogen *Aeromonas hydrophila* in Nile tilapia in the context of aquaculture. The study demonstrated that *B. subtilis* supplementation in the diet significantly reduced the mortality rate associated with *A. hydrophila* infection. This research contributes to the expanding body of knowledge on probiotic applications in aquaculture, emphasizing the importance of strain-specific efficacy against specific aquatic pathogens. The findings have implications for sustainable aquaculture practices by mitigating disease risks and improving overall fish health.

Growth Promotors in Livestock

The exploration of probiotics as growth promoters in livestock has been a subject of considerable research, aiming to enhance animal performance, feed efficiency, and overall productivity. Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts, are recognized for their potential to positively influence the growth and development of livestock. Numerous studies have investigated the impact of probiotics on growth promotion in various livestock species. A study by Mountzouris et al. (2010) evaluated the effects of a multi-strain probiotic on growth performance. The research demonstrated that broilers supplemented with the probiotic exhibited improved body weight gain, feed conversion ratio, and overall feed efficiency. The probiotic intervention contributed to enhanced nutrient utilization, promoting better growth rates in poultry.

Similarly, research in pig farming has explored the application of probiotics to enhance growth performance. A study conducted by Liu et al. (2017) investigated the effects of a *Bacillus*-based probiotic on the growth parameters of weaned piglets. The findings revealed that piglets receiving the probiotic supplementation showed increased average daily gain and improved feed conversion efficiency compared to the control group. This indicates the potential of probiotics in optimizing nutrient utilization and supporting efficient growth in swine.

In addition to poultry and swine, the application of probiotics as growth promoters has been examined in cattle. A study by Shokryazdan et al. (2017) investigated the effects of *Lactobacillus*- based probiotics on the growth performance of dairy calves. The research demonstrated that calves receiving the probiotic supplementation exhibited higher body weight gain and improved feed efficiency, suggesting a positive impact on growth rates in young dairy cattle. The mechanisms underlying the growth-promoting effects of probiotics are diverse. Probiotics contribute to improved nutrient absorption, modulation of the gut microbiota, and enhanced immune function.

Prebiotics: Supporting the Microbial Ecosystem

Prebiotics, as feed additives, play a crucial role in supporting the microbial ecosystem within the gastrointestinal tract of livestock. Unlike probiotics, which are live microorganisms, prebiotics are non-digestible compounds that selectively promote the growth and activity of beneficial bacteria already present in the gut. These compounds serve as a nutritional source for beneficial microbes, fostering their proliferation and enhancing their metabolic activities. By positively influencing the composition of the gut microbiota, prebiotics contribute to improved gut health, nutrient utilization, and overall animal well-being (Yadav and Jha, 2019).

One of the primary functions of prebiotics is to selectively stimulate the growth of beneficial bacteria such as *Bifidobacteria* and *Lactobacilli*. These bacteria are known for their probiotic properties, including the production of short-chain fatty acids (SCFAs) through the fermentation of prebiotic substrates. SCFAs, particularly butyrate, serve as an energy source for the intestinal epithelial cells, promoting gut health and integrity. Additionally, the increased abundance of beneficial bacteria can create a competitive exclusion effect, limiting the colonization of harmful pathogens and contributing to a balanced microbial ecosystem (Topping, 2016).

Research in this field has demonstrated the positive effects of prebiotics on the gut microbiota composition and overall animal performance. For instance, a study by Liu et al. (2018) investigated the impact of dietary supplementation with FOS on the intestinal microbiota of weaned piglets. The research revealed shifts in microbial composition, with an increase in beneficial bacteria and a decrease in potential pathogens, suggesting the prebiotic's potential in modulating the gut microbiota to enhance intestinal health. Another study by Pourabedin et al. (2017) focused on the use of MOS as a prebiotic in broiler chickens. The research indicated that MOS supplementation positively influenced the cecal microbiota, promoting the growth of beneficial bacteria and contributing to improved performance and gut health in broilers. Prebiotics serve as valuable tools in livestock nutrition by promoting the growth of beneficial bacteria in the gut. The inclusion of prebiotics in animal diets contributes to a balanced microbial ecosystem, supporting overall health, nutrient utilization, and performance (Yadav and Jha, 2019).

Challenges and Considerations in Livestock Probiotics

The application of probiotics in livestock faces several challenges and requires careful considerations to ensure their stability, viability, and effectiveness in diverse agricultural environments. Addressing these challenges is essential to harness the full potential of probiotics for enhancing animal health and performance. One significant challenge is the stability and viability of probiotic supplements throughout the feed production process and storage. Factors such as temperature, humidity, and processing conditions can impact the survival of probiotic microorganisms.

Research by Buntyn et al. (2016) highlights the importance of selecting probiotic strains with inherent resistance to environmental stressors, as well as employing appropriate encapsulation techniques to protect these microorganisms during feed processing. Ensuring the viability of probiotics until the point of consumption is crucial for realizing their benefits in the gastrointestinal tract of livestock. Formulation considerations also play a pivotal role in the effectiveness of probiotic supplements. The choice of carriers, binders, and additives in the formulation can influence the stability and delivery of probiotics to the target sites in the gut. Studies, such as that conducted by Bedford (2000), emphasize the need for formulating probiotic supplements that provide sustained release and protection of microorganisms in the gastrointestinal environment.

Furthermore, the effectiveness of probiotics in diverse livestock environments is influenced by various factors, including diet composition, host genetics, and the overall health status of the animals. Dietary components, such as the presence of antimicrobial substances or the ratio of fibrous to non-fibrous materials, can impact the growth and activity of probiotic microorganisms. Research by Mikkelsen and Jensen (2014) highlights the interaction between diet and probiotics, emphasizing the need for tailored approaches based on the specific nutritional characteristics of different livestock species. Host genetics also play a role in determining the response to probiotics. Variability in the gutmicrobiota composition among individual animals may influence the colonization and persistence of probiotic strains. Additionally, the health status of the animals, including the presence of diseases or stressors, can affect the receptivity to probiotic interventions. Studies, such as those by Bailey et al. (2010), underscore the importance of considering host-related factors when designing probiotic strategies for livestock.

Regulatory Frameworks and Industry Adoption of Probiotics and Prebiotics in Livestock

The use of probiotics and prebiotics in livestock is subject to regulatory frameworks that vary across regions. Regulatory agencies, such as the United States Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), play key roles in evaluating and approving the safety and efficacy of these feed additives. In the United States, for example, probiotics and prebiotics are generally classified as feed additives and are subject to approval by the FDA. The regulatory process involves assessing the safety of the microbial strains, their intended purpose, and the supporting scientific evidence. The European Union follows a similar process through EFSA, ensuring that products comply with stringent safety and efficacy standards before entering the market (Chatzopoulou et al., 2020).

Industry Trends and Adoption

The livestock industry has witnessed a growing trend towards the adoption of probiotics and prebiotics in modern management practices. Several factors contribute to this trend:

1. Antibiotic Alternatives

With increasing concerns about antibiotic resistance and the drive towards more sustainable and responsible livestock practices, there is a heightened interest in alternatives to traditional antibiotics. Probiotics and prebiotics offer a viable solution by promoting gut health, improving immunity, and enhancing overall animal performance without the drawbacks associated with antibiotic use (Haque et al., 2020).

2. Nutritional Efficiency

Probiotics and prebiotics contribute to improved nutrient utilization and absorption, leading to enhanced feed conversion rates and better growth performance. Livestock producers are recognizing the economic benefits of incorporating these additives into feed formulations (Popp et al., 2016).

3. Advancements in Research and Technology

Ongoing research in microbiology, genetics, and animal nutrition has provided deeper insights into the mechanisms of probiotics and prebiotics. This knowledge has facilitated the development of more effective formulations, contributing to increased industry confidence in adopting these practices (Zoumpopoulou et al., 2018).

4. Strategic Marketing Initiatives: Livestock producers and feed manufacturers are increasingly incorporating probiotics and prebiotics into their product lines, recognizing the market demand for products promoting animal health and sustainability. Strategic marketing initiatives highlight the benefits of these additives, further driving their adoption in the industry (Stanton et al., 2001).

Future Directions and Research Needs in Livestock Nutrition

1. Microbiome Precision Nutrition: Understanding the unique microbial communities in different species and optimizing diets to support specific beneficial bacteria can enhance overall animal health and performance (Hughes et al., 2019).
2. Host-Microbe Interaction Studies: Advancements in technology, such as metagenomics and metabolomics, will enable deeper exploration of host-microbe interactions. Investigating how probiotics and prebiotics influence the host's immune response, gene expression, and metabolic pathways can provide insights into the mechanisms behind their beneficial effects (Lamichhane et al., 2018).
3. Multi-Omics Approaches: Integrating genomics, transcriptomics, proteomics, and metabolomics will allow researchers to comprehensively analyze the impact of probiotics and prebiotics on the entire biological system. This holistic approach can reveal intricate details about the molecular and physiological responses to these additives (Yang et al., 2024).
4. Precision Farming and Data Analytics: The application of precision farming principles, coupled with data analytics, can optimize probiotic and prebiotic interventions based on real-time data (McClements et al., 2021).

Potential Innovations and Advancements

1. Synthetic Biology: The field of synthetic biology holds potential for designing customized probiotics with enhanced functionalities. This may involve engineering microorganisms to produce specific metabolites or enzymes that further benefit the host's health or improve nutrient utilization (Yadav and Shukla, 2020).
2. Advanced Encapsulation Techniques: Innovations in encapsulation technologies can enhance the stability and targeted delivery of probiotics and prebiotics. Microencapsulation methods that protect these additives during feed processing and improve their survival in the gastrointestinal tract will be crucial for efficacy (Martin et al., 2015).
3. Personalized Nutrition Strategies: Tailoring probiotic and prebiotic interventions based on individual animal requirements, health status, and environmental conditions will likely become a standard practice. Precision nutrition approaches can optimize the effectiveness of these additives in diverse livestock systems (Chassard et al., 2011).
4. Gut-Brain Axis in Livestock: Investigating the gut-brain axis in livestock can unveil connections between gut health and stress responses. Understanding how probiotics and prebiotics influence neural signaling and stress resilience can lead to innovative strategies for improving animal welfare (Kraimi et al., 2019).

Conclusion

Throughout this chapter, the emphasis has been on the role of probiotics and prebiotics in promoting livestock health and productivity. The gut microbiota plays a crucial role in livestock health, influencing digestion, immune function, and overall well-being. Probiotics and prebiotics contribute to a balanced gut microbiota, fostering a favorable environment for nutrient absorption and disease prevention. Prebiotics provide substrates for beneficial microbial growth, contributing to a stable and diverse gut ecosystem. A balanced microbial community is essential for efficient digestion and nutrient absorption in livestock. Probiotics offer a promising avenue for enhancing livestock nutrition. The sources of probiotics vary, and their mechanisms of action involve competitive exclusion of pathogens, production of antimicrobial substances, and modulation of the immune system. Foster collaboration between researchers, industry stakeholders, and regulatory bodies. Support ongoing research efforts to advance the understanding of probiotic and prebiotic applications, leading to continuous improvements in livestock management practices. By implementing these recommendations, livestock managers can harness the potential of probiotics and prebiotics to optimize health, enhance productivity, and contribute to sustainable and responsible livestock production.

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Chapter 05

Dietary Probiotic and Prebiotic Supplementation: their Effects on Broiler Chicken Performance

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ABSTRACT

Increasing the demand on poultry meat necessitate the improvement in production. Broilers that were not given any medicine performed poorly. Dietary measures may be used to control the height of the intestinal villus, which is linked to the ability to digest and absorb nutrients. Various feed additives, including oligosaccharides, enzymes, and antibiotics, are used in poultry feed to promote growth through possibly improving feed intake. While extended usage of antibiotics has resulted in the emergence of resistant bacteria, they also cause an accumulation of antibiotic residue in poultry feed. Prebiotic substrates, probiotic bacteria, or symbiotic prebiotic-probiotic combinations can be used as a substitute to sub-therapeutic antibiotics. Probiotics are living microorganisms that are used in the diet as feed additives or supplements. Also known as a direct-fed microorganism. Probiotic supplementation in the diet can improve host health and performance by enhancing gut health and nutrient utilization. While Prebiotics are defined as 'a non-digestible feed element that benefits the host by increasing the quantities of health-promoting bacteria in the intestinal tract. When the prebiotic enters the colon, it is selectively fermented by members of the indigenous microbiota. Subtherapeutic antibiotics are commonly used to prevent illness and promote body weight growth. The antibiotics subtherapeutic uses have a negative reputation among some customers, because there is emerging evidence that antibiotic resistance genes can be passed from animals to people.

KEYWORDS
Prebiotic, Probiotic, Broiler, Gastrointestinal tract

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INTRODUCTION

Over the last few years, the poultry business has improved its standing within the agri-food sector (Krysiak et al., 2021). Broiler chickens face a variety of stressors due to the high demands of production; these stressors negatively impact the overall health and productivity of the birds. In these situations, using synthetic antimicrobials and antibiotics is normal practice to lessen stress while simultaneously promoting development and feed efficiency (Dhama and Singh, 2010). Since chicken feed makes up over 70% of the overall production costs, the long-term profitability of the business depends on its assessment. Therefore, it's essential to increase feed efficiency while minimizing costs (Agawane and Lonkar, 2004).

Antibiotics have been used for more than 50 years to improve animal health, growth, and efficiency. However, as early as the 1950s, researchers found concern over the development of resistance bacteria for the tetracycline used in broilers. These results paved the way for agricultural regulators to apply more stringent guidelines on the use of antibiotics in chicken feed (Abd El-Hack et al., 2020). Antibiotic growth promoters (AGPs) are being used less or not at all in poultry production. As a result, the poultry industry has experienced a number of challenges, including poor performance and general health problems (Hafez and Shehata, 2021). Oxidative stress, diarrhea, and enteritis are examples of production-related illnesses (Lin et al., 2006; Lauridsen, 2019). Poultry farms often face the difficulty of inflammation, which is linked to innate immunological responses and can result in substantial financial losses (Shah et al., 2020).

Applying feed additives without antibiotics has similar benefits. Like enhancing broiler development and feed consumption (Mountzouris et al., 2007), since the health of consumers is at risk when in-feed AGPs are used in chicken diets (Abudabos et al., 2015). A growing number of probiotics, prebiotics, and phytogenic compounds are among the substitute supplements for antibiotics that have been created, examined, and applied to the production of chickens (Gernat et al., 2021).

Probiotics are live bacteria that are added to animal feed as supplements or additives and have the ability to improve the host's health, mostly through the gastrointestinal system (Abd El-Hack et al., 2020) by enhancing the native microflora's characteristics or the microbial equilibrium (Wang et al., 2018). Prebiotics are indigestible feed additives that preferentially encourage the development and activity of useful bacteria in the intestine, hence providing a favorable effect on the host. The primary prebiotics include mannose, galactose, fructose, and glucose (Hume, 2011). Probiotics are thriving in an excellent environment created by prebiotics (Sekhon and Jairath, 2010). Synbiotics comprise a mixture of probiotics and prebiotics (Yang et al., 2009).

Probiotics and prebiotics decrease the load of pathogens by boosting the host's mucosal immunity and establishing resistance to bacterial colonization (Sugiharto et al., 2017; Azad et al., 2020). Numerous investigations have demonstrated that probiotics, and prebiotics together have combined benefits on immune function, beneficial native bacterial development, and directed probiotic strains in the colon (Mookiah et al., 2014).

Better regulation of intestinal pathogens can be achieved by maintaining a healthy digestive tract through a balanced microbial population (Konstantinov et al., 2006).

Probiotics have demonstrated numerous advantageous attributes, including the capacity to enhance immunity, intestinal architecture, and gut barrier performance in broiler chickens. These elements may enhance absorption and digestion, which in turn may enhance performance under heat stress (Larsson et al., 2012). The demonstrated advantages include elevated peripheral immunoglobin synthesis and enhanced IgA secretion (Villena et al., 2008). The digestive tract is the largest producer of immunity; its activated mucosal B cells create a significant amount of IgA, acting as the body's first line of defense against pathogens (Lycke and Bemark, 2017).

Probiotics benefit chickens of all ages and classes in terms of immunity, health, and growth. They enhance maturation and intestinal integrity, boost immunity, reduce inflammation, improve feed consumption and digestion by lowering the activity of bacterial enzymes and raising the activity of digestive enzymes, decrease ammonia production and neutralize enterotoxins (Rehman et al., 2020). Probiotics and prebiotics have been shown to have growth-promoting properties, which suggests that they can alter the gut ecosystem by boosting the quantity of lactic acid bacteria, Bifidobacteria, and other anaerobic bacteria and lowering the quantity of enteric Bacilli and other aerobic bacteria (Schrezenmeir and de Vrese, 2001). Li et al. (2008) demonstrated that synbiotic (pre- and probiotic) combinations are frequently more beneficial than single supplements. Food digestion, intestinal health, and broiler performance are all improved by symbiotics (Patterson and Burkholder, 2003).

Intestinal Microbiota

The gastrointestinal system of birds is home to a vibrant and diverse community of microorganisms that coexist symbiotically with their hosts. Nutrition, immunity, and metabolism of the host depend on this mutualistic interaction. To support host homeostasis, the intricate ecosystem functions as a virtual organ system (Al-Khalaifa et al., 2019).

Under normal circumstances, intestinal health is largely determined by the symbiotic interaction that exists between the host's gut microbiota and itself. However, an unbalanced host-microbe connection known as "dysbiosis" can result from a disruption in the gut microbiota (Zoetendal et al., 2008). The gut microbiota can be disrupted by a number of factors, including heavy metals, toxic compounds, bacterial toxins, herbicides, and antibiotics. These effects could result in widespread infection, regional inflammation, or even intoxication (Ackermann et al., 2015).

The intestinal tract's permeability regulates not only the movement of non-digested materials but also the intake of nutrients and undesirable external substances like bacteria and xenobiotics. For this reason, the pathophysiology of many intestinal illnesses depends critically on gut health. The gut flora, digesting secretions, physical barriers (mucin, intestinal epithelial cells lining and tight junctions), and chemicals like cytokines regulate the permeability of the intestine (BisBischoff et al., 2014).

The disorder known as "leaky gut," or decreased intestinal barrier function, is characterized by damage to the small intestine's lining, which allows toxins and other luminal materials, such as bacteria, to penetrate between epithelial cells. Following these disorders, the intestines may become inflamed or damaged, resulting in elevated blood levels of endotoxins produced by bacteria. As a result of this inflammatory process' heavy nutritional consumption, metabolic responses particularly immunometabolic and endocrine responses are negatively impacted. Animal performances consequently suffer greatly (Abuajamieh et al., 2016).

Dysbiosis, or the modification of the composition of the gut microbiome, is caused by a number of things, including antibiotics, illnesses, stress, and food. Currently, a variety of techniques are used to alter the gut microbiome, including dietary modifications, the use of antibiotics and antimicrobials, probiotics, prebiotics, postbiotics, and synbiotic (Takáčová et al., 2022).

The activity of intestinal epithelial cells can be modulated by commensals and probiotics in a number of ways, including indirect effects on microbial biofilms (Vastano et al., 2016), and direct effects on the synthesis of mucin and tight junctions, which improve barrier function of intestinal epithelial cells (IECs) (Zyrek et al., 2007), raising the synthesis of heat shock protein and antimicrobial peptides (AMPs) (Liu, 2017), and interference with pathogenesis and pro-inflammatory and immunoregulatory cytokine modulation (Chen et al., 2006).

The majority of the microbial communities in chickens were composed of Bacteroidetes and Firmicutes, which are known to be important for energy production and metabolism (Yan et al., 2017; Pandit et al., 2018).

Based on studies carried out primarily in the last ten years, several roles are being assigned to the gut microbiota, for example (i) preservation of the barrier epithelium, (ii) suppression of intestinal surface pathogen adhesion, (iii) immune system regulation and appropriate maturation, (iv) degradation of carbon sources that would otherwise be indigestible, like plant polysaccharides, and (v) creation of several metabolites, including short-chain fatty acids (SCFAs) and vitamins (S´anchez et al., 2017).

Probiotics are made up of both bacterial and yeast cultures, which encourage the growth of microorganisms that can improve feed efficiency and change the gastrointestinal environment to a healthy state (Abudabos et al., 2015). The pathogenic bacteria that use toxins to degrade the intestinal wall were inhibited by the use of probiotics and prebiotics (Hassan et al., 2012).

Antibiotic Growth Promoters

The use of antibiotic growth promoters (AGPs) and synthetic growth promoters (SGPs) peaked many years ago (Broom, 2017). For several decades, the poultry industry has routinely used subtherapeutic dosages of antibiotics as antibiotic growth promoters (AGP) to increase feed efficiency (FE) in broiler chickens additionally lower illness incidence (Paul et al., 2022). Their range of activity encompassed antibacterial mechanisms, mostly aimed at Gram-positive bacteria (Broom, 2017).

The diversity of gut bacteria has been demonstrated to be altered by AGPs., such as beneficial lactic acid bacteria (LABs) (Neumann and Suen, 2015; Fasina et al., 2016). The modification of the animal immune system is another area where the AGP's methods of action extend; however, depending on the substance used, these responses differ, and for example, avilamycin influences the inhibition of bacterial protein synthesis, which results in a reduced release of proinflammatory compounds (Kabploy et al., 2016).

Furthermore, the quantity of amino acids, nucleosides, vitamins, or fatty acids metabolized is affected by the use of these feed additives; surprisingly, studies have indicated an increase in these levels. On the other hand, the information about the rise in polyunsaturated fatty acids (PUFA) is the most startling (Gadde et al., 2018). The usage of AGPs is involved in the development of poultry, and their gradual discontinuation can lower finishing weights. When combined with the detrimental effects of heat stress (HS), this can significantly reduce productivity (Lin et al., 2007).

Due to proven residues in soil, water, and animal products, as well as adverse effects on allergies and antibiotic resistance, AGPs have been discontinued (Ronquillo and Hernandez, 2017). The European Union outlawed using antibiotics as growth enhancers in 2006 (Castanon, 2007). Subsequently, the FDA requested in 2009 that medically significant AGPs be removed voluntarily from animal feed in the US (Thanner et al., 2016). The less effective usage of antibiotics has been replaced by more effective dietary supplements, such as probiotics and/or prebiotics. It is said that these substitute elements will strengthen immunity to all pathogenic agents and improve growth (Al-Khalaifah, 2018).

Since antibiotics reduce the number of beneficial bacteria in the intestine, such as *Lactobacillus* and *Bifidobacteria*, they produce reduced villi height when taken in supplements (Oliveira et al., 2008). In addition to eliminating pathogenic bacteria, antibiotics for treatment purposes change the overall microbiota of the host population. Leading to bacterial dysbiosis as well as future infections that are difficult to treat. Although taking probiotics with antibiotics not only stops diarrhea but also maintains the proper balance of gut bacteria without compromising the effectiveness of the medicine (Yousaf et al., 2022). It is anticipated that the antibiotic growth promoters would be taken out in the not too distant future due to the potential for major health effects from drug-resistant bacterial strains and antibiotic residues in chicken products (Yousaf et al., 2022).

Prebiotics and probiotics may be used in poultry diets as an alternative to AGPs. Prebiotics are oligosaccharides that has the ability to specifically stimulate particular gut bacterial species, perhaps improving the host's health but they are not digested by animal enzymes. Prebiotics are supposed to specifically promote the beneficial bacteria that are already present in the gut, whilst probiotics are designed to introduce helpful bacteria to the gut (Yang et al., 2009).

The Concept of Prebiotic and Probiotics

The Greek term "pro bios," which means "for life," led to the creation of the term "probiotics," which describes bacteria that are good for the body (Bansal et al., 2011). The name "probiotic" was not defined until recently, despite the fact that the concept of probiotics seems ancient. The term "probiotic" was first used in a 1965 in a science paper by Lilly and Stillwell, who described probiotics as "growth promoting factors produced by microorganisms." Probiotics are described by Parker as "organisms and substances which contribute to intestinal microbial balance". A decade later, the term "live microbial feed supplements which improve the intestinal microbial balance of the host animal" was added to the definition. Additional definitions were put up, such as "microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host," to describe both dead bacteria and bacterial components (S´anchez et al., 2017). The current definition of a probiotic by FAO/WHO is that it cannot be used with dead or mostly dead bacterial cells; instead, it must include live, viable bacteria. The International Scientific Association for Probiotics and Prebiotics (ISAPP) is one of the organizations that have accepted this description (Hill et al., 2014).

According to the Food and Drug Administration (FDA), probiotics are classified as Generally Recognized as Safe (GRAS) substances, both side effects and aftereffects are absent. By regulating the gut's microbial environment, preventing pathogenic gut bacteria, and reducing digestive disturbances, probiotics enhance live weight gain, improve feed conversion ratio, reduce mortality, and in layers increasing feed conversion ratio and egg production (Bansal et al., 2011).

Since the AGP was outlawed, probiotics have been used more frequently to treat bacterial illnesses. This can be attributed in great part to prior understanding of bacterial contact, wherein microbes compete with one another for survival mechanisms and substrates (Hernandez-Patlan et al., 2020). A probiotic need to possess the following qualities: It is appropriate for bacteria to be a part of the gut microbiota, be able to readily cling to the gut epithelium and resist acidic environments (Kabir, 2009) as well as preserve the intestinal microbiota at the proper physiological level (Krysiak et al., 2021). Probiotics are typically made from a variety of microorganisms, including yeasts like *Candida* spp., and bacteria like *Bifidobacterium* spp., *Lactococcus* spp., *Lactobacillus* spp., *Bacillus* spp., and *Streptococcu*s spp. (Park et al., 2016).

Prebiotics are polysaccharides and oligosaccharides that the animal cannot properly digest them, but can be easily digested by helpful of anaerobic colonic bacteria (Zhang et al., 2003). They alter the environment inside the gut by lowering the pH, providing digestive enzymes, and boosting gastrointestinal enzyme activity (Kabir, 2009). Compared to probiotics, prebiotics have the advantage of that they activate bacteria that normally reside in the animal's digestive system, and have so evolved to thrive in such environment (Snel et al., 2002). Mannan oligosaccharides, produced from the cell wall of yeast, are common type of prebiotics, which are elements of the yeast cell walls' outer layer, and mannose, glucans, proteins, and phosphate radicals are among their constituents (Klis et al., 2002).

Prebiotics might take the place of AGPs as non-microbial performance enhancing feed proponents. Although the goal of probiotics is to bring beneficial bacteria into the intestine. Prebiotics might function by specifically triggering the beneficial microorganisms already present. Furthermore, prebiotics support the endogenous microbiota by providing energy, metabolic substrates, and essential micronutrients to the host (Murshed et al., 2024). Prebiotics typically offer a fermentation substrate, increasing the survivability of probiotic organisms. Prebiotic and probiotic preparations work better together than they do separately most of the time (Mookiah et al., 2014).

Synbiotics are a combination of prebiotics and probiotics. Which consist of substrates and advantageous microbes, it might have complementary effects on animal digestive systems. Through improving the viability and intestinal implantation of food supplements containing live microorganisms, synbiotics have a positive effect on the host. These outcomes are caused by either selectively encouraging the development of one or few health-promoting microorganisms, which enhances the host's well-being, or both (Gibson and Roberfroid, 1995).

Diarrhea and a lack of appetite are among the symptoms that could arise from bacteria proliferating in the host's digestive tract. There will be a reduction in the effectiveness and immune capacity of chicken production because of the elimination of the natural microbiome causing significant financial losses for poultry producers. To preserve the health and balance of the poultry's microbiota, regular and timely addition of a probiotic supplement to the meal is advised (Bar-Shira and Friedman, 2006).

Increased daily increments, better feed conversion ratio (FCR), and enhanced laying and egg quality are among the nutritional benefits shown in flocks given probiotics. The quality of meat has also improved. This shows that using probiotics can help producers achieve better production results. Bird immunity is enhanced in addition to these production benefits by enabling the organism to more effectively defend itself against infections and stress (Krysiak et al., 2021).

The selection of prebiotics and probiotics, methods of preparation, dose administration, food composition, bird age, and hygienic conditions can all be factors in the variations in growth performance. (Mountzouris et al., 2007). Conversely, the observed improvement in body weight gain (BWG), and feed conversion ratio (FCR) may be associated with a reduced microbial population within the broiler's gastrointestinal tract (Thongsong et al., 2008).

Immunomodulatory Potential of Probiotics

The statement "immunity comes from the intestines" has become more important in the poultry industry since zoonoses and bacterial illnesses have been demonstrated to be effectively combated by probiotics (Krysiak et al., 2021). The establishment of passive and active resistance to avian intestinal illnesses are facilitated by the immune system, gut microbiota, and epithelial cells. There is little information available regarding how the bacteria in an avian's stomach differentiate between "bad" and "good" bacteria and regulate the immune system (Bomba et al., 2002).

Because different cell types, including bacteria in the gut lumen, epithelium, or lamina propria, and members of the innate and adaptive immune systems, are constantly interacting, intestinal enterocytes monitoring the epithelial surface area for potential pathogens in the gut. The precise intestinal lay out and the inter-digitation of immune cells across epithelial tissue enable the balance between hyper- reaction and non-reaction. Partly due to the fact that the gut has the highest density of lymphocytes than any other organ, moreover, because its surface area and size in relation to both autochthonous and allochthonous probiotics are significant variables. The intestinal epithelium's enterocytes serve as a barrier to keep pathogens from obtaining nourishment and help the immune system identify potential infections in the lumen. Consequently, the greatest immunological organ is occasionally applied to the gut (Bouzaine et al., 2005).

Raising cell-mediated immunity could potentially help fight viral infections and potentially alleviate some of the symptoms of infection-associated diseases for example chicken infectious anemia, infectious bursal disease, Marek's disease, reoviral infections, mycotoxins, and other immune-suppressive conditions. Because of the immunomodulatory effects of their metabolites, chicks are shielded from a wide range of infectious diseases. Probiotic-treated chicks may have greater antibody levels against viral diseases including ND and IBD, which are prevalent in the industry (Boirivant and Strober, 2007).

Probiotics have positive effects on the immune system, such as enhanced natural killer (NK) cell, macrophage, and lymphocyte function, increased production of immunoglobulins (IgG, IgM, and IgA), as well as enhanced oxidative burst in heterophils. The use of probiotics, which aid in immune system regulation and stomach stabilization, may support the maintenance of a balanced level of pro- and anti-inflammatory cytokines. Based on these findings, probiotics have been shown to have the ability to raise the quantity of lamina propria lymphocytes (LPL) and intestinal epithelial lymphocytes (IEL) in the small intestine, as well as preventing the growth of harmful bacteria (Dhama and Singh, 2010).

Probiotics have the ability to decrease inflammation caused by pathogen-infected cells or microflora by blocking signaling pathways including MAP kinase and NF-kappa beta that are responsible for immune response activation. Probiotics also strengthen the immune system by raising the lumen's IgA levels, the quantity of cells that make IgA, IgM, and IgG, and the quantity of T cells in the cecal tonsils (Cavit, 2003). When probiotics are taken orally, the stomach and bloodstream produce more natural antibodies to a greater variety of antigens (Chichlowski et al., 2007).

For poultry farms, inflammation linked to innate immunological responses is a frequent problem that results in large financial losses (Shah et al., 2020). Malondialdehyde (MDA) is the end result of lipid peroxidation, and antioxidant enzymes glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) are involved (Yu et al., 2022).

Probiotics support the immune system and screen for or avoid developing intestinal infections when suffering from viral infections or immunosuppressive circumstances (bacterial, coccidian) to reduce the risk of secondary infections in birds. As many infectious illnesses are transmitted by bacteria, fungi, protozoa, and viruses, a multi-strain probiotic ought to be a regular part of the diet (Hajati and Rezaei, 2010).

How Probiotic and Prebiotics Produce their Positive Effects

It has been demonstrated in multiple scientific investigation that probiotics and direct-fed microbial feed supplements can modify the gut microflora's composition by effectively competing with pathogens through an alternative mechanism (Mountzouris et al., 2007). Two potential defense mechanisms used by probiotic bacteria to keep viruses out of the digestive tract include competitive exclusion and bacterial antagonism such as intestinal villus and colonic crypts, which are favored habitats of enteric pathogens (Yousaf et al., 2022).

A phenomenon known as "competitive exclusion" happens when pathogens and probiotics compete for gut adhesive receptors that are essential to the adhesion and growth of microorganisms. According to this method, probiotics have an impact on how bacteria colonize disorders. Pathogens are unable to establish themselves in the gastrointestinal system due to probiotics quick colonization (the development of a thick layer of microflora) (Yousaf et al., 2022). Additionally, they alter the environment inside the gut by lowering pH, providing digestive enzymes, and boosting gastrointestinal tract enzyme activity (Kabir, 2009).

Preventing the infectious agents from getting the food and energy they need to proliferate in the gut environment is one of the key objectives of probiotics. Bacterial pathogens such as *Salmonella* and *E. coli* cannot thrive in an environment where primary and secondary metabolites such as lactic acid, volatile fatty acid (VFA), and organic acid have changed the pH of the gut. There is evidence that a class of substances called bacteriocins can effectively eradicate or stop harmful bacteria from colonizing an area (Yousaf et al., 2022).

Animal growth and development can be aided by probiotics' ability to create digestive enzymes, preserve intestinal structure, reduce the growth of harmful bacteria, and improve nutrient absorption (Kabir 2009). According to Sakata et al. (2003), probiotic bacteria actually accelerate the breakdown of indigestible carbohydrates, which raises the rates at which volatile fatty acids (VFA), lactic acid, and occasionally succinic acids are produced. The fermentation products produced by the oligosaccharides in the colon may help prevent the growth of harmful bacteria such as *Salmonella* spp., *Campylobacter* spp., or putrefactive bacteria such as *Clostridium perfringens* (Gibson and Wang, 1994).

Probiotics have a significant impact on the oxidative state of the gut because they directly possess antioxidant qualities and stimulate intrinsic organisms that communicate antioxidant defense (Zolotukhin et al., 2018). By lowering the amount of malondialdehyde (MDA) and increasing the amount of glutathione in the colon, probiotics may be able to withstand oxidative stress brought on by LPS (Chorawala et al., 2021). In response to repeated LPS stimulation, the peripheral blood immune organs, such as the thymus and spleen, extensively proliferated, producing inflammation and proinflammatory cytokines (TNF-a) (Zhong et al., 2018).

In addition, pathogenic bacteria with type-1 fimbriae, such *E. coli*, are prevented from adhering to the gut wall by the prebiotic mannanoligo saccharides, which also serve to push them out of the wall (Abdel-Raheem et al., 2012).

Advantages of using Probiotics and Prebiotics

The effects of antibiotics on microorganisms can be reduced with the use of probiotics. Nevertheless, the growth of drug-resistant bacteria and residues in chicken products could be caused by the common usage of subtherapeutic dosages of antibiotics. In addition to eliminating harmful bacteria, antibiotics used to treat illnesses also alter the general microflora of the host, resulting in bacterial dysbiosis and future infections that are challenging to treat. When probiotics are used with antibiotics to prevent diarrhea, the balance of gut microbiota is maintained without compromising the medication's effectiveness (Farnell et al., 2006). Probiotics successfully prevent pathogens from obtaining access to resources by maximizing their utilization of the accessible substrate, which is a result of their higher colonization aids in the gut (Yousaf, et al. 2022).

The health benefits of probiotics have previously been studied at the cellular level, where they were shown to modify gene expression and reduce heat stress (Krysiak et al., 2021). Probiotics have been shown to provide defense against a variety of cellular stressors, including oxidative stress-mediated apoptosis (Tao et al., 2006). Additionally, they improve barrier performance by stopping intestinal paracellular permeability from being destroyed (Llewellyn and Foey, 2017).

Effective probiotic use has been demonstrated to benefit chicks and poults (Bansal et al., 2011). As they improve feed conversion, growth rate, efficiency, nutrient absorption, and microbial balance (Elam et al., 2003). Preventing the growth of harmful microorganisms particularly those that cause digestive problems due to bacterial invasions also lowers the mortality rate of chicks. The performance of layers may also be improved by increased egg production, weight/size, and food uptake ratio (Al khalf et al., 2010). Probiotics have enhanced the flavor and quality of poultry products in addition to improving avian health (Krysiak et al., 2021). They increase the quality of egg yolk cholesterol content, egg albumen quality, egg fertility, and hatchability (Elam et al., 2003). Probiotic supplements also have a major impact on the carcass yield, and live weight gain, and prominent cut up meat parts (Soomro et al., 2019).

Prebiotics are not digested in the small intestine, consequently, increase the likelihood that bacteria will exit the intestine without adhering to the epithelium, which reduces or prevents of unwanted bacteria from colonizing the small intestine (Spring et al., 2000). Improved probiotic survival in the gut requires prebiotics. With the aid of prebiotics, probiotics may thrive in the digestive tract and withstand anaerobic conditions, such as low oxygen, low pH, and low temperature (Hanamanta et al., 2011).

The primary cause of the reduction in meat quality and the shortened shelf life of meat and meat products is the lipid macronutrients' susceptibility to various medications. Prebiotics can alter lipid metabolism and increase the proportion of polyunsaturated fatty acids (PUFAs) in chicken meat, which is beneficial to human health but shortens the meat's shelf life (Maiorano et al., 2017).

Probiotics and prebiotics work together to give the body greater benefits than either alone. Through targeted growth stimulation and/or metabolic activation of certain beneficial bacteria, synbiotics enhance the host's defenses and facilitate the implantation of feed supplements containing living microbes in the channel of digestion, improving the host's overall health. This combination also has the advantage of increasing probiotic bacterial survival since it provides particular substrates for fermentation (Gibson and Roberfroid, 1995).

The potential to reduce rooster semen quality and the variety of production methods that could impact the probiotic's durability are only two of the few disadvantages that outweigh the many benefits (Krysiak et al., 2021).

Supportive Effects of Probiotic and Prebiotics against Disease Conditions

For improving the health of broilers both inactivated and live probiotics are effective (Hajati and Rezaei, 2010). When added to a bird's diet on a regular basis, probiotics can help keep their microbiome balanced and healthy, which can enhance the bird's overall health and productivity. Probiotics are strongly recommended for use in the care of new hatch chicks, during stressful times, and as a broiler chicken substitute for antibiotic growth boosters (Duggan et al., 2002).

Successful probiotic colonization depending on several variables, including dosage and frequency of use, stability of the microbes and their long-term relationships with hosts (Yousaf et al. 2022). Gut microbiota disruption can occur due to a variety of stresses and pathogenic microorganisms found in all poultry raising facilities, potentially leading to an unbalanced microbiome in the gut, and a decrease of the body's defenses (Balevi et al., 2001). Wilson et al. (2005) clarified that the generation of toxic compounds that irritate the gut mucosa is the cause of the growth-suppressive impact of intestinal bacteria, so restricting the absorption of nutrients.

Through the competitive exclusion process, probiotics have been demonstrated to prevent the gastrointestinal tract from becoming colonized with harmful bacteria (Teo andTan, 2006; Abudabos et al., 2013).

The removal of pathogenic microorganisms, especially enteric pathogens, can potentially avert early chick mortality as well as gastro-intestinal abnormalities such scouring, lack of appetite, and incorrect digestion. This could lead to an increase in productivity and a reduction in significant losses for chicken breeders (Duggan et al., 2002). Probiotic therapy significantly improves birds' gut immunity, and is quite successful against parasitic coccidian and bacterial intestinal infections. Supplementing chicken with probiotics has been demonstrated to suppress a number of infections, including *Salmonella enteritidis, E. Coli, Clostridium perfringens, Listeria monocytogenes, Campylobacter jejuni*, and *Candida albicans* (Dhama and Singh, 2010).

A subclinical condition linked to necrotic enteritis may harm the intestinal mucosa, impairing absorption and digesting leading to poor performance (Kaldhusdal et al., 2001). Feighner and Dashkevicz (1987) elucidated that the growth depression resulting from a *C. perfringens* infection was connected to the pathogen's high level of bile salt hydrolase activity. Probiotic supplementation alone or as a part of synbiotics inhibited the growth of *C. perfringens* in the ileum. This elimination may account via competitive exclusion and immune system activation (Abudabos et al., 2015).

Probiotic use might help reduce the production of litter ammonia, which would reduce the danger of keratoconjunctivitis, an eye condition brought on by an overabundance of ammonia in the environment (Yousaf et al., 2022).

Lipopolysaccharide stimulation in addition to readily causing intestinal inflammation, it frequently resulted in severe liver injury (Baranova et al., 2016; Stephens and von der Weid, 2020). Probiotic therapy prevented an increase in LPS induced pro- and anti-inflammatory (IL-1b, TNF-a and IL-6) peripheral cytokines. Decreased the mRNA expression of central cytokines in the hypothalamus, prefrontal cortex, and hippocampus, stopped the alterations in the gut microbiota brought on by LPS (Murray et al., 2019).

Prebiotics have demonstrated potential in suppressing pathogens like *Salmonella* and *E. coli* while promoting the growth of *Bifidobacteria* and *Lactobacilli*. Mannan oligosaccharides (MOS) are frequently utilized prebiotics. They consist of 12.5% protein, 30% mannan, and 30% glucan. Serine, aspartic acid, glutamic acid, and methionine are abundant in the protein (Song and Li, 2001). The inclusion of MOS in the diets of broiler chickens may improve their growth performance (Rosen, 2007). MOS function through modifying the bacteria communities within the gastrointestinal system, which sets them apart from other oligosaccharides. MOS offer a competitive binding site and a ligand with strong bacterial affinity. Therefore, pathogens pass through the intestine without colonizing because they adhere to the MOS rather than the intestinal wall (Benites et al., 2008). Furthermore, MOS raised the level of IgA in the serum (Kim et al., 2009).

Conclusions

The supplemented diet with probiotic and prebiotic can enhance chicken growth performance through changes in intestinal flora. Dietary combination of various compounds can help in body weight gain and modulation of immune system. Using of probiotic and prebiotic as potential alternative of antibiotic is recommended in broiler diet. They have been shown to have beneficial profits both directly in the gastrointestinal tract and indirectly in the immune system immunomodulation of chickens. Applying of probiotic and prebiotic, will help in balancing of gut microbiota encourage growth and boost immunity in the bird population. Even though synbiotic seemed to be superior in improving broiler performance. By using natural means, the occurrence of diseases in poultry can be reduced, strengthening their immune systems and contributing to higher levels of chicken production. Probiotics are a less expensive and more beneficial feed additive or growth stimulant than antibiotics because they have no known adverse consequences.

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Chapter 06

Use and Impact of Prebiotics and Probiotics in Poultry Broiler Performance Growth

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ABSTRACT

The poultry industry is depending more and more on different and new methods to maximize the health and productivity of broiler birds. Probiotics and prebiotics have become important participants in this search, providing various advantages for host immunity and gut flora. Prebiotics, like fructo-oligosaccharides and inulin, provide as nutritious substrates for gut flora that support the growth and function of the microbiome. Through their ability to withstand digestion in the upper gastrointestinal system, withstand lower pH levels, and encourage the growth of advantageous bacteria, they support gut health. Furthermore, prebiotics have been connected to higher feed conversion rates and daily weight increase in chickens. Concurrently, probiotics, encompassing organisms such as Lactobacillus and Bacillus, exert a direct impact on gut health via mechanisms that include immunomodulation and intestinal barrier reinforcement. Probiotics support host defences and help to maintain a healthy gut ecology by balancing pro- and anti-inflammatory cytokines in the body. Probiotics also show promise for improving the uptake and utilization of nutrients, which would maximize the performance of chickens. Prebiotics and probiotics have long been known to have health advantages, but less is known about the intricate relationships that exist within the chicken gut microbiota and how these relationships affect general health and productivity. In order to direct the creation of focused interventions suited to certain poultry species and production systems, future research efforts must concentrate on clarifying these connections.

KEYWORDS		
Probiotics, Prebiotics, Immunomodulation, Cytokines, Microbiota	Received: 23-May-2024 Revised: 10-July-2024 Accepted: 09-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Prebiotics are indigestible feed elements that work by improving the poultry birds' health by slightly affecting the action of one or more bacteria of colon (Gibson and Roberfroid, 1995). Prebiotics affect the intestinal bacteria and defense system of the bird (Kim et al., 2011; Bozkurt et al., 2014). Prebiotics are likely to possess characteristics like:
1) not absorbed in upper gastrointestinal tract.
2) resistant to lower pH
3) enhance the growth of beneficial microbiota
4) alternate the host defensive mechanism in favor of beneficial bacteria (Patterson and Burkholder, 2003).

Prebiotics commonly used in poultry birds are oligosaccharides such as fructo-oligosaccharides (FOS), inulin, mannan-oligosaccharides (MOS) and xylo-oligosaccharides. Probiotics are living microorganism in a mono or mixed culture that improve the intestinal bacterial balance of the birds (Fuller, 1989). According to FAO/WHO, probiotics are living microorganisms which, when given in sufficient proportions boost the host's health and is beneficial for its production. Following are the characteristics of good probiotics:

1) Strains that benefits the host animal.
2) Should not be harmful or pathogenic for the host.
3) Should be live cell and capable to grow
4) Able to survive and metabolize the gastrointestinal environment.
5) Should be able to persist as live cells for long period of time inside the host's cells and also in storage form (Fuller, 1989).

Another name of probiotics is "direct fed materials". Most frequently used probiotics are: *Lactobacillus* (*L. bulgaricus, L. plantarum, L. acidophilus, L. helveticus, L. lactis, L. salivarius, L. casei, Bacillus subtilis*), *Enterococcus* (*E. faecalis, E. faecium*), *Bifidobacterium* spp., *Steptococcus*, *Enterococcus*, *Lactococcus*, *E. coli* and fungi and yeast (*Aspergillus oryzae, Saccharomyces cerevisiae*) (Huang et al., 2004). The widely utilized species of LAB and *bifidobacterium* have also been employed for human use. *Bacillus*, *Enterococcus*, and *Saccharomyces* yeast are also extensively used in livestock animals (Ferreira et al., 2011). Different strains act of different sites in GIT and each have its own distinct function and they also form synergism acting together and are more beneficial than single specie for the nourishment of micro flora and growth of the host organism (Sanders and Huis in't Veld, 1999; Timmerman et al., 2004; Klose et al., 2006).

Importance of Gut Health in Poultry Broiler Performance

Like humans, birds also have sophisticated defense mechanism that comprise of different cell and complexes working together and some soluble substances that along with these cells produce the productive immune response (Yegani and Korver, 2008). The gut microbiota plays an important role in maintaining the health and promoting the growth and maturity of the chicken innate and adaptive immune systems (Muir et al., 2000; Haghighi et al., 2006; Brisbin et al., 2008). On the basis of studies, certain commensal bacteria play a crucial role in development and stimulation of the immune cell of GIT and also increase their number (Kogut, 2013). For instance, it has been demonstrated that bacteria from phylum bacteroidetes, namely *Bacteroides fragilis*, are linked in the maturation of helper T cells that produce interlukin-17 (IL-17) (Mazmanian et al., 2005). Lactobacilli are long recognized due to their capacity to boost immune system against illness and stimulate the gastrointestinal immune cells. This effect is attributed to their ability to produce low molecular weight peptides that trigger the immunological response (Muir et al., 2000).

Studies show that these bacteria are also involved in the production of bacteriostatic short chain fatty acids (SCFAs) and bacteriocins that are either microbicidal or mircobistatic. Additionally, these bacteria also act by decreasing the pH of GIT and altering the receptors associated with the pathogenic bacteria hence reducing the colonization of pathogenic bacteria (Adil and Magray, 2012; Rinttilä and Apajalahti, 2013). Short chain fatty acids help in the regeneration of GI's epithelium and also support its barrier function against pathogenic bacteria (Kogut, 2013). The gut is the home of potentially harmful bacteria *lkesalmonella*, *Escherichia coli* and clostridium specie as well as good bacteria like *bifidobacteia* and gram positive *lactobacilli*. It is important that there should be homeostasis in the concentration of good and harmful bacteria. It is reported that almost 85% of good bacteria in the gut are beneficial for maintaining a healthy gut. Disproportion in the population of both bacteria have great impact on chicken health (Choct, 2009). Removing antibiotics from feed can have an effect on the normal microbiota and the pathogenic bacteria. (Choct, 2009). It is easy to alter the gut microbiota through nutrition, which also helps for the proliferation of beneficial bacteria in the gut (Adil and Magray, 2012). These bacteria also regulate GI function and also play a vital role in the proliferation of gut epithelium. Studies have shown that commensal bacteria have integral part in the digestion mechanism their action modulate crucial pathways like bile acid synthesis and regulating the breakdown and absorption of lipids. They are also involved in the production of vitamins (Brestoff and Artis, 2013).

Additionally, these bacteria also affect the mucosa of the gastrointestinal tract and the action of the digesting enzymes. (Lan et al., 2005). The gut microbiota has a potential benefit on the undigested feed but the condition become unfavorable when feed is given that is digestible for the chicken. This creates an imbalance between the microbiota ecosystem and the host and it leads to insufficient energy extraction and utilization by the host (Lan et al., 2005). As there in no indigestible substrate/ carbohydrate for the microbiota and there will be a competition between the host and the bacteria for the substrate. It this condition the microbiota will be nothing more than a burden on the host and especially in broiler birds that are quick growing birds (Yang et al., 2009). To support healthy gut and maximize the production, the balance of intestinal bacteria is crucial (Lutful-Kabir, 2009). Changes in the intestinal bacteria can affect the structure of the gastrointestinal tract and trigger immunological responses which can then effect the chicken development and nutrition requirements (Humphrey and Klasing, 2004). Similarly, pathogen colonization of the gut can trigger an immunological response that ultimately redirects resources and energy from growth to the urgent need to fight infections (DiAngelo et al., 2009).

In poultry, while addressing the microbial infection the inflammatory response play a significant role (Kogut, 2013). But, if left unchecked this immunological activity might lead to severe damage to internal lining of GIT and activate inflammatory responses. This subsequently affect the ability of intestine to absorb or digest the feed material and it might pass undigested (Brisbin et al., 2008). Additionally, when the inflammation is severe it also cause hindrance in host metabolic activities (Kogut, 2013). It is found that intestinal microbiota plays a crucial role in sustaining the homeostatic environment of the gut and also aid in digestion by maintaining host and microbiota relation (Lan et al., 2005). As these commensal microbiotas are involved in the formation of SCFAs and other products have an anti-inflammatory action of the gut surface, reducing gut damage (Brestoff and Artis, 2013). Overall, the gut microbiota is involved in supporting the gut by maintaining its normal functions including digestion and absorption along with immunological responses. Hence, these play a vital role in the homeostasis of chicken's gut.

Better Characterization of the Microbiome for Poultry Production

By the fact it has been found that the microbes are 10 fold greater in the gut than host's own cells. This confers the importance of maintaining a healthy gut microbiota (Savage, 1977; Bengmark, 2002). The GIT of chicken contain large number of cell as compared to any other system. These are approximately in a ratio of 107 to 1011 bacteria per gram of gastrointestinal material (Apajalahti et al., 2004). The gut environment is supplied by large quantity of microbiota including mostly bacteria (Sergeant et al., 2014), often yeast (Koneman et al., 1978; Laubscher et al., 2000; Kano et al., 2001). Microbiota form a complex interaction with host cells and due this these microbiota plays a crucial role in the growth and performance of the host (Tremaroli and Bäckhed, 2012).

In fact, the significance of the microbiome's make up in poultry species has long been recognized (Samli et al., 2007; Torok et al., 2008), moreover, there is proof that some bacteria populations may have a beneficial relationship with feed AME in broiler chickens (Torok et al., 2008). Unfortunately, little is known about the majority of commercial poultry species' microbiomes or how they relate to health and productivity. Thus, more study is required to functionally characterize the microbiome of poultry species and link the microbial composition to the birds' observed health and performance. Collaborations across public and private sectors as well as between institutions might be essential for the large-scale microbiome characterization of poultry species that is required.

Prebiotics: Nourishing the Gut Microbiota

Prebiotics have an enhanced immunological response because of their direct contact with gut immune cells (Janardhana et al., 2009) and they maintain the health of the birds' digestive systems in a similar pattern to that of probiotics (Huyghebaert et al., 2011). Among the dietary constituents, non-digestible carbohydrates (polysaccharides and oligosaccharides), some proteins and peptides, and certain lipids are potential prebiotics (Sinovec and Marković, 2005). According to reports, oligosaccharides can create volatile fatty acids (VFAs), which can negatively impact digestibility by inducing peristalsis and shortening the time it takes for food to move through the gut (Sinovec and Marković, 2005). Trevino et al. (Treviño et al., 1990) revealed that the length of the chicken's jejunum, ileum, and caecum had increased, with the ileum's villi growing longer. It has been observed that using prebiotics increases daily weight gain by around 8–10% and lowers conversion by 10%–15% (Sinovec and Marković, 2005). Prebiotic supplements in the diet are responsible for the birds' increased efficiency and use of energy (Yang et al., 2008; Choct, 2009; Nabizadeh, 2012).

Kim et al. (Kim et al., 2011) said that although there were no changes in feed intake, feed conversion, or mortality, there was a considerable increase in weight gain when compared to the control group (Waldroup et al., 1995; Canibe et al., 2001; Gauthier, 2002; Ao, 2005; Biggs and Parsons, 2007; Abdel Fattah et al., 2008; Liem et al., 2008; Yang et al., 2008; Chotikatum et al., 2009; Chowdhury et al., 2009; Józefiak et al., 2010). Oligosaccharides, such as inulin, fructo-oligosaccharides (FOS), mannan-oligosaccharides (MOS), galacto-oligosaccharides (GOS), soya-oligosaccharides (Coutinho et al.), xylo-oligosaccharides (XOS), pyrodextrin, iso-malto-oligosaccharides (IMO), and lactulose, are the most widely utilized prebiotics in poultry (Kim et al., 2011).

Probiotics: Direct and Indirect Effects on Gut Health

Probiotics and antibiotics both offer antimicrobial compounds at a degree of efficacy that is quite similar to that of organic acids, bacteriocin, short-chain fatty acids (SCFA), or hydrogen peroxide (with slight alteration in the intestinal pH and also in combination with glucose) (Alloui et al., 2013). Particularly, the investigation on well-known Bacillus species revealed immunomodulatory effects; there was a rise in the expression of TJ protein adhesion molecules (zonulin 1 and occludin). As a result, the intestinal barrier functions more effectively and is more intact. Probiotic bacteria, such as IL-10 and TGF-β, can increase the level of anti-inflammatory cytokines and balance pro-inflammatory cytokines (Kim and Lillehoj, 2019). The amount of immunoglobulins M and A have a lot of beneficial effects by the administration of these feed additives. Additionally, there has been an increase in serum's total antioxidant capacity (TOAC) % (Wang et al., 2018). Furthermore, studies have shown that *Lactobacillus rhamnosus* possesses the capacity to activate the receptor that are responsible for the growth of intestinal epidermal and their proliferation. This leads to a decrease in intestinal epithelial apoptosis which play a crucial part in the defense mechanism against gastrointestinal disorders (Menconi et al., 2014).

The microbiota, or diverse population of bacteria, found in the digestive system of fowl, is remarkable. It is estimated that there are 1010–1011 CFU/g of intestinal content of bacteria in the gastrointestinal system. The most prevalent bacteria include *Clostridium* spp., *Ruminococcus* spp., *Bifidobacterium* spp., *Lactobacillus* spp., and *Bacteroides* spp. These microbes have broad roles that maintain the body's equilibrium. Each kind of gut bacteria has a very distinct purpose. Among other things, their job is to use fermentation to boost feed's energy efficiency. It yields SCFA or breaks down substances that are indigestible, such polysaccharides into monosaccharides. Ten percent of the energy in feed is thought to come from gut microorganisms. This results in a more efficient utilization of feed in terms of energy and facilitates the absorption of critical nutrients. The term "metabolic organ" refers to the microbiota, which is an intrinsic component of the intestinal ecosystem and adjusts to the host organism's physiology. The function and structural structure of the gut are affected by bacteria; these microbes cause the intestinal crypts and villi to expand. Its shape can be influenced by the gut microbiome, which is especially important for controlling immunological activities. Antibiotics have been linked to intestinal mucosal damage, including altered mucus layer composition and an increase in abnormalities at the tips of the intestinal villi.

However, the probiotic's administration had the reverse impact; that is, a diet supplemented with a probiotic preparation induced the intestines to develop (Park et al., 2016). The gene reservoir that contains the enzymes required for metabolic alterations is the most significant part of the microflora. The distribution of polysaccharides depends on glycosidic hydrolysis and polysaccharide lyase genes, neither of which are present in poultry. Consequently, the existence of bacteria promotes and permits this activity (Mousavi et al., 2018). When using probiotics, the metabolism of bacterial microflora is essential. Every person's experience is different. Its composition becomes more variable as a result of probiotics, which aids in the removal of infections. Other variables, such as the quantity and quality of nutrients or the composition and balance of the meal itself, can also affect the makeup of the microbiome (Fuller and Freter, 1992). The most common bacteria in the makeup of the gut microbiome, *Betabacterium* and *Lactobacillus* species, are often found in greater numbers as a result of this little alteration. Additional consequences include a drop in stool pH and bacterial enzyme activity (Ashraf and Shah, 2014). Probiotic bacteria interact with mucosal epithelial cells to induce particular CD-206 and toll-like receptor (TLR)-2 cells, which is the basis for the mechanism of action (Al-Khalaifah, 2018). The study found that higher quantities of lactic acid, acetic acid, and volatile fatty acids (VFA) were responsible for the decline in the pH of the feces and the intestinal environment. The development of intestinal microbes is facilitated by the acidified environment, which also strengthens the organism's natural defensive systems and aids in the fight against harmful germs (Park et al., 2016).

Conclusion

In conclusion, the complex relationship that exists between the administration of prebiotics or probiotics and gut health and performance of chickens highlights the critical need of preserving a healthy microbiome. There are promising ways to improve gut health and increase bird productivity with prebiotics (like oligosaccharides) and probiotics (like yeast and other bacterial strains). These feed additives improve optimal health and productivity in chicken farming by stimulating the growth of beneficial microorganisms, regulating immunological responses, and facilitating nutrient absorption, among other methods. Additionally, as we learn more about the makeup of poultry species' microbiomes, we discover the possibility of focused therapies to improve health outcomes. It is essential for the public and commercial sectors to work together to enhance knowledge and turn it into workable plans for sustainable chicken production. More than just aiding in digestion, prebiotics and probiotics are essential for strengthening the host's defences, maximizing the absorption of nutrients, and building a healthy gut environment. Utilizing the potential of these bioactive substances presents a viable route to attaining ideal health and performance results in chicken farming as we work to continuously improve the production of poultry. In other words, we ensure the sustainability and success of the chicken business for future generations by fostering the symbiotic link between birds and their gut bacteria. This leads to healthier and more resilient flocks.

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Chapter 07

Functional Aquafeeds: A Comprehensive Approach to Probiotics, Amino Acids and Feed Additives Integration for Sustainable Production

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ABSTRACT

Fishmeal is a very important protein source in fish feed, but its limited availability compared to the growing demand forces fish nutritionists to explore other protein sources. However, increasing the amount of alternative proteins in fish feed to a certain limit can sometimes decrease fish performance. To mitigate the adverse impacts of including plant proteins in aquafeed, the researchers are investigating various approaches, such as altering the processing of feed ingredients, feed formulation, and feed supplementation. Supplementation with functional feed additives (FuFAs) including organic acids, nucleotides, prebiotics, probiotics and particular amino acids, have shown promise in improving the effectiveness of incorporating alternative protein, enhancing fish growth and health. Nonetheless, there are still many unknown things about how FuFAs affect digestion, absorption, species variations, age-related responses, metabolism and physiological responses in fish fed with alternative protein-based feed. To enhance fish performance, it is necessary to use bio-chemical and molecular technologies to better comprehend the function of FuFAs in fish feed. Additionally, actions are required to develop cost-effective production technologies for functional feed supplements. Furthermore, further research is necessary to explore the potential of functional feeds in disease prevention and reducing dependence on chemical interventions and antibiotics in aquaculture, thus promoting environmentally sustainable practices.

KEYWORDS		
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INTRODUCTION

Production of fish increased globally through aquaculture (FAO, 2016) and it has gained importance and expanded in volume (Maas et al., 2020). With the rising global demand for fish and other aquatic species, aquaculture helps to meet this demand while simultaneously enhancing nutritional security on a global scale (Fiorella et al., 2021). Fish production and environmental stress have increased because of the diversification and intensification of aquaculture technologies used to sustain high production levels, which is thought to be the main constraint in the cultivation of fish. As a result, the need for growth promoters and environment-friendly alternatives has become a significant factor and extremely necessary for sustainable aquaculture (Boyd et al., 2020). Fish cultivation and nutrition have received significant attention during the last 10 years (Okomoda et al., 2017).

Fish is one of the important and cheap sources of lean meat, as over half of the world's population depends on it as a supply of dietary protein. Aquaculture produces a substantial amount of fish for consumption by humans (Ogunkalu, 2019). Live food is the most effective for fish since it is both natural and nutritious (Oramary, 2016). The ability of fish to exhibit its genetic potential for growth and reproduction is primarily determined by its nutrition. Nutritional deficiencies might weaken immunity, increase disease vulnerability, hinder physical and mental development and decrease performance (Mohanty et al., 2019).

Evolution of Aquafeed Formulation

The primary feed used in fish farming is either formulated aquatic feed or sources of nutrients. In aquaculture, feed accounts for 50 to 80% of total production expenses. A cheap cost of production and a diet rich in nutrients are essential for successful aquaculture. The price and nutritional expenses of the feed additives and ingredients used in feed formulation determine the feed's nutritional price and quality. Feed ingredients are made up of both inorganic and organic components (Yousefi et al., 2018). Fishmeal has been employed as a significant source of protein for both carnivorous and omnivorous species. A larger percentage of fishmeal is found in many aquaculture feeds than in the diets of other animal species (Raswiswi et al., 2021).

Fishmeal has typically made up the majority of the protein used in aquaculture feed, making up roughly 68% of fishmeal produced worldwide (Tacon and Metian, 2015). Fishmeal inclusion levels in aquaculture feeds have, however, been steadily declining in recent years; this could be due to a stagnant supply, rising costs, or ethical concerns. Furthermore, using a lot of fish meal in aquatic feed may cause a lot of environmental problems (Han et al., 2018). Fluctuations in fishmeal supply, price and quality pose serious risks to fish health. The recognition, development and exploitation of alternatives to fishmeal has gained great attention to reduce the risks associated with fishmeal supply (Glencross et al., 2020). To be a competitive feed substitute for fishmeal in aquaculture feeds, a candidate component needs to have the following qualities: full availability, ease of handling, shipping, storage and application in feed production. The necessity for more study into alternatives has arisen as a result of the rising costs and limited availability of fish feed (Raswiswi et al., 2021). As a result, additives must be given to fish feed (Ogunkalu, 2019).

Feed Additives in Aquaculture: Enhancing Growth and Reducing Mortality in Fish

In aquaculture, one of the most significant objectives is to improve fish growth performance. Several investigations have been conducted on fish feed formulation. Few studies investigate the influence of natural immune-stimulant additives in fish feed. Feed additives are edible ingredients that are very sparingly added to fish feeds to enhance the feeds, which in turn enhances fish growth and lowers the fish mortality rate (Dada, 2015). The source material and the method of extraction determine which of these components are used. Feed additives improve the growth performance of the species being cultivated as well as feed consumption and protein utilization rates when added to the diets of aquatic animals, particularly fish. Probiotics, plants and some algae are among the living things used as feed supplements (Ogunkalu, 2019).

Feed additives are applied throughout feed processing to increase feed quality, the health of fish and feeding efficacy. Antioxidants, immunostimulants, probiotics and antibiotics are among the many possible non-nutritious sources of Functional feed additives (FuFAs) (Bharathi et al., 2019). These elements in fish feed also raise the price of production. To combat rising expenses, feed industries have adopted the use of FuFAs which have emerged as an alternative to antibiotics and chemotherapeutics (Yousefi et al., 2018). FuFAs perform better than standard feed additives in terms of growth, physiology, immunological response and fish overall health (Alemayehu et al., 2018).

Functional Feed Additives

Functional feed additives are nutritive and non-nutritive substances added to fish diets for particular uses, such as improving the feed's physico-chemical qualities or the target species's performance (Bai et al., 2015). Several functional feed additives are available that fulfill these functions; which can be divided into various groups based on their origin and chemical composition. The effects, contribution price and commercial availability of prospective feed additives should all be taken into consideration while choosing them. Potential feed additives include sodium butyrate (an organic acid), gamma-aminobutyric acid (an amino acid that is not found in proteins), selenium-yeast (a yeast extract with added minerals), nucleotides (a yeast extract), yucca meal (a plant extract), song-gang stone (a natural mineral) and protease (a digestive enzyme). The feed additives improve the growth, immunological response and intestinal histology of aquatic animals (Wang et al., 2019). It is imperative to acknowledge that the efficacy of functional feed additives is contingent upon the target species and production environment. Similarly, the practicability of their implementation is contingent upon market availability and cost (Bae et al., 2020).

Functional feed additives (FuFAs) are various feed additives used in aqua-feeds to improve diet's nutritional value, are the range of feed additives used in aqua-feeds to enhance the diet's nutritional quality, increase pellet binding efficiency, stop sensitive nutrients from oxidizing, increase nutrient availability, get rid of anti-nutritional factors, elevate the quality of product and lengthen the lifespan of aquafeeds (Hossain et al., 2024). In addition to offering vital nutrients, FuFA supplements are among the dietary additives that promote health and growth. The development of aquafeed has adopted a new paradigm with the use of FuFAs. Sustainable and economically feasible aquafeeds are essential. Fishmeal's usage as the only source of protein in aquafeeds is restricted by rising market demand and diminishing supply (Hossain and Koshio, 2017).

Classification of Functional Feed Additives

Fishmeal must be reduced, if not eliminated while preparing fish feed to increase the amount of plant-based protein concentration and by-products of meat and fish processing industries. On the other hand, fish performance may suffer from plant-based alternative protein sources, particularly if they exceed a particular dietary percentage. The addition of functional feed additives (FuFAs) can lessen the detrimental effects of increased inclusion in fish diets and increase the utilization of substitute plant proteins (Hossain and Koshio, 2017). Furthermore, using a comprehensive approach to create functional aquafeeds may lead to enhanced immunity, growth and stress tolerance against illness challenges, which would improve the present antibiotic and chemotherapeutic treatment methods for cultured organisms. Numerous substances have the potential to be FuFAs, including vitamins, minerals, immunostimulants, probiotics, prebiotics, seaweed extracts, acidifiers and phytogenics (Gomez and Balcazar, 2008).

Enhancing growth, improving reproduction, extending feed shelf life, minimizing anti-nutrient effects, optimizing nutrient usage, enhancing the quality of the final product and providing general health advantages to cultured organisms are the primary goals of feed additives. FuFAs fall into two main categories. First, nutrients (such as nucleotides, amino acids, carotenoids, n-3 polyunsaturated fatty acids, vitamins and minerals) are described as supplements that include both macro and micro nutritional components and provide essential nutrition while also enhancing animal health and stress resilience. Non-nutrients and various other substances do not provide animals with primary nutrition but have a positive impact on their health by modifying different physiological responses including chitosan, peptidoglycan, β-glucans, lactoferrin, organic acids, plant extracts and essential oils (Hossain et al., 2024).

Probiotics

Probiotics are micro-organisms such as bacteria, fungi and yeast. Meanwhile, in rare circumstances, thermal inactive forms when ingested as a food ingredient or nutritional feed additives beneficially affect the formation as well as maintenance of the host's microflora and hinder disease invasion (Zorriehzahra et al., 2016). Supplementing animals with probiotics is good for their health since they affect the gut microbiota. Due to their capacity to alter the immune systems and gut microbiota of humans and animals, probiotics have been the subject of extensive research. In clinical and veterinary settings, probiotics are used both as therapeutic agents and as preventative measures. When it comes to improving the performance of farmed species, probiotics are seen to be a promising and effective substitute for antibiotics in fish diets (Alayande et al., 2020).

Research on the use of feed additives primarily synbiotics, probiotics and prebiotics, in aquaculture species feeds became a problem in the latter half of the 20th century (Okey et al., 2018). The use of synbiotics, prebiotics and probiotics in culture species also results in additional costs for the aquaculture industry because, before adding synbiotics, prebiotics and probiotics to feeds or diets, a thorough evaluation of the new strains should be conducted, evaluating for efficiency and welfare. Once more, to produce safe and high-quality synbiotics, prebiotics and probiotics, companies producing them must strictly enforce the use of modern methodologies; this is also likely to result in higher production costs (Rohani et al., 2022). Table 1 showed different probiotics and their impact on fish health.

Table 1: Effects of probiotics on fish health (Bharathi et al., 2019)

Probiotics	Impacts on Fish Health
Bacillus subtilis and *Streptomyces*	Enhanced the ornamental fish growth and survival
Bacillus cereus	Boosted the growth response of fishes
Bacillus coagulans and *Rhodopseudomonas palustris*	Enhanced the Specific Growth Rate and weight gain
Lactobacillus rhamnosus	Provided immunological enhancement
Enterococcus faecium	Improves the growth response and immune status

Amino Acids

The structural components of tissue protein are called amino acids and serve an important function as metabolic intermediates. Twenty amino acids are required for healthy animal function, especially fish and are classed as essential or non-essential based on their ability to be produced. Fish nutrition studies have also shown that several amino acids are regarded as functional amino acids (FAA), such as leucine, arginine, methionine, proline, cystine, glutamine, glutamate taurine, tyrosine, tryptophan and aspartic acids, are associated with and regulated by significant metabolic pathways that are connected to fish health, growth, development, reproduction, antioxidant defense and survival (Wu, 2013). Studies have shown that adding functional amino acids to aquafeed improved the overall performance of a variety of aquatic species and that reducing FAA in aquafeed stunted fish development and health, particularly when the fish were fed a diet heavy in plant ingredients (Hossain et al., 2024).

Taurine is classified as a functional amino acid (FAA) and is an amino sulphonic acid. It is missing from plant feed ingredients but plentiful in fishmeal (Lall and Dumas, 2022). Taurine supplements are required for marine fish-fed diets. Taurine is regarded as an essential amino acid (EAA) in certain species, or as a conditionally essential amino acid in others (Salze and Davis, 2015). Dietary taurine addition has been shown to improve fish performance in some aquatic species with low fishmeal feeds (Koven et al., 2016) or diets consisting solely of plant protein. Taurine should be supplemented to the diets of aqua-cultured species. Equally enhanced results have been recorded in freshwater carnivorous fish such as Rainbow Trout and Yellow Catfish (*Pelteobagrus fulvidraco*) except for omnivorous Nile Tilapia (*Oreochomis niloticus*) and herbivorous Common Carp (*Cyprinus carpio*) (Koch et al., 2016). Furthermore, including taurine in a sparse fishmeal diet has been shown to improve lipid peroxidation levels, enhance the functioning of important enzymes of intermediate metabolism and boost antioxidant activity (Li et al., 2016).

Other Feed Additives (Vitamins and Minerals)

Vitamins and minerals are micro-nutrients that are required in minute amounts for proper reproduction, development, health and survival of particularly shrimp and fish species (Upadhaya and Kim, 2020). Because animals cannot synthesize the majority of vitamins and minerals, they must be obtained from food. Several studies have looked at the link between fish health and immunological function, as well as vitamin and mineral supplements. Vitamins and minerals supply the substrates and co-factors essential for the physiological defense mechanism to operate properly. In contrast, the depletion of particular minerals and vitamins is believed to cause diseases and immunosuppression, hence their replenishment as feed additives in a diet is critical. Vitamins and minerals are usually provided in aqua feed as vitamins and minerals premixes, although in some situations, they may be delivered separately on the nutritional composition. In aquatic animal studies, iron, selenium and vitamins C, E and A have attracted the most interest for their possible practical features (Hossain et al., 2024).

Carotenoids

Carotenoids are natural pigments belonging to the extensive family of xanthophyll and carotenes. Carotenes such as β-carotene and lycopene are hydrocarbons without O_2 and xanthophylls include O_2 like cryptoxanthin, lutein and zeaxanthin. Carotenoids are naturally abundant and produced by microorganisms like phytotrophic bacteria, fungi and algae. Chicken, shrimp and fish being vertebrates are unable to manufacture carotenoids and must get them through food as an additive. Carotenoids have several roles, including visual pigments, antioxidants and colorants. Pro-vitamin A carotenoid (such as β-carotene) are transformed into retinol. Carotene and xanthophyll are distinct from each other as they produce orange and yellow colors respectively. The beneficial effects of carotenoids on aquatic species are the process of metabolism and may boost the utilization of nutrients, leading to enhance development and persistence, immunity and resistance to stress (Wang et al., 2019).

Astaxanthin is the carotenoid that is most frequently added to the feeds to enhance the color of different marine species. Other physiological advantages of astaxanthin supplementation have also been reported, including enhanced growth, survival rate, capacity for reproduction, resilience to stress and enhanced immune and antioxidant systems in shrimp and fish. Because of low production prices, almost all astaxanthin (more than 95%) is produced from synthetic sources instead of natural sources like yeast, fungi, algae and bacteria. However, astaxanthin is more necessary for biological sources than for synthetic ones. Most studies investigated the efficiency of astaxanthin administration and employed synthetic astaxanthin supplies in refined and semi-purified feeds (Lim et al., 2018). Whereas the benefits of administering astaxanthin in HAPP-based functional diets for fish and shrimp are quite restricted. The need for astaxanthin and carotenoids in fish diets varies by species and nutritional composition (Wang et al., 2021).

Enzyme Supplementation

The use of enzymes as an ingredient in fish feed formulation is growing in importance. Fish raised in aquaculture systems require their feed to be optimally digested by the appropriate enzymes to supply the necessary quantities of calories and essential nutrition. According to several studies, pre-treating plant-derived raw components with enzymes as functional feed additives increased the rate of fish development and feed digestibility (Maas et al., 2020). The majority of enzymes utilized in fish feed are hydrolases, with proteases, glucosidases and lipase having the most functions (Ghosh et al., 2019). These enzymes can enhance the breakdown of antinutritional factors (ANF) found in fish feed, including phytic acid, indigestible oligosaccharides such as stachyose and raffinose and antigen proteins, which impair fish development, digestion and malnourishment (Liang et al., 2022).

Exogenous enzymes, including phytases, which are required for the digestion of phytates produced from plants, can be supplemented into the diet to improve the bioavailability of phosphorus and other minerals as well as overall growth performance (Lemos and Tacon, 2017). To reduce aquaculture diseases and improve the health of farmed fish, several enzyme preparations that are used to improve intestinal health and suppress harmful bacteria have also gained attention. This holds significance as it has the potential to mitigate antibiotic use, enhance environmental quality and guarantee food safety in aquaculture. In contrast, the two most often utilized exogenous enzyme feed additives are lysozyme and glucose oxidase. They are employed for a variety of reasons, some of which are as follows: Improve feed consumption and digestibility and numerous studies have demonstrated the benefits of adding enzymes to fish diets (Liang et al., 2022). Fig. 1 shows the role of enzymes in fish feed.

Marine Seaweed

Marine seaweed (microalgae) is the most significant natural source of bioactive components. Many types of polysaccharides found in seaweed have the potential to be employed as prebiotics (Doan et al., 2019). In recent times, seaweed phenolics have gained popularity as a sustainable and appealing antioxidant source with a variety of bio-functional qualities. They have the potential to replace current aqua-feed additives. Although several phenolic compounds from various seaweeds have had their biological characteristics extensively studied, there have been few attempts to use these compounds as bio-functional components in aqua-feed formulations. Asia has utilized seaweeds mainly as a traditional food source, especially in China, Japan and Korea. Less than 20% are utilized in a variety of industrial applications, including fertilizers, bioplastics, cosmetics and feed ingredients in fish and animal feed (Gunathilake et al., 2022).

Seaweed has been used as aqua-feed in the form of a meal or an extract (Teves and Ragaza, 2016). This has been indicated to improve animal's overall physiological performance, including expansion rate, consumption of feed, disease resistance, reaction to stress, fillet quality, natural pigmentation, protein accumulation during the winter and elevated long-chain polyunsaturated fatty acids levels in fillets (Kamunde et al., 2019). As a result, developing aqua-feed formulations with specific seaweed-derived compounds might improve the wellness of fish at an affordable price. Seaweed phenolics offer substitute components that are compatible with artificial additives utilized for aquaculture, with a wide range of bioactive effects including prevention from microbes, viruses, fungi and stress also serve as an antioxidant, anti-inflammatory, immunostimulant and hunger stimulation (Freitas et al., 2015). Furthermore, their antioxidant activities reduce lipid oxidation, protect feed quality and extend shelf life. Silver seabream fed on diets enriched with seaweed demonstrated substantial Bromopheol found in fish gut and meat, giving the fillets a "sea-like flavor" (Gunathilake et al., 2022).

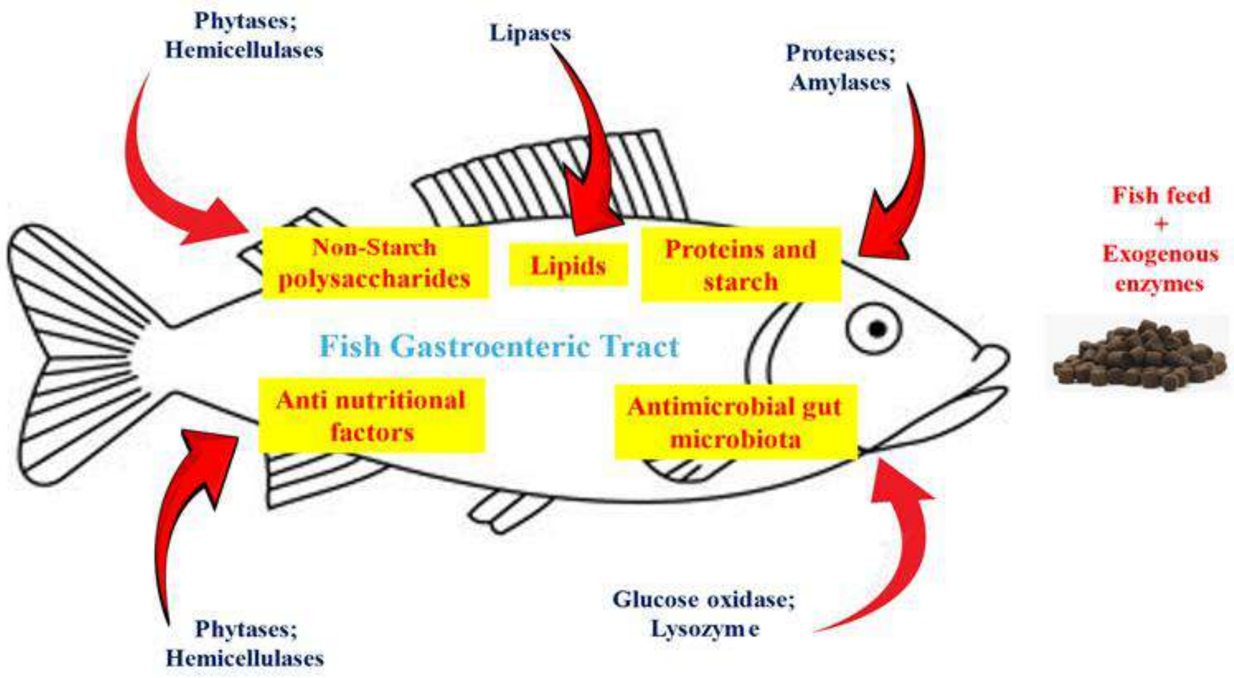

Fig. 1: Role of exogenous enzymes in fish feed (Liang et al., 2022)

Plant Derivatives (Phytobiotic)

Plant derivatives known as phytobiotic or phytogenic compounds are added to fish feed to enhance fish health and growth performance. These plant components contain a variety of qualities, including antioxidant, antibacterial, anticarcinogenic, analgesic, insecticidal, antiparasitic, anticoccidial, growth promoter, appetite enhancer, stimulator of bile secretion and digestive enzyme action (Bharathi et al., 2019). These phytobiotic substances are diverse feed supplements derived from many parts of the plants, including leaves, roots, tubers, fruits and spices. These ingredients can be utilized as an extract, powder, or oil (Alemayehu et al., 2018). When compared to a synthetic antibiotic, adding a combination of phytobiotics, such as pepper rosemary, red thyme and volatile oils of thyme, to the diet enhances fish resistance to *Aeromonas hydrophila* challenge, oxidative stress, immune and hematological responses and growth performance (Rezende et al., 2021). Table 2 showed the effect of different phytobiotic compounds on fish health.

Table 2: Impacts of phytobiotic compounds on fish health (Bharathi et al., 2019)

Phytobiotic Compounds	Effects
Astragalus radix	Provides excellent results against *Aeromonas hydrophila* infection and transportation
Astragalus radix	Root extract enhanced the leucocytic phagocytosis and lysozyme activity
Allium sativum	improved the blood indices and resistance against *Aeromonas hydrophila* infection
Psidium guajava	Controls *Aeromonas hydrophila* infection
Ipomoea batatas	Increased the growth response and feeding efficacy

Bioactive Immunostimulants

Bioactive immunostimulants have been employed to enhance aquatic animal health in a variety of ways, including protection from microbes, oxidants and inflammation and serve as growth-promoting immunostimulants. The incorporation of bioactive compounds in aquatic feed is an innovative method and this bioactive immunostimulant approach is accurate and reproducible. Furthermore, dietary administration of several bioactive immunostimulants (soybean isoflavones, butyric acid, polyphenol, lipoteichoic acid, propionic acid, chitosan, lentinan, lactoferrin and fucoidan) displays multiple advantages in aquatic organisms, including enhanced immunity, survival, growth and resistance to disease towards harmful microbes. Natural bioactive immunostimulant food additives technology is an innovative approach to improving aquatic productivity (Vijayaram et al., 2022).

Problems with Practical Application

The functional additives created for practical use methodologies must undergo an impeccable and precise methodology. This involves characterizing the additives through extremely careful investigatory conditions, uniform selection standards, a productive rough guide methodology and continuous production and reevaluation. Once more, factors like dosage and strain must be acknowledged as having an impact on the occurrence and magnitude of the reaction. Limits are determined by the particular preparation processes used and the organisms or species engaged or under consideration, which must be not only recognized but also overcome. Because the positive results obtained in the research laboratory are probably not appropriate or applicable to outdoor situations, field testing and challenge are quite important (Amenyogbe et al., 2020).

It is undeniable that using functional feed additives in the aquaculture sector is beneficial. The recent investigations devoted to exogenous carbohydrate enzymes (Castillo and Gatlin, 2015), prebiotics (Hoseinifar et al., 2017) and probiotics demonstrate the remarkable amount of knowledge that has been obtained within the previous 15 to 20 years. It appears that there is a clear divide between the understandings that are "science-based" and the successful practical applications, which call for the essential closure. Without a doubt, functional feed additives are a good alternative to fish diets due to their apparent benefits. However, accepting feed additives in the widely used aquaculture, also known as fish farming, presents logistical challenges (Amenyogbe et al., 2020).

Implication of Functional Aquafeed for Sustainable Production

Rising feed costs are a significant factor limiting the usage of Functional Feed Additives (FuFAs) in aquafeed, although there is a wide variation in feed cost increases depending on the type and quantity of FuFAs. The variety, processing techniques, the supplier’s commercial marketing plan and product purity all affect the costs of FuFAs. Without significantly increasing production, the relative costs of different FuFAs reduce additional feed costs. Specific FuFAs can offer better performance and be less expensive in specific situations. For instance, by utilizing inexpensive, locally sourced agricultural items or by-products, fermentation can be used to create helpful probiotic bacteria in the farmer's house/pond surroundings (Hossain et al., 2024).

The aquaculture industry's long-term survival depends on the development of functional feeds that are both aesthetically pleasing to consumers and ecologically sustainable. Due to an increased understanding of food safety and consumer health consciousness, a greater number of customers are knowledgeable about how fish are produced, including details about the cultured aquatic habitat and the quality of feed supplied. Furthermore, consumers believe that aquaculture producers have the major duty of ensuring healthy farmed fish. The primary goal of employing functional aquafeed in fish culture is to produce healthy fish without the need for medicine or antibiotics, therefore its usage in aquaculture will affect consumers' perception of fish that have been cultivated. However, any health advantages of raised animals associated with functional feeds should be based on good and legitimate scientific standards, as well as comprehensive investigations of their safety and effectiveness (Hossain et al., 2024).

Determining dietary combinations with other components and potential side effects with different therapeutic agents is crucial. Furthermore, these claims should also be extensively researched to assist consumers in comprehending the scientific foundation for claims made regarding the possible health advantages of consuming cultured fish produced with functional feed. Aquafeed is the key input and the main source of operational costs for aquaculture operations. However, maintaining a healthy aquatic environment, continuous feed management, illness prevention, stress reduction, high-quality fish seed and general culture monitoring are other essential inputs that are directly connected to increased output and a profitable aquafarm operation. Only when all of these problems are effectively addressed and bolstered by the dietary inclusion of functional feed additives can functional feeds be a part of an effective strategy to maximize fish production and health and minimize the risk of disease and deterioration of water quality in aqua-cultured organisms (Hossain et al., 2024).

Conclusion

Functional feed additives (FuFAs) are used to boost fisheries productivity and fortify resistance to infectious diseases, two things that aquaculture needs to be sustainable over the long term. Understanding how the animal's physiological and biochemical systems interact with the functional feed additives in the feeds is crucial for the further development of functional feeds. Additional functional feed additives are safe for the environment and may not negatively impact aquaculture. The development of ecologically and financially attractive functional feeds is essential to the aquaculture industry's long-term survival. Aquafeed is the major operational expense and input for aquaculture enterprises. However, maintaining a healthy aquatic environment, continuous feed management, illness prevention, stress reduction, high-quality fish seed and general culture monitoring are other essential inputs that are directly connected to increased yield and successful aquafarm operations. Functional feeds can only be included in a plan that will maximize fish health and production while minimizing the risk of illness in aquaculture organisms and declining water quality, provided that all of these issues are successfully managed and FuFAs are added to the diet. To determine whether FuFAs are most beneficial for a range of aquatic species, experimental meals were used rather than actual diets with high concentrations of replacement components. Further study is required to determine how functional additives fit into the molecular and physiological mechanisms governing the eating behavior of farmed fish on diets high in plant ingredients. Most aquafeed applications cannot employ numerous functional additives, like fucoidan, astaxanthin and nucleotide (NT), because of their high cost. However, if further research is on low-cost manufacturing techniques that employ several sources (such as industrial by-products) of different FuFAs, this restriction will most likely be eliminated. A wide range of co-products from the production of agro-industrial and fisheries items can be used as FuFAs sources.

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Chapter 08

Use of Probiotics in Ruminants

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ABSTRACT

Probiotics are microorganisms that have nutritional, immunological, bacteriostatic, and bactericidal effects on their host animal. In ruminants, the supplementation of probiotics has been demonstrated to promote the proliferation of advantageous rumen microorganisms, thereby improving their nutrition, meat and milk production, reproductive health, and feed efficiency. Various strains of probiotics exhibit diverse mechanisms of action, contributing to their positive effects on health. Some commonly used probiotics in ruminants are *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Lactobacillus rhamnosus*. All these microorganisms help their host in different ways, highlighting the importance of selecting the adequate strain for a specific application in animal production and veterinary medicine. To select a potential probiotic to be used in ruminants, it is possible to isolate strains from the rumen and gut of the host animal. These strains must be metabolically active, promote animal health, and be safe for animals and humans. The effects of probiotics in ruminants are influenced by the specific microbial strain or mix of strains used, as well as the dosage, timing, and frequency of administration.

KEYWORDS
Probiotics, Ruminants, Microorganisms, Mechanisms of action, Production systems

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INTRODUCTION

The gastrointestinal tract (GIT) of ruminants harbors over 500 species of microorganisms in a complex environment. The symbiotic relationship between the ruminant and its gut microbiota is well-documented, playing an essential role in animal health and development. Several studies have focused on improving the gut microbiota of ruminants to enhance their growth performance and feed, especially by investigating the rumen (Abd El-Tawab et al., 2016; Khan et al., 2016; Alawneh et al., 2020; Lambo et al., 2021; Reuben, et al., 2022; Robles-Rodríguez et al., 2023). Preserving an optimal balance in both rumen and gut microbiota provides suitable growth to the host animal and enhances the development of animal food products (Robles-Rodríguez et al., 2023).

Nowadays, the administration of probiotics in ruminants has been associated with various benefits, such as providing overall protection by mitigating gastrointestinal diseases, inflammation, and diarrhea, modulating the gut microbiota, and acting as growth promoters. (Saha et al., 2023). Probiotics are "live microorganisms which, when administered in adequate amounts (at least 10^6 viable CFU/g), confer a health benefit to the host" (FAO and WHO, 2001). This definition includes the key aspects of probiotics: microorganisms, viability, and beneficial effects. They are characterized as not being toxic and pathogenic and having a status generally recognized as safe (Shokryazdan et al., 2017).

Nearly 40 commercial strains are used as single- or multi-strain probiotics for livestock production. These microbial food and additive supplements are endorsed by the Food and Agriculture Organization of the United Nations (FAO, 2016). Likewise, probiotics are being used as alternative antibiotics, as demonstrated by a growing number of researchers making considerable efforts to combat the global issue of antibiotic resistance (Leistikow et al., 2022). Their primary mechanisms of action include enhancing the function of the mucosal barrier and directly antagonizing pathogens. They also inhibit bacterial adherence and the capacity for invasion in the intestinal epithelium, enhancing the immune system and regulating the central nervous system (Ma et al., 2018; Fu et al., 2023).

Fig. 1 displays a classification of probiotics used in animal nutrition and production. The first classification distinguishes between bacterial and non-bacterial probiotics. The majority are bacterial, and a few yeasts and fungi are classified as non-bacterial probiotics (Table 1). The second classification pertains to the ability of probiotics to form spores. The third group is based on the number of species present, categorized into single- or multi-strain. The last classification refers to whether these microorganisms are autochthonous or allochthonous (FAO, 2016). Nonetheless, it is recommended to obtain potential probiotic strains from the autochthonous target host for a better understanding of their microbiota and microbiome (Shokryazdan et al., 2017; Robles-Rodríguez et al., 2023).

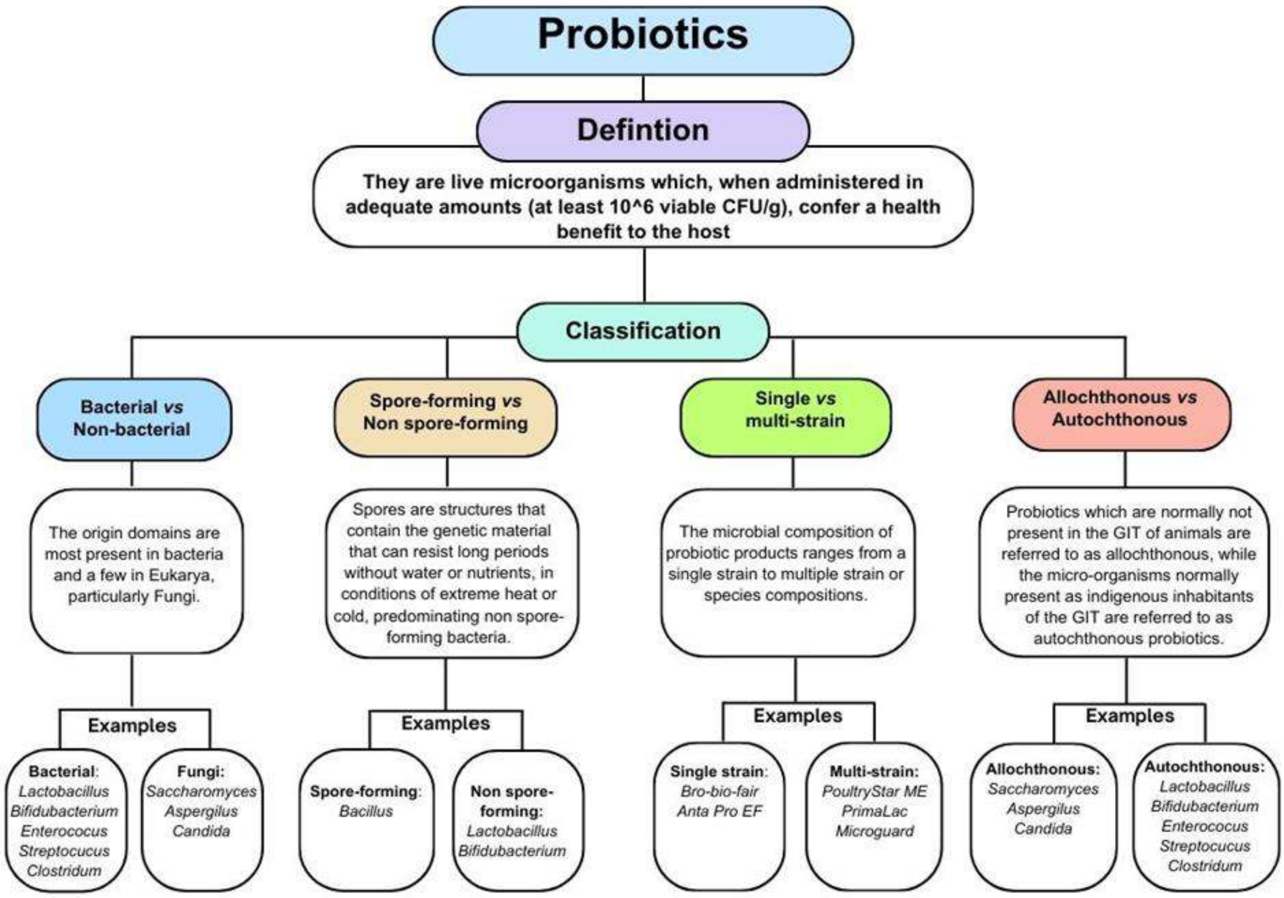

Fig. 1: Classification of probiotics used in animal nutrition and production (adapted from FAO, 2016)

The supplementation of probiotics in ruminants has been shown to enhance the growth of beneficial rumen microbes, thereby improving their nutrition, milk production, reproductive health, and feed efficiency. Nevertheless, the positive effects of probiotics could be inconsistent due to various factors such as their source, probiotic stability during storage and feeding, dosage, feeding frequency, and animal-related factors like age, health, and nutritional status of the host (Kulkarni et al., 2022). Thus, exhaustive research is imperative to evaluate the optimal probiotic strain and conduct a cost-benefit analysis.

Microorganisms used as Probiotics in Ruminants

Probiotics offer nutritional, immunological, bacteriostatic, and bactericidal effects to host animals, as previously mentioned (Robles-Rodríguez et al., 2023). Wochner et al. (2018) and Saha et al. (2023) stated that probiotics positively impact the microbial ecosystem, nutrient synthesis, growth performance, carcass weight, muscle production, meat and milk quality, prevention of intestinal diseases, and immunity. Different strains of probiotics demonstrate a range of ways in which they affect animals, thereby leading to their beneficial impact on health. *Saccharomyces cerevisiae*, a type of live yeast, is widely used as a probiotic in bovine nutrition due to its diverse range of functions in maintaining a stable rumen environment. This is crucial for the optimal performance of the microbial community, specifically the bacteria that can break down fibrous materials. Addition of *S. cerevisiae* to ruminant feed supplies organic acids and vitamins, which, in turn, promote the proliferation of lactic acid bacteria (LAB) (Khan et al., 2016; Arowolo and He, 2018). Shah et al. (2018; 2021) showed that bacterial probiotics can improve the conditions in the rumen; increasing dry matter intake (DMI), feed efficiency, and weight gain (Arowolo and He, 2018). One example is *Lactobacillus acidophilus*, known for its ability to bolster the immune system and facilitate digestion by the secretion of enzymes that help to break down lactose. *Bifidobacterium bifidum* maintains and promote a favorable gut microbiome by outcompeting pathogenic bacteria for essential nutrients and attachment sites. A study has demonstrated that *Lactobacillus rhamnosus* strengthens the integrity of the intestinal barrier, thereby preventing that toxic substances and disease-causing microorganisms enter the circulatory system (Song and Kim, 2019). These examples demonstrate the various ways in which various probiotic strains interact with the body of animals through distinct processes. This underscores the significance of carefully choosing strains for various purposes in animal production and veterinary treatment (Fu et al., 2023).

Table 1 lists various microorganisms that have been used as probiotics in ruminants. Each microorganism targets different aspects to exert its effects and yields varying results in young or adult animals, influencing animal production and intestinal health differently.

Table 1: Microorganisms frequently employed as probiotics in ruminants

Genus	Species
Bacteria	
Lactobacillus	*L. acidophilus*
	L. alimentarus
	L. amylorvous
	L. animalis
	L. brevis
	L. bulgaricus
	L. casei
	L. delbrueckii sub sp. Bulgaricus
	L. fermentum
	L. gallinarum
	L. helveticus
	L. johnsonii
	L. mucosae
	L. plantarum
	L. reuteri
	L. rhamnosus
	L. sakei
	L. salivarus
	L. sporogenes
Lactococcus	*L. cremoris*
	L. lactis
Bifidobacterium	*B. animalis*
	B. bifidum
	B. breve
	B. lactis
	B. longum
	B. pseudolongum
	B. ruminantium
	B. thermophilum
Enterococcus	*E. faecalis*
	E. faecium
Pediococcus	*P. acidilactici*
	P. pentosaceus
Bacillus	*B. amyloliquefaciens*
	B. cereus
	B. coagulans
	B. licheniformis
	B. mesentericus
	B. natto
	B. subtilis
	B. toyoi
	B. toyonensis
Paenibacillus	*P. sp*
Prevotella	*P. bryantii*

Escherichia	*E. coli (*EHEC O111:NM, EHEC O157:H7)
	Escherichia coli Nissle 1917, (ECN 1917 O6:K5:H)
Streptococcus	*S. thermophilus*
	S. bovis
	S. faecium
Megasphaera	*M. elsdenii*
Butyrivibrio	*B. fibrisolvens*
Propionibacterium	*P. shermanii*
	P. freudenreichii
	P. acidipropoinici
	P. jensenii
Fungi and Yeast	
Aspergillus	*A. niger*
	A. oryzae
Candida	*C. ethanolica*
	C. pararugosa
	C. rugosa
	C. tropicalis
Magnusiomyces	*M. capitatus*
Debaryomyces	*D. hansenii*
Saccharomyces	*S. boulardi*
	S. cerevisiae
Pichia	*P. kudriavzevii*
Galactomyces	*G. sp.*

Adapted from Seo et al. (2010), Abd El-Tawab et al. (2016), Nalla et al. (2022), Fu et al. (2023), and Cabral and Weimer (2024).

How to make a Probiotic for Ruminants

Strain Selection

Probiotics have demonstrated beneficial effects on ruminants (Shokryazdan et al., 2017). Thus, several studies have isolated strains from the rumen and feces of ruminants as potential probiotics (Rodríguez-González et al., 2023a; Ruvalcaba-Gómez et al., 2023b). Selecting a strain to be considered as a probiotic must have specific characteristics: the ability to colonize, be metabolically active, promote animal health, and be safe for animals and humans (Saha et al., 2023). A potential strain should also demonstrate stability and resistance to absorption in the upper GIT (Abd El-Tawab et al., 2016). It must be non-toxic, non-pathogenic, and well-identified at a molecular level (genus and species). 16S deoxyribonucleic nucleic acid sequencing is a viable approach for accurate identification (Shokryazdan et al., 2017). The Food and Agriculture Organization stated in 2002 that the strain must meet specific criteria for a probiotic to obtain GRAS status. These criteria include undergoing *in vitro* assessment for pH, temperature, and bile acid tolerance, adherence to mucus, antibiotic resistance, and the ability to reduce pathogens. Additionally, *in vivo* studies are necessary to confirm its functionality (Shokryazdan et al., 2017).

Culture Media

It is crucial to consider the characteristics of the strain when selecting the optimal culture media. For instance, LABs are grown best at pH 5.5-5.8 in a complex environment of nutrients (Miranda and Nader-Macías, 2023). The most used laboratory media to culture probiotic bacteria is Man Rogosa and Sharpe, although other media can be used (Hayek et al., 2019). At an industrial level, using conventional media is expensive and impractical. Therefore, it is necessary to explore alternatives that enhance biomass production. Substitutes such as industrial residues (soybean residue flour, corn syrup, whey protein concentrate, molasses, and commercial nutritional supplements) have been used to cultivate microorganisms to enhance production of biomass (Coghetto et al., 2016). Additionally, food-grade ingredients have been shown to be effective in culturing probiotics (Boontun et al., 2020).

Preservation Methods

The stability and viability of probiotics are crucial to ensure their functionality. Dried probiotics have better long-term storage capabilities, enabling transportation without refrigeration (Celik and O´Sullivan, 2013).

Spray-drying and freeze-drying are common long-term preservation methods (Celik and O’Sullivan, 2013). Spray-drying involves the atomization of a liquid in a hot air chamber with controlled inlet temperature and airflow, whereas freeze-drying involves water removal through the sublimation principle (Baral et al., 2021). Spray-drying is more economical than freeze-drying, but the latter is more commonly used to preserve probiotics due to low-temperature exposure. The critical point in both methods is the extreme temperatures applied (freeze-drying at -20 to -80°C, while spray-drying uses an inlet temperature of up to 170°C) (Tang et al., 2020). Nonetheless, the use of protectants such as proteins, carbohydrates, lipids, and gums minimize the damage caused by temperature fluctuations (Bircher et al., 2017;

Baral et al., 2021). Sugars induce shrinkage of the cells, reducing intracellular ice formation (Bircher et al., 2017). Skim milk is also used as a protectant (Rodríguez-González et al., 2022); it stabilizes the membranes of bacterial cells and facilitates rehydration by generating a porous structure with a large surface area (Selmer-Olsen et al., 1999).

Other methods of drying probiotics include vacuum drying and fluidized bed drying (FAO, 2016). Therefore, it is crucial to take into account the attributes of the probiotics when selecting the appropriate drying method.

Mechanisms of Action of Probiotics in Ruminants

Probiotics operate within the host systems through the three primary mechanisms described as follows:

Competitive Exclusion

This term refers to the occurrence when one species outcompetes and eradicates another species in a particular ecological niche. Probiotics can offer advantages by effectively preventing or inhibiting the growth of harmful or disease-causing microorganisms in the GIT. They engage in competition for nutrients and adhesion sites, which impedes the formation of pathogens and decreases the incidence of infections. Through the process of colonization, probiotics contribute to the preservation of a harmonious and robust gut microbiota (Dos Reis et al., 2017; Fu et al., 2023).

Colonization Resistance

Refers to the body's capacity to inhibit the colonization and proliferation of pathogenic bacteria. Probiotics can strengthen the natural defense systems of the host by supporting the ability to resist colonization. This refers to the capacity of the indigenous gut microbiota and the provided probiotics to withstand the colonization of detrimental bacteria. Probiotics have the ability to fortify the intestinal barrier, boost immunological responses, and generate antimicrobial substances that hinder the proliferation and infiltration of detrimental microorganisms (Molska and Reguła 2019; Fu et al., 2023).

Regulation of Physiological Processes

Probiotics can exert influence on multiple facets of the body's health and physiological processes. They could exert effect on the immune system by promoting the generation of advantageous immune cells and augmenting immunological reactions. Probiotics can also impact the process of decomposing, assimilating, and metabolizing nutrients, resulting in enhanced nutritional utilization and overall animal performance. It is crucial to acknowledge that, although these pathways offer a thorough comprehension of how probiotics may operate, the precise mechanisms of action can differ based on the probiotic strains and the species they interact with. Further investigation is required to comprehensively understand and reveal the intricacies of the biological impacts of probiotics within the host system (Oelschlaeger, 2010; Molska and Reguła, 2019; Fu et al., 2023).

Probiotics in ruminants can be used:

1) To manipulate ruminal fermentation. The primary purpose of selecting probiotics for adult ruminants is to enhance the ability of rumen microbes to digest cellulose. These probiotics have beneficial impacts regarding several digestion processes, including cellulolysis and the synthesis of microbial proteins. The predominant type of probiotic typically employed in dairy cows is a diverse array of yeast strains, primarily *S. cerevisiae*. Regarding the use of probiotics in adult ruminants, lactate-producing bacteria such as *Enterococcus* and *Lactobacillus* are more effective in maintaining a consistent level of lactic acid compared to *Streptococcus bovis*. This could potentially help prevent acidosis in animals that are fed high-concentrate diets, especially feedlot cattle. *Megasphaera elsdenii,* or *Propionibacterium* species, which metabolize lactate, have also been introduced as direct-fed microbials (DFM) to prevent lactate buildup in the rumen (Uyeno et al., 2015).

2) To modify intestinal permeability in order to manipulate absorption and/or alter bacterial populations, thereby improving intestinal health. Probiotics can directly interact with host intestinal epithelial cells (IECs) and dendritic cells (DCs), influencing signals that lead to the formation of mucus and defensin. They also enhance the function of tight junctions and the intestinal barrier while preventing apoptosis triggered by cytokines (Schlee et al., 2008; Ma et al., 2018). IECs and DCs engage with and respond to gut microbes through their pattern recognition receptors (PRRs). The macromolecules found in the bacterial cell wall, such as peptidoglycans in Gram-positive cells and lipopolysaccharides in Gram-negative cells, serve as important binding sites for probiotics. These ligands have the ability to interact with PRRs and initiate signaling pathways, facilitating the communication between the probiotics and the host organism. Pili on the surface of bacteria have a crucial role in attaching bacteria to host PRRs (Ma et al., 2018). Moreover, probiotics can inhibit the proliferation of harmful microorganisms through various mechanisms: a) synthesis of toxic or antimicrobial substances such as bacteriocins, organic acids, and hydrogen peroxide, which directly inhibit the proliferation of harmful microorganisms; b) competition with pathogens for limited nutrients and energy, thereby inhibiting their growth and reproduction in the gut; c) adherence to the surface of the intestinal lining and competing with pathogens for attachment sites; and/or d) acidifying the stomach by producing lactic or acetic acid to hinder the proliferation of specific bacteria such as *Salmonella* and *E. coli* (Bermudez-Brito et al., 2012; Kumar-Bajaj et al., 2015; Ma et al., 2018). An effective probiotic should be free from pathogens and toxins, capable of surviving gastric acid, adhering to the intestinal lining, and

producing chemicals that combat microorganisms. Furthermore, it is important for it to remain in the GIT for an extended duration in order to demonstrate a positive impact (FAO, 2016).

Application of Probiotics in different Ruminant Production Systems

Intestinal Health in Calves

Intestinal digestive functions are crucial, involving chemical and enzymatic digestion, peristaltic movements, nutrient absorption, and excretion of waste products. In addition to its digestive functions, the intestine serves other important roles. It acts as an immune organ, regulates nervous and endocrine signals in a gut-brain axis often referred to as the "second brain", controls pathogens, and creates a favorable environment for the complex relationship of mutual benefits or symbiosis between the microbiota and the host (Rodríguez et al., 2020).

In young calves, common risks and factors that lead to stress frequently include environmental conditions and husbandry practices, such as separation from the dam, vaccination, tagging, dehorning, delaying colostrum intake, and inappropriate use of antibiotics (Stecher et al., 2013; FAO, 2016). As a result, animals may suffer dysbiosis or an inadequate microbial balance in the GIT (FAO, 2016). Weight loss is a significant risk for producers (Berge et al., 2009). Evaluation of health in calves, including common outcomes, such as fecal consistency score, clinical examination, mortality, blood parameters, and gastrointestinal microbiota, was the focus of a scoping review on trials evaluating probiotic supplementation in dairy calves. The review mentioned that out of 110 trials, 52 reported the use of various genera: *Bacillus* (11.5%), *Enterococcus* (7.7%), *Lactobacillus* (34.6%), *Saccharomyces* (13.5%), multiple genera (26.9%), and other genera (5.8%) (Branco-Lopes et al., 2023). Based on the research conducted by Alawneh et al. (2020), a review and meta-analysis of probiotic supplementation in calves, there is sufficient evidence to confirm that probiotics promote accelerated digestive development and significantly improve animal performance and productivity parameters.

Milk Production

The application of probiotics or DFM in dairy cattle provides benefits, highlighting the improvement in feed efficiency, quality and quantity of milk production, increase of the digestibility of the nutrients in the feed, enhancement of the immune system, reduction of infection, and diarrhea (Uyeno et al., 2015; Nalla et al., 2022).

The results of several studies on the administration of probiotics (*Bacillus subtilis*, *Enterococcus faecalis*, *Prevotella bryantii*, *P. acidilactici*, *L. plantarum*, *L. acidophilus*, *L. casei*, *B. thermophilum*, *E. faecium*, and *S. cerevisiae*) in dairy domestic ruminants, including cows, goats, and ewes, support these benefits (Kritas et al., 2006; Nocek and Kautz, 2006; Chiquette et al., 2008; Desnoyers et al., 2009; Boyd et al., 2011; Salvedia et al., 2015; Ma et al., 2020). However, controversial results have been found in other studies, which showed no effects on the mentioned production variables for the DFM, such as *L. acidophilus*, *Propionibacterium freudenreichii*, and *S. cerevisiae* (Boga and Gorgulu, 2007; Raeth-Knight et al., 2007).

The use of the yeast *S. cerevisiae* in adult ruminants has been widely researched to date, so that the meta-analysis of 110 papers made by Desnoyers et al. (2009) shows the main effects in digestibility, ruminal parameters with increases in rumen pH ($p<0.05$), volatile fatty acids (VFAs) ($p<0.01$), and organic matter digestibility ($p<0.05$), as well as higher DMI ($p<0.05$) and milk yield ($p<0.001$), as compared to the group without probiotics.

Meat Production

The use of probiotics in beef has the purpose of providing protection against stress for the calves and during the feedlot cattle process. Besides, the effect of stimulating the immune system and improving animal efficiency and performance has been mentioned in several works. The latter effect is due to the production of VFAs, which could increase the energy availability. It has also been suggested that the DFM that inhabit the intestine improve the feed conversion ratio and growth rate to increase nutrient digestibility and feed intake (Arowolo and He, 2018).

Some works on domestic ruminants (cattle and goats) have shown that the administration of probiotics, such as *Limosilactobacillus fermentum*, *Limosilactobacillus mucosae*, *L. acidophilus*, *L. salivarius*, and *L. reuteri*, increased the daily weight gain or the body weight (Chiofalo et al., 2004; Mansilla et al., 2023).

The effect of stimulating the immune system reported by Barreto et al. (2021) on the review and meta-analysis of 19 works of the use of probiotics in cattle did not show any significant positive effect on cattle immunity and disease prevention. The previous results were attributed to several factors in the studies and to those inherent to the animals, which could be possible as Uyeno et al. (2015) mention and, as they analyzed, the probiotics efficacies might not be consistent due to numerous factors, especially because of the dynamics of the GIT community.

Small Ruminants

In recent years, small ruminant production has become an important source of livestock products for human consumption. The addition of feed additives is a positive contribution. However, the use of antibiotics can lead to dysbiosis in the GIT, causing microorganisms to become resistant. Due to these harmful effects, probiotics are emerging as a viable alternative (Abd El-Tawab et al., 2016; Reuben et al., 2022). Therefore, the utilization of microorganisms as probiotics in small ruminants depends on the objectives of the producers, such as growth promotion, health enhancement, disease mitigation, milk or meat production, and the improvement of livestock product quality. The enhancement of performance and productivity parameters of ruminants depends on a variety of factors, such as ruminant species and breeds, different strains of microorganisms, dosage, environmental conditions, feeding strategies, farm management, and others (Reuben et al., 2022).

Wild Ruminants

To date, only a few studies have been done on wild ruminants, but this area requires more research. Some works on wild ruminants, like the moose (*Alces alces*), have demonstrated that the administration of probiotics, such as *Bacillus foraminis*, *B. firmus*, *B. licheniformis*, *Staphylococcus*, and *Saprophyticus* bovis increased the dietary efficiency (Ishaq et al., 2015). Additionally, the administration of *L. casei*, *L. plantarum*, and *Bifidobacterium*, significantly improved the health of musk deer (Yang et al., 2021).

The use of the yeast *Saccharomyces cerevisiae* in buffaloes resulted in significant ($p<0.05$) improvement of DMI, milk yield and milk components, digestibility of nutrients, and body weight gain (Ali et al., 2023).

Conclusions

Probiotics in ruminants can be used as an alternative to improve the production of volatile fatty acids at the rumen level and to enhance and promote intestinal health. In any case, these effects will contribute to improving the production of meat and milk, the reproductive efficiency, and the ruminant health.

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Chapter 09

Use of 6-Gingerol as Potential Prebiotics to Promote Gut Health

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ABSTRACT

Zingiber officinale (ginger), a common spice has been used for medicinal and culinary purposes, treating various ailments ranging from gut health to various other problems. 6-gingerol (6G), a significant bioactive component of ginger has been interlinked with several properties including analgesic, anti-oxidant, anti-inflammatory, chemo-protective, gastrointestinal, neuro-protective, cardio-protective, and anti-diabetic effects. Ginger chemical constituents contains more than 400 compounds. It comprises both volatile and non-volatile components. Non-volatile compounds include zingerone, paradols, shogaols, and gingerols. Volatile compounds comprises monoterpenes (geranial, borneol, linalool, neral, and cineole), as well as sesquiterpenes (curcumene, zingiberene, and β-sesquiphellandrene). Of them, shogaol and gingerol are more abundant than the others. Ginger extract contains anticancer properties that include cytotoxicity, chemoprevention, and cellular protection. Several studies contended that utilizing ginger in diet may assist in treating and preventing gastrointestinal disorders such as gastrointestinal cancer, nausea and vomiting, Irritable bowel syndrome (IBS), and other digestive problems. This chapter aims to provide general facts about 6-gingerol and its beneficial effects on gut health.

KEYWORDS
Zingiber officinale, 6-gingerol, Prebiotics, Gut health, Gastrointestinal cancer

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INTRODUCTION

The tropical flowering plant known as *Zingiber officinale*, (Ginger) belongs to Family: Zingiberaceae consumed as a spice in cooking all over the world because of its distinct flavor and aroma (Kubra and Rao, 2012). The Greek word "zingiberis," which itself derives from the Sanskrit name of the spice "singabera," is the source of the generic name Zingiber. As a result of its root's likeness to a deer's antler, the Sanskrit name singabera means "shaped like a horn" (Sharma, 2017; Srinivasan et al., 2019). Gingerol-like compounds and gingerol, as well as antioxidants like alkaloids, polyphenols, ascorbic acid, terpenoids, and beta-carotene, are among the several identified bioactive constituents of ginger, which is widely used for its anti-inflammatory, antimicrobial and bio-absorption properties (Peterson et al., 2019).

6-gingerol, the primary functional ingredient of gingerol, is a type of phenolic compound that has been shown to have strong anti-apoptotic, anti-inflammatory and antioxidant properties as well as to control blood lipid and blood sugar levels (Li et al., 2017; Choi et al., 2018; Rahmani et al., 2014). It has also been found that the length of 6-Gingerol present in the colon was upto 12 hours, that may improve their antioxidant capacity and beneficial benefits (Majdoub et al., 2019). 6-gingerol also ameliorated bleeding and ulceration in the gastrointestinal tract induced by aspirin (Zhu et al., 2017).

According to reports, 6-gingerol intervention has a positive impact on colon health (Jiang et al., 2020). 6-gingerol a significant bioactive component of ginger has been recorded to possess antiemetic, antitumor, antioxidant, antiviral and anti-inflammatory properties (Promdam and Panichayupakaranant, 2022). Thus, 6-gingerol is anticipated to be a prospective prebiotic that enhances gut health to benefit human health (Wang et al., 2020). Prebiotics are referred to as probiotic food because they support the development of bacteria that create a balanced gut flora. They also lessen the possibility of oxidative damage and intestinal irritation. The main prebiotic sources include foods high in fiber that contain carbohydrates, such as inulin. Prebiotics are a crucial component of a balanced diet, although the amount of their main sources is restricted (Anand et al., 2022).

Chemical Constituents of Ginger

Most tropical areas of the globe are home to the cultivation of the rhizome of ginger (*Zingiber officinale*), a member of *Zingiberaceae* family that is used globally as a spice and herbal remedy (Seran, 2013). Ginger rhizome are composed of non-volatile pungent phytochemicals made up of shogaols, gingerols, zingerone, and paradols that are biologically active in nature, as well as volatile compounds such as β-phellandrene, β-elemene, linalool, zingiberol, β-bisabolene, β-sesquiphellandrene, α-zingiberene, camphene, borneol, limonene, geraniol, terpineol, α-farnesene, geranyl acetate, cineole, and curcumene (Wang et al., 2014). The strong phenolic compounds found in ginger are referred to as gingerols. The primary pharmacologically active ingredient in ginger is one of them, 6-gingerol (Fig. 1) (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy-3 decanone), whose aliphatic chain moiety with a hydroxyl group is what makes the molecule active. 6-A range of biological characteristics, including anti-platelet aggregation, antifungal, anti-inflammatory, antioxidant and anticancer effects, have been linked to gingerol (Wang et al., 2014). (Fig. 2) provides an overview of medicinal properties of ginger.

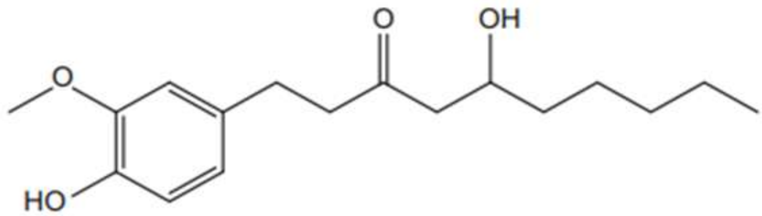

Fig. 1: 6-gingerol structure (Srinivasan et al., 2019)

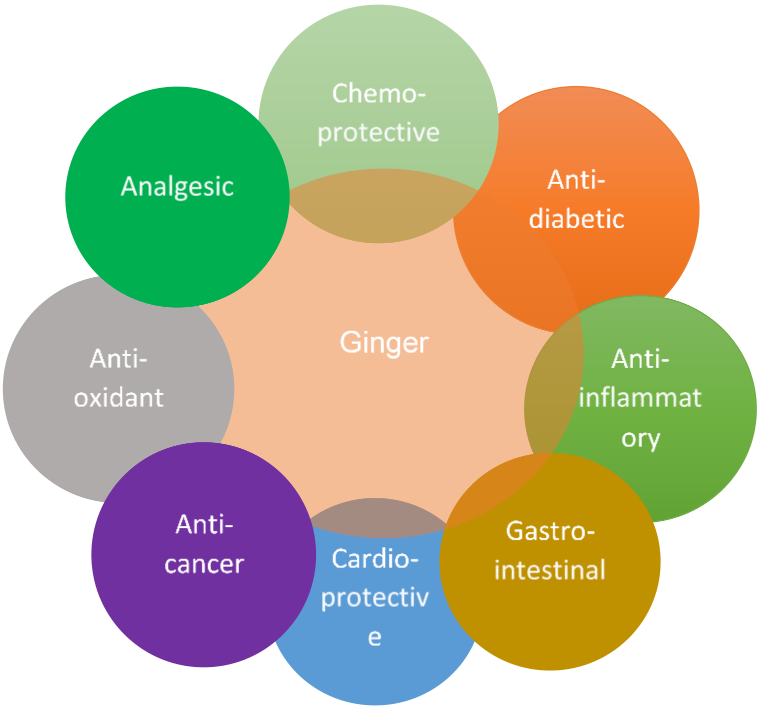

Fig. 2: Ginger medicinal properties (Menon et al., 2021)

Nutritional and Phytochemical Composition of Ginger

A powdered specimen of ginger has the following nutrients: Dietary fiber, vitamin C, calcium, iron, carotene, proteins, fats and carbohydrates (Kumari and Gupta, 2016). Its phytochemical analysis also shows the occurrence of anthocyanidins, total polyphenols, and flavonoids (Trinidad et al., 2012). In fact, the types and amounts of organic acids play a major role in how they are used in innovative functional foods. The rhizome of ginger plants contain 5 organic acids: oxalic, malic, succinic, tartaric and citric (Yeh et al., 2014; da Silveria Vasconcelos et al., 2019). Ginger's phytochemical composition, as determined by chemical analysis, includes phenolic chemicals like paradols, shogaol, and gingerol as well as terpenes inclusive of α-curcumene, zingiberene, β-sesquiphellandrene, α-farnesene, and β-bisabolene. The main ingredients of ginger dietary supplements have been identified as the bioactive chemicals 10-gingerol, 8-gingerol, 6-gingerol, and 6-shogaol. Its distinctive smell and strong taste are attributed to the volatile oils gingerols and shogaols, as well as to bisabolene and zingiberene (da Silveira Vasconcelos et al., 2019). Phytochemical and nutritional composition of ginger are enlisted in Table 1.

Ginger Importance in Gut Health

The gut microbiota, a diverse and ever-changing population of microorganisms found in the human gastrointestinal (GI) tract, has a significant impact on the host during both health and illness (Thursby and Juge, 2017). An essential food ingredient, ginger has a carminative action, eases intestinal cramps, lowers the pressure on the lower esophageal sphincter, and guards against bloating, flatulence, and dyspepsia (Nikkhah Bodagh et al., 2019).

Table 1: Phytochemical and Nutritional composition of ginger (Trinidad et al., 2012; Kumari and Gupta, 2016; da Silveira Vasconcelos et al., 2019)

Phytochemical and Nutritional Composition of ginger		
Composition	Ingredients	Powder sample/100g
Phytochemical	Anthocyanidins	22mg
	Flavonids	37mg
	Total polyphenols	55mg
Nutritional	Total carotene	76.7μg
	Dietary fiber	20.1g
	Iron	9.8mg
	Calcium	88.7mg
	Vitamin C	9.2mg
	Protein	6.08g
	Carbohydrate	39.35g
	Fat	3.6g

Since ancient times, ginger has been widely used to treat gastrointestinal symptoms like dyspepsia and gastrointestinal hemorrhage. It is primarily used to treat indigestion because it helps the liver and gall bladder produce and secrete more bile and absorbs certain toxins from the stomach. Because bile facilitates the breakdown of fats, cholesterol levels are lowered. Ginger extract has been shown in numerous preclinical and clinical investigations to effectively reduce a variety of gastrointestinal issues, including indigestion, nausea, vomiting, gastritis, belching, bloating, and constipation (Srinivasan et al., 2019). Apoptosis is reportedly induced by 6-gingerol, which is useful against stomach cancer (Mansingh et al., 2018).

Furthermore, it protects the stomach by preventing the formation of *Helicobacter pylori*, a significant ulcerogenic, and the related inflammatory lesions (Mahady et al., 2003; Gaus et al., 2009). Additionally, studies using various animal models of ulcerative colitis or colitis have demonstrated the efficacy of gingerols and ginger volatile oil (Rashidian et al., 2014; Zhang et al., 2017a).

Anti-ulcer and Anticholinergic

As a consequence of its antioxidant qualities, ginger serves to protect the gastric mucosa from a variety of substances that can induce ulcers. It is particularly helpful in cases where ulcers are developing. Because prostaglandins have been demonstrated to have gastro-protective and housekeeping role by maintaining the integrity of the stomach mucosa, this has several advantages as well as disadvantages. By increasing intestinal motility and blocking serotonin receptors, ginger exhibits potent antiemetic properties. It has been observed that ginger antagonizes 5-hydroxytreptamine receptors in the gastrointestinal tract and stimulates anti-histaminic and anti-cholinergic receptors (Imo and Za'aku, 2019).

Antimicrobial Properties

Significant antifungal and antibacterial effects are demonstrated by ginger. Ginger's active ingredients support the colon's in vitro colonization of proliferating bacteria, including Salmonella, proteus species, *E. coli, Streptococci*, and *Staphylococci.* By digesting undigested carbohydrates, which ginger can regulate, colon bacteria cause flatulence. Aspergillus, often known as fun gas, is inhibited in its growth by ginger and produces aflatoxin, a carcinogen. When kept at room temperature, fresh ginger juice extract demonstrated inhabitation against *L.acidophilus* (14%), *Mycoderma* spp. (12%), *A. niger* (4%), and *S.cerevisiae* (10%) (Zadeh and Kor, 2014; Mahmood, 2019).

Prebiotics and their Function

Regaining gut homeostasis can be achieved by consuming prebiotics, which are substrates that beneficial bacteria specifically use to promote the host's health (Sanders et al., 2019). Verified prebiotics are mostly indigestible carbohydrates from food, like fructans and galactans that pass through the upper gastrointestinal tract without being broken down and enter the colon or large intestine intact.

While fructans are naturally occurring fructo-oligosaccharides (FOS) and inulin found in various plant-based foods including onions, garlic, leek, bananas, chicory root, and Jerusalem artichokes, galactans are synthesized galactooligosaccharides (GOS) from lactose.

When dietary prebiotics enter the colon, beneficial bacteria like lactobacilli and bifidobacteria use them specifically for metabolism (Gibson et al., 2017). The substrate is fermented during this process, turning it into an energy source that modifies and improves the microbiota (Sanders et al., 2019). Therefore, prebiotics can be used as meals or supplements to address dysbiosis brought on by stressors such poor diet, sickness, antibiotic usage, surgery, lifestyle choices, and aging (Gibson et al., 2017).

Digestive Health

Additionally, ginger can assist in the bloodstream's delivery of nutrients and phytochemicals to the body's cells. Gastrointestinal illnesses brought on by dietary variables include gastroesophageal reflux disease (GERD), diverticular disease, and Crohn's disease. Ginger increases the activities of pancreatic lipase and amylase. Ginger is a key ingredient in enhancing the gastrointestinal tract's function. One gastrointestinal condition marked by aggressive reactions is ulcerative colitis. TNF-α (cancer necrosis factor) increases the seditious response by triggering an unaffected reaction flow. Additionally, ginger aids in attracting the growth of bacteria that reside in the gastrointestinal tract. The conclusion that ginger helps to civilize the strength of the gastrointestinal system is very definitive as of late (Naureen et al., 2022).

Gastrointestinal Cancer

Cancer of the digestive system, encompassing the whole alimentary canal including esophagus, stomach, pancreas, liver, gallbladder, small intestine, large intestine, rectum, anus, is referred to as gastrointestinal (GI) cancer. High-fat diet, alcohol consumption, smoking, infection, geographic location, family history, gender, age, and race are among the main risk factors for GI cancer. In wealthy nations, GI cancer is extremely common. Twenty percent of recently diagnosed cancer cases in the United States are related to gastrointestinal cancer. The most prevalent and second-leading cause of mortality among the many GI cancers is colorectal cancer (Prasad and Tyagi, 2015). Dysphagia, constipation, vomiting, chronic nausea, and early satiety are a few of the most upsetting symptoms that patients with advanced cancer reports having. The degree of the symptoms has been classified as moderate or severe in 60–80% of these patients, and they have significantly reduced the quality of life (Bhargava et al., 2019).

Preclinical studies have shown that ginger extract and its constituents have chemopreventive and antitumor properties against stomach cancer. 6-gingerol induces stomach cancer cells to go through apoptosis, according to an in vitro study. It facilitates apoptosis triggered by TRAIL (TNF-related apoptosis-inducing ligand) via increasing caspase-3/7 activation. 6-Gingerol caused apoptosis by inhibiting trail-induced NF-κB (nuclear factor-kappaB) activation and downregulating (cIAP)-1 (cytosolic inhibitor of apoptosis). Moreover, 6-shogaol damaged microtubules, reducing the potency of stomach cancer cells, in addition to 6-gingerol. In Sprague-Dawley rats with acetic acid-induced stomach ulcers, ginger extract significantly reduced the ulcer's surface area. Furthermore, ginger extract decreased the ulcerated mucosa's high levels of malondialdehyde (MDA), xanthine oxidase, and myeloperoxidase. As a result, ginger extract protects the stomach mucosa and functions as an antioxidant to aid in ulcer repair (Darekar et al., 2023). Benefits of ginger in patients with gastrointestinal cancer have also been summarized in Table 2.

Table 2: Benefits of ginger and its components in patients with gastrointestinal cancer (Prasad and Tyagi, 2015)

Effects	References
Boost the lymphocyte levels in people suffering from colorectal cancer.	(Khiewkhern et al., 2013)
lessen chemotherapy-induced delayed nausea and stomach dysrhythmia	(Levine et al., 2008)
Reduce PGE2 levels and inhibit COX in colorectal cancer	(Zick et al., 2015)
Reduce the number and frequency of adenomas	(Zick et al., 2015)
Reduce the expression of hTERT, MIB-1, Bax, and hTERT throughout the colon's crypts.	(Citronberg et al., 2013)
Reduce colon cancer's MIB-1, hTERT, and p21waf1/cip1 proliferation and differentiation	(Stoner, 2013)
Reduce the colorectal cancer mean percent change in 5-HETE and PGE-2 levels.	(Zick et al., 2011)
Reduce the expression of COX-1 protein in those who are more susceptible to colorectal cancer	(Jiang et al., 2013)

Inflammatory Bowel Disease (IBS)

With an increased risk of colorectal cancer (CRC), inflammatory bowel disease (IBD), which encompasses Crohn's disease (CD) and ulcerative colitis (UC), is a chronic inflammatory illness of the small intestine and colon that is extremely debilitating. Millions of people worldwide are afflicted by IBD, which mostly affects genetically predisposed individuals who have dysregulated immunological responses to a variety of environmental factors (Xavier and Podolsky, 2007; Cazarin et al., 2014; Beloqui et al., 2016). Frequent abdominal pain, diarrhea, vomiting, fever, blood in the stool, and weight loss with an increased risk of colon cancer are among the most typical symptoms (Bribi et al., 2016; Zhang et al., 2017b; Khare et al., 2020).

Ginger reduces the inflammatory response in inflammatory bowel disease (IBD) by suppressing the mTOR, nuclear factor kappa B (NF-κB), TNF-α, Nod-like receptor family proteins (NLRP), TLR, signal transducer of activators of transcription (STAT), and several proinflammatory cytokines (IL-6, IL-1β), as well as myeloperoxidase enzyme (MPO) (Ajayi et al., 2018; Zhang et al., 2018). Many writers have noted that the rhizome's gingerols and shogaols reduce inflammatory indicators in the liver by blocking NF-κB activation following a high-fat meal (Li et al., 2012).

The suppression of TNF-alpha results in the control of the inflammatory response, which in turn causes the downregulation of NF-κB signaling (Grzanna et al., 2005). In an experimental model of mice with ulcerative colitis caused by dextran sulfate sodium, oral administration of gingerol, a bioactive component of ginger, lowered the values of cytokines (IL-1beta, IL-6), TNF-alpha, NF-kB (p65), and elevated IL-10. Additionally, it reduced the activity of the enzyme cyclooxygenase-2 (COX-2) and monocyte chemoattractant protein-1 (MCP-1) (Ajayi et al., 2018).

For Nausea and Vomiting Treatment

Ginger is frequently used to treat nausea and vomiting. Moreover, it is an antiemetic. It breaks up and helps eliminate intestinal gas because of its carminative properties (Viljoen et al., 2014; Sharifzadeh et al., 2018). Ginger can reduce pregnancy-related nausea and vomiting (NVP). Approximately eighty percent of pregnant women have morning sickness, often known as nausea and vomiting during pregnancy (Quinla and Hill, 2003). According to one study, pregnant women who have mild to moderate vomiting and nausea can lessen their symptoms by taking a dose of ginger (Hu et al., 2020). When ginger is used before 16 weeks of gestation, it is more effective in treating mild to moderate nausea and vomiting (Saberi et al., 2013). Ginger binds to 5-HT3 receptors and increases detoxifying enzymes, which is predicted to promote antiemetic effects and fight oxidative damage to tissues (Geiger, 2005).

Conclusion

One of the world's healthiest and most popular nutritional condiments is ginger. In addition, ginger has been used as herbal treatment for numerous illnesses. Ginger food supplements could be viewed as a cutting-edge dietary strategy to lower the risk of chronic illnesses. Though ginger has numerous bioactive compounds, 6-gingerol is the major active ingredient. It has biological and physiological benefits, including antitumor, antioxidant, anti-inflammatory, and antiemetic effects. Because of its hypotensive, antioxidant, antibacterial, hypoglycemic, lowering lipid content, antiplatelet aggregation, chemo-preventive, and anti-inflammatory qualities, ginger has shown significant efficacy. Consequently 6-gingerol has crucial role in promoting gut health by preventing gastrointestinal issues. The ability of ginger and its polyphenols to target a variety of signaling molecules supports the use of the herb against complex human diseases like cancer. Ginger food supplements could be viewed as a cutting-edge dietary strategy to lower the risk of chronic illnesses. Further clinical research is necessary to determine the long-term effects and safe dosages of ginger supplements, though.

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Chapter 10

Commercially Available Human Probiotics and Prebiotics

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ABSTRACT

Probiotics and prebiotics have a significant importance in animal and human diet. It depends on the population and functions of the microbes that take over human bodies. Live bacteria and yeast are virtuous for our health, mainly for our digestive system, are known as probiotics. Probiotics can be found in different forms, like capsules, powders, tablets, and in some foods (like yogurt and kefir). Most common commercially available probiotic microorganisms are Lactobacillus, Bifidobacterium and yeasts, Saccharomyces. The non-digestible fibers that help in the growth of valuable bacteria are prebiotics. Starch, glucose-derived oligosaccharide and miscellaneous oligosaccharide in human and animal nutrition used as prebiotics. These are found in foods like whole grains, onion, garlic, asparagus and banana. Probiotics and prebiotics are available in the form of supplements. These supplements contain concentrated doses of prebiotic fibers for the support of beneficial bacterial growth in the gut. Both probiotics and prebiotics work synergistically when we take them together, they are involved in the upgrading of gut health. The probiotic organisms and prebiotic sugars in commercial products are often called synbiotics. Commercially available probiotics or prebiotics intricate in the improvement of immunity decreased the antibiotic-associated gastrointestinal disturbances or irritable bowel syndrome and downregulate the allergic response. It's advisable to check with a physician before starting any new supplements, particularly when you are taking medications, or you have underlying health issues.

KEYWORDS
Probiotics, Synergistically, Intestinal Infections, Supplements, Nutrition, Prebiotics.

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INTRODUCTION

Probiotics are characterized as alive microbes that are helpful in maintaining the host's health and therefore gaining popularity in a variety of dietary applications. Probiotics such as *Lactobacillus* species, *Bifidobacterium* species, *Streptococcus* species, *Enterococcus* species, and *Saccharomyces boulardii* are the most often used. Probiotics have a difficult ride through the GI system due to a number of physiological obstacles that can significantly impair their survival, including low pH, bile salts, enzymes, peristaltic motion (Roobab et al., 2020).

In the last few years, probiotics have captivated the interest of medical professionals due to their potential use in the management and escaping of various illnesses. Probiotics work primarily through enhancing the function of the mucosal barrier, directly opposing pathogens, preventing bacterial adhesion and invasion in the epithelium of the intestine, stimulating immunity, and controlling the central nervous system (CNS). Probiotics act as an accessary therapy that seems to be a capable means of preventing and mitigating the symptoms of clinical illnesses and boosting immunity by maintaining the balance of the gut microflora (Stavropoulou and Eugenia, 2020).

Mode of Action of Probiotics

The quality of life can be improved by the beneficial health effects of probiotics. It directly interactss with the immune cells and tries to maintain the immune balance of the GI tract. Probiotics are used as a tool to respond dysbiosis by flushing out the bad microorganisms with beneficial ones (Kechagia et al., 2013). The harmful microflora upset through the firming of the intestinal epithelial barrier, secretion of antimicrobial substances, competing for bonding to the mucosa and intestinal epithelium and by modifying the natural defense. The harmful bacteria try to control the immune system in case of different disorders such as obesity, infections and autoimmune diseases. The microflora of the human body has a strong relationship with the immune system, health and diseases.

Probiotics inhibits the expansion and colonization of the harmful microbes in the digestive tract, it also secretes the bioactive metabolites, and decreases the pH of the colon. Other modes of actions include enzymatic activity and the neutralization of toxin, synthesis of vitamins in the gastrointestinal (GI) tract. The helpful bacteria compete with the bad microorganisms for the nutrients. It is also involved in the increase of absorption of electrolytes in the intestine, suppressing gram-negative bacterial growth. Probiotics reduces production of cytokines and also strengthens the immune system (Guo et al., 2019) (Fig. 1).

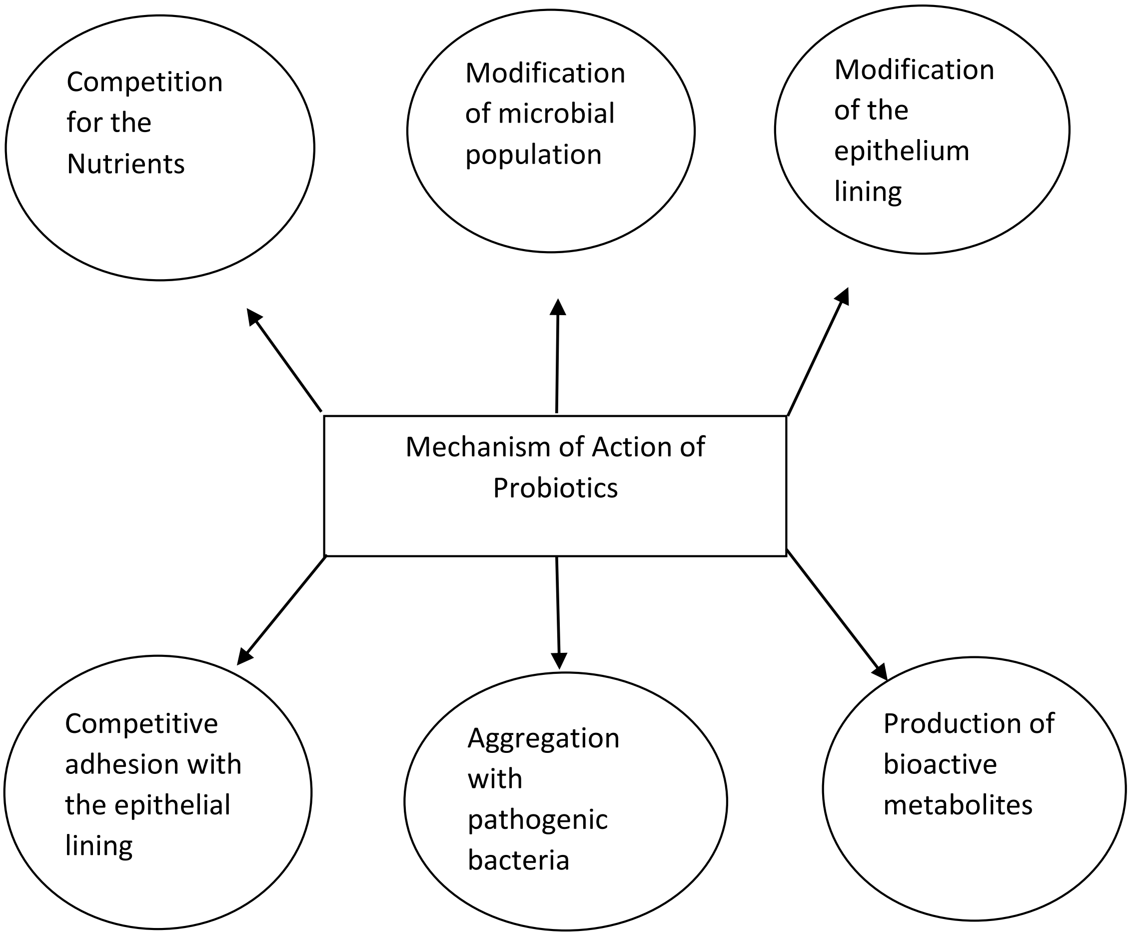

Fig. 1: Mode of Action of probiotics

Role of Probiotics in Human Health

Probiotics offer a range of potential health advantages that includes:

- **Enhanced Digestive Health**

Probiotics can assist in reducing the symptoms of gastrointestinal conditions like diarrhea, spastic colon and inflammatory bowel disease (IBD) (Ding et al., 2021).

- **Enhanced Immune Function**

The gut is closely linked to the immune system, and probiotics can aid in support of immune function by enhancing the growth of beneficial microbes and inhibiting the growth of pathogens (Wang et al., 2021).

- **Prevention of Antibiotic-Related Side Effects**

Side effects can result from antibiotics upsetting the gut's bacterial balance, such as squirts and yeast infections. Probiotics can aid in mitigating these effects by replenishing beneficial bacteria (Liao et al., 2021).

- **Maintenance of Vaginal Health**

Certain probiotic strains have been shown to support vaginal health by preventing and treating yeast infections and bacterial vaginosis (Lehtoranta et al., 2022).

- **Advantages of Mental Health**

A link between gut health and mental health is being suggested by emerging research; probiotics are also helpful in reducing anxiety symptoms, for example depression, and anxiety (Yang et al., 2023).

Commercially Available Probiotics

We can use alive microbes in human nutrition and sold them commercially, include yeasts like Saccharomyces and the bacterial genera *Bifidobacterium*, *Lactobacillus* and *Streptococcus*. Some examples of commercially available probiotics are given below (Table 1).

1) Culturelle

It contains *Lactobacillus rhamnosus* GG, which is known for its digestive health benefits. It's often used to support gut health and alleviate symptoms of digestive issues like diarrhea (Goldstein et al., 2014). It provides strong fence against non-beneficial bacteria in the intestinal wall, also have the ability to survive in the harsh stomach acid which is helpful in the digestion.

2) Align

It contains *Bifidobacterium infantis* 35624, which is believed to aid in regulation of bowel movements and decrease signs of irritable bowel syndrome (IBS) (Kumar et al., 2018). Align support your gastrointestinal health that contributes to a natural healthy intestinal flora. It can be used as a daily dietary supplement.

3) Florastor

It provides a range of probiotic formulations which provides digestive support, women's health, and immune system support. It typically includes strains like *Lactobacillus* and *Bifidobacterium* culture. It helps in protection of your natural flora and digestive balance. It is also helpful in order to flush out the bad bacteria from your body.

4) Hyperbiotics PRO-15

It offers a blend of 15 probiotic strains, including *Lactobacillus* and *Bifidobacterium* and *Streptococcus* species, with a delayed-release capsule designed to deliver the probiotics to the intestines for maximum effectiveness (Ballini et al., 2019). It is helpful in prevention and recovery of bacterial and yeast infections, including acne and atopic dermatitis, gum disease and cavities, urinary tract and vaginal infections (UTIs).

5) Dr. Ohhira's Probiotics

A fermented probiotic supplement containing a blend of various beneficial bacteria strains, along with prebiotics and post-biotics, to support gut health and overall well-being (Pelton, 2020). It is helpful in maintaining the digestive pH for the digestion of food and absorption of nutrients.

Table 1: Commercially Available Human Probiotics

Probiotic Brand	Manufacturer	Probiotic Strain
Culturelle	Allergy Research Group	*Lactobacillus rhamnosus* GG
Microbiol Platinum	Vitals	*Lactobacillus rhamnosus* GG
Actimel (dairy product)	Danone	*Lactobacillus casei* DN-114001
Probioticum	Wapiti	*Saccharomyces boulardii*
Winbiotic Pro-AD	Win-clove	*Bifidobacterium bifidum* W23
Lactinex	Becton, Dickinson & company	*Lactobacillus gasseri* *Lactobacillus bulgaricus*
Floranex	Rising Pharmaceuticals	*Lactobacillus acidophilus*
Florastor	Bio-codex	*Saccharomyces boulardii*
Bacid	Prestige Consumer Healthcare	*Bifidobacterium bifidum* *Lactobacillus acidophilus* *Lactobacillus bulgaricus* *Streptococcus thermophilus*
Probiotic 123	Pure encapsulations	*Bifidobacterium bifidum* *Bifidobacterium lactis* *Lactobacillus acidophilus*
Hyper-biotics PRO-15	Hyper-biotics Probiotics	*Lactobacillus Fermentum* *Bifidobacterium Lactis* *Streptococcus thermophilus*
Dr. Ohhira's Probiotics	Essential Formulas	*Bifidobacterium breve* *Lactobacillus brevis* *Streptococcus thermophilus*

Introduction of Prebiotics

Prebiotics are inedible sugar, boosting particular bacterial activity to improve an individual's health. Unlike probiotics, which are live beneficial bacteria, prebiotics are essential food elements for the probiotic bacterial growth. The primary purpose of these prebiotics is to support and provide a normal balance of microorganisms in the gastrointestinal tract, particularly in the colon (Holzapfel 2006). Selectively fermented compounds were first considered prebiotics in 1995 by Gibson and Roberfroid (Gibson and Roberfroid, 1995).

Prebiotics exist in various forms like oligosaccharide carbohydrates (OSCs), a subset of short and long chain beta-fructans like inulin, fructo-oligosaccharides (FOS), and galacto-oligosaccharides (GOS) and lactulose. There are different conditions for a compound to be categorized prebiotic as these compounds are unbreakable by human gastric enzymes, go to the colon without degradation, where they act as a source of nourishment for the beneficial bacteria (*Bifidobacteria* and *lactobacilli)*, this will improves host's health (Plamada and Dan, 2021). Additionally Fibers, may serve as prebiotic, unable to digest by gastric enzymes (Howlett et al., 2010).

Mode of Action of Prebiotics

Prebiotics serve as an energy source, capable of modify the formation of the gut microbiota. In the human intestine, these prebiotics are not dissolved by the enzymes and escaped from digestion. These can reach in the colon where the beneficial bacteria (*Lactobacilli & Bifidobacterium*) start fermenting these compounds. These prebiotics are used to modulate the gut microbiota that are involved in pathogenesis of different infections by fabrication of SCFAs (including butyrate, acetate and propionate), used as food for bacterial growth and protection of beneficial fermentation substances like SCFAs which improves the immune system and formation of pro-inflammatory cytokines (van der Beek et al., 2017).

Prebiotics like fructans used as an energy source by Lactobacilli and Bifidobacteria and produced lactate and acetate as the fermentation product which further used by Eubacterium, Feacalibacterium and Roseburia as a source of energy and produced butyrate, the fermentation product, by the mechanism of cross-feeding. The process showing the production of butyrate by the use of fructans, which increases the growth of microorganism (Holscher, 2017).

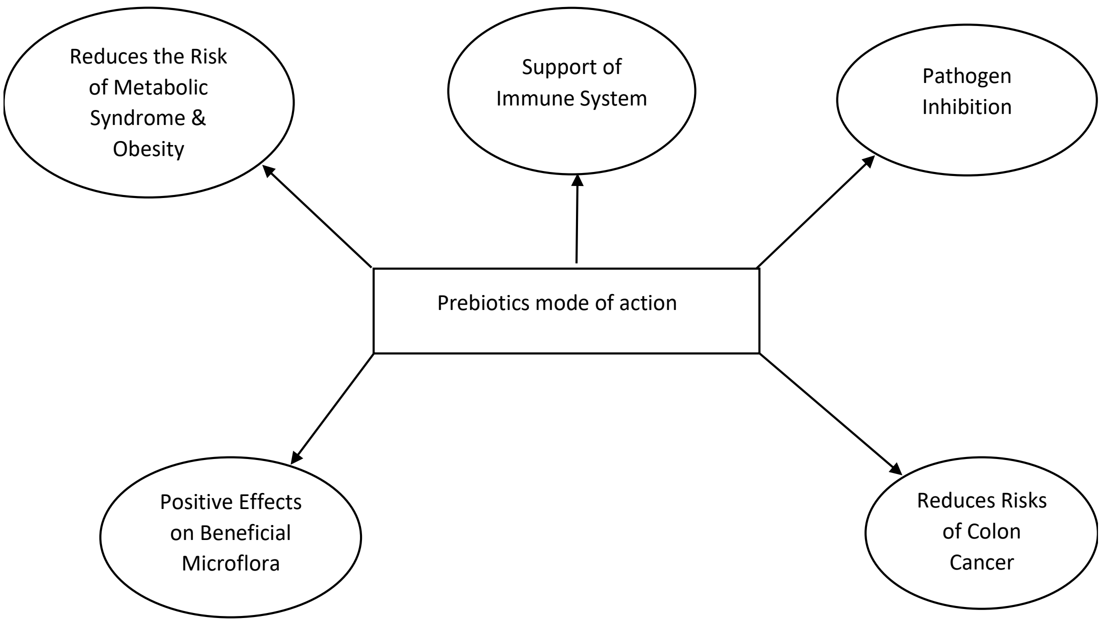

Fig. 2: Mod of Action of Prebiotics

Role of Prebiotics in Human health

- **Beneficial Gut Bacterial growth**

Many Prebiotics like as inulin and polyphenols are specifically used by GM, producing metabolic products such as SCFAs also other compounds that may lower pH of the intestinal lumen, stop the formation of infections, and improve the absorption of minerals and vitamins. Probiotic microorganisms may enhance the intestinal barrier's integrity and boost the microbial diversity of genetically modified organisms (GM), which would reduce both pathologic and baseline inflammation (Ballan, et al., 2020).

- **Improved Digestive Health**

As gut microbiota, these supplements have a capacity to ferment dietary nutrients and assist host body to perform various functions like the synthesis of vitamins and nutrients, defense against infections and maintain immune system. Additionally, it has already been established that prebiotics may play roles in the gut ecosystem (Azad et al., 2020).

- **Healthy Defense System**

The gut microbiota contains a sizable amount of defense system. Prebiotics promote the immune system's function by assisting in the maintenance of a gut microbiome. They stimulate the formation of short-chain fatty acids (SCFAs) such as butyrate having anti-inflammatory properties and support immune function (Ashaolu, 2020).

- **Reduced Risk of Chronic Diseases**

Research suggests that prebiotics may help in reduction of chances of persistent diseases such as obesity, type 2 diabetes, cardiovascular diseases, and even some forms of cancer by influencing gut microbiota composition and metabolic processes (Zheng et al., 2021).

- **Improved Mineral Absorption**

Prebiotics such as insulin and fructo-oligosaccharides (FOS) may improve the absorption of minerals such as calcium and magnesium, leading to better bone health (Karakan et al., 2021).

- **Screening of Irritable Bowel Syndrome (IBS)**

Numerous researches have been done to examine the result of different probiotic strains, prebiotics, and its synergistic effect on individuals with irritable bowel syndrome. A few investigations were carried out using animal models, which may be useful if the caused disease's etiology is as close to that of people as feasible (Chlebicz-Wójcik and Katarzyna, 2021).

- **Weight Management**

Prebiotics may help in weight management by boosting the beneficial gut bacterial growth, associated with reduced obesity risk. They also contribute not only formation of hormones that maintain appetite but also reduce the calorie intake (Thompson et al., 2022).

- **Management of Metabolic Disorders**

Prebiotics have been studied for their potential role in maintaining metabolic disorders such as type 2 diabetes and metabolic syndrome, Additionally, upgrade insulin sensitivity, maintain blood sugar levels, reduce inflammation, which are all factors associated with these conditions (Gheorghita Puscaselu et al., 2020).

- **Reduced Risk of Colon Cancer**

Prebiotics are foods that may be useful in reducing inflammation and colorectal cancer (CRC) by preserving intestinal microbial equilibrium and mitigating dysbacteriosis. By promoting the development of probiotics involved in the generation of short-chain fatty acids (SCFAs), intestinal epithelial barrier balance, pro-apoptotic processes, and other cellular mechanisms, these nutrients might mitigate the effects of dysbiosis (Mahdavi et al., 2021).

- **Support for Mental Health**

New studies point to a connection between mental and digestive health. Prebiotics may indirectly support mental health by modulating the gut brain point, influencing neurotransmitter production, and reducing inflammation, which are all implicated in conditions like depression and anxiety (Bistas et al., 2023).

- **Prevention of Allergies**

Early exposure to certain types of bacteria may help prevent the development of allergies. Prebiotics support the production of beneficial bacteria in infants' guts, potentially decreasing the risk of allergic conditions like eczema and food allergies (Sestito et al., 2020).

Prebiotics play a key role in promoting health gut by:

Commercially available human Prebiotics

The carbohydrates and dietary fibers that are available commercially as prebiotics are mentioned below (Table2).

1) Insulin

Insulin is a hormone primarily used for the management of diabetes by regulating blood sugar levels. However, there is ongoing research exploring the potential therapeutic applications of insulin and its analogs beyond glycemic control, including its effects on gut health and the gut microbiota. Insulin is one of the most commonly used prebiotics. Form of soluble fiber present in many plants, including chicory root, garlic, onions, and bananas. Inulin is widely used as a prebiotic supplement, often mixed with various food items such as yogurt, cereal bars, and beverages (Klancic et al., 2021).

Now a days, understanding of the intricate relationship between gut health and overall well-being has expanded significantly. Alongside this, there's a growing interest in utilizing various compounds, including hormones like insulin, as potential prebiotics to modulate gut microbiota composition and function. While insulin is traditionally known for its role in glucose metabolism, emerging research hints at its untapped potential as a prebiotic agent (Mitchell et al., 2021).

2) Fructo-oligosaccharides (FOS)

FOS are short-chain carbohydrates that occur naturally in fruits and vegetables such as bananas, onions, and asparagus. Commonly available prebiotic supplements can stimulate the growth of beneficial bacteria in the colon (Kherade et al., 2021). Fructo-oligosaccharides are short-chain carbohydrates made up of fructose molecules connected together by β (2→1) bonds, with a glucose molecule at the terminal end. They occur naturally in many fruits, vegetables, and grains, but they are also produced commercially from sources such as sucrose or inulin extracted from chicory root (Rahim et al., 2021).

FOS act as selective permeable and growth booster, such as *Bifidobacteria* and *lactobacilli*. These microbes ferment FOS in the colon, producing (SCFAs) such as acetate, propionate, and butyrate, which provide energy for colonocytes and contribute to gut health (de la Rosa et al., 2022). Furthermore, FOS can be used as an ingredient in various food products and beverage, including yogurt, dairy alternatives, cereal bars, beverages, and dietary supplements. They are often added to improve the nutritional profile of products and promote gut health (Nobre et al., 2022).

3) Galacto-oligosaccharides (GOS)

A group of carbohydrates, commonly found in dairy products and certain plants. They consist of chains of galactose molecules of varying lengths, typically ranging from two to eight units. GOS are considered prebiotics because they restrict digestion in the upper GI tract and reach the colon intact, where they selectively promote the growth and activity of beneficial bacteria (Mei and Li, 2022).

GOS can be drawing out from various sources like lactose. The main source of commercial GOS production is lactose, a disaccharide found in milk. Plant Sources GOS can also be extracted from plant-based sources such as legumes and certain root vegetables. There are several processes involved in the manufacturing of GOS, which include lactose conditioning, enzymatic reaction, thermal inactivation (or enzyme removal), product purification. For any commercial GOS product, there may be differences in the particular setup requirements to finish each phase (Ambrogi et al., 2023).

4) Lactulose

A synthetic, non-digestible disaccharide composed of galactose and fructose. It is primarily used as a laxative due to its ability to increase fecal bulk and promote the growth of beneficial gut bacteria. Recently, lactulose has got attention for its potential role as a prebiotic; a substance, selectively promotes the growth and/or gut activity of beneficial microorganisms (Karakan et al., 2021).

5) Resistant Starch

A portion of starch known as linear a-1,4-d-glucan (RS) is resistant to being broken down by human pancreatic amylase in GI tract, meaning it passes through to the colon undigested. Intestinal microorganisms ferment RS in the colon (Cichońska and Malgorzata, 2021). Prebiotics, including certain types of RS, play a critical role in promoting gut health (Johnson et al., 2020).

Here are some common sources of resistant starch like raw or cooked and cooled potatoes, green bananas. Unripe bananas are high in resistant starch as bananas ripen, the resistant starch content decreases, and the sugar content increases. Legumes like beans, lentils, chickpeas, and other legumes are rich sources of resistant starch. Whole grains like oats, barley, and brown rice contain resistant starch, especially if they are consumed in their whole form or slightly undercooked. A resistant starch is formed by cooked and cooled rice, cooked and cooled pasta, this process is called starch retrogradation. Certain seeds, such as chia seeds and flaxseeds, contain resistant starch along with other beneficial nutrients. Some of the tubers and roots, high-amylose starches and processed foods, which include certain types of bread, pasta, and snacks (Bede and Lou, 2021). Several variables have been proposed in the literature as the cause of RS's resistance to enzymatic hydrolysis. These variables allow RS to be categorized as RS1, RS2, RS3, RS4, and RS5 (Kaimal et al., 2021).

6) Arabinoxylan

In the cell walls of many plants like cereal grains such as wheat, corn, barley, rye and rice bran, arabinoxylan is present as dietary fiber. It consists of a backbone of xylose sugar molecules with arabinose side chains attached. Arabinoxylan has gained attention for its prebiotic properties. Commercially available arabinoxylan as a prebiotic typically comes as functional food ingredients or in the form of supplements (He et al., 2021).

Arabinoxylan is extracted from the cell walls of cereal grains, primarily wheat and corn bran. The extraction process involves the isolation of arabinoxylan through mechanical and chemical means. The resulting arabinoxylan extract contains a high concentration of soluble and insoluble fiber, with arabinoxylan being the predominant component (Rudjito et al., 2023). Commercial arabinoxylan products vary in their composition and structure based on the source and extraction method. However, they typically contain a mixture of xylose and arabinose sugars arranged in a polymer backbone with arabinose side chains. The ratio of xylose to arabinose and the degree of branching can influence its prebiotic properties and fermentability by gut bacteria (Izydorczyk, 2021).

7) Pectin

Heteropolysaccharide was found in the cell walls of fruits and vegetables. Commercially available pectin, derived from natural sources such as citrus fruits, apples, and sugar beet, can serve as an effective Prebiotic (Freitas et al., 2021). Pectin is a heteropolysaccharide composed of D-galacturonic acid units linked by α-(1→4) glycosidic bonds. The side chains may contain other monosaccharides, such as arabinose, rhamnose and galactose (Ropartz and Marie, 2020).

8) Beta-glucans

Beta-glucans are famous for their diverse health benefits, including immune modulation, cholesterol reduction, and prebiotic effects. They are found in the cell walls of bacteria, fungi, yeasts, algae, lichens, and plants such as oats, barley, and mushrooms. As prebiotics, beta-glucans stimulate the growth and functionality of microbiota in the gut. Several commercially available beta-glucans are utilized as prebiotics, offering various advantages and applications (Jan et al., 2021).

β-glucans (BGs) have garnered significant attention as dietary fibers and involved in manipulation of GMB, fermentation in the intestine, production of numerous metabolites, and may have therapeutic effects in gut health. The food industry is becoming more and more interested in using BG as a bioactive ingredient in commercial food compositions (Karimi et al., 2023). There are different types of beta-glycan like oat beta-glucan, mushroom beta-glucans, yeast beta-glucans and algal beta-glucans.

9) Xylo-oligosaccharides (XOS)

The non-digestible carbohydrates that enhance the growth or activity of microflora in the colon. XOS consists of short chains of carbohydrates linked together by glycosidic (β-(1→4)) bonds. They are derived from the hydrolysis of xylan, a complex hemicellulose found abundantly in plant cell walls, particularly in hardwoods and cereal grains. They occur naturally in different plants like bamboo shoots, fruits, vegetables, and honey (Yoo et al., 2012). Typically, 2 to 10 xylose units linked by β-(1→4) glycosidic bonds and form the Xylo-oligosaccharides. The number of xylose units in the molecule (Degree of polymerization) are helpful in its categorization (Bhatia et al., 2019).

Degree of Polymerization of Prebiotics

Probiotics utilize prebiotics on the basis of their degree of polymerization and it is closely related to the activity of probiotics. Prebiotics with lower molecular weight and lower degree of polymerization have active groups that make the probiotics stronger. The degree of polymerization (DP) of three fiber substances, carboxymethylcellulose, beta-glucans and GOS investigated on gastrointestinal tract bacteria (*Lactobacillus, Bifidobacterium* and *Streptococcus*) which indicates that lower DP showed greater effects (Chen et al., 2020). Insulin is the most common example of prebiotic and its effect depends on DP. Short chain insulin with low DP is more effective for the activation of *Bifidobacterium* that utilizes oligosaccharides and inhibits the secretion of endotoxins instead of long chain insulin with high DP (Zhu et al., 2017).

Table 2: List of Commercially Available Prebiotics

Prebiotic Compound	Composition	Degree of Polymerization
Fructans	Glucose, Fructose	
• Linear		
Insulin		10-60
Fructo-oligosides (FOS)& Oligofructose (OF)		2-9
• Connected		
Graminans		unknown
Lactulose	Galactose, Fructose	2
Galacto-oligosaccharides (GOS)	Glucose, Galactose	2-5
Xylo-oligosaccharides (XOS)	Xylose	2-9
Polydextrose	Poly-D-glucose	12
D-tagatose	Tagatose	1
Starch resistant	Glucose	>1000
Soy-oligosaccharides	Galactose, Fructose, Glucose	3-5
Isomaltooligosides	Glucose	2-5
Oligolaminarans	Glucose	5-25

Conclusion

Human intestinal microflora is maintained and steady with the help of probiotic microorganisms.These probiotics have positive impact on host's health by attributing high therapeutic potential in obesity, type 2 diabetes and insulin resistant syndrome. Probiotics can also work as therapeutics of gastrointestinal disorders, eczema, atopic neurodermatitis, and bacterial infections. In the treatment of neoplastic diseases, these live microbes have positive impact (Nowak et al., 2010). As an additional support of probiotics and as an alternative of probiotics, we can use prebiotics. When probiotics and prebiotics work together as synbiotics for the development of bio-therapeutic formulas and having positive effect on the health of intestine and colon. These enhanced products maybe more effective for the protection and stability of the human health. (Bomba et al., 2002). These supplements seem to be safe for healthy people but may have risks or side effects for people with weaker immune system. Foods are the best source of probiotics and prebiotics (like yogurt, cheese, kimchi and kefir etc.) as they provide good nutrition, including antioxidants, vitamins and essential minerals.

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Chapter 11

Probiotics and Prebiotics use in Humans

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ABSTRACT

Various human diseases may have benefited from the prebiotics and probiotics use, like diarrhea, infectious disorders and inflammatory bowel infections. Enteric bacteria produce vitamin k and folate. Probiotics are live microorganisms that provide health benefit when taken in proper amount. Probiotics and prebiotics recover the nutritional and health status. In healthy people, the use of probiotics and fermented foods provide them the dietary approach of enhancing health and proper functioning of gut microbiota. These help to overcome the sensation of anxiety, stress, depression, brain functions, vaginal health, and metabolism homeostasis. Prebiotics are confirmed plant-based extracts.

KEYWORDS
Prebiotics bacteria, Probiotics bacteria, Human health, Fermented food

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INTRODUCTION

Nutrition plays a crucial role in maintaining health and combating diseases, alongside other essential aspects that are becoming increasingly important in the maintenance of health and frustrating diseases (Wiertsema et al., 2021). Probiotics and prebiotics have earned intensifying attention in different fields of science and health (Zommiti et al., 2020). Various Scientific reports are focusing upon the probiotics and prebiotics for their health benefits (Salminen et al., 2021). For decades, these have been consumed by the people for health benefits particularly intestinal health (Kumari et al., 2020). Some babies get the bacterial infection on their first feeding, born with sterile intestinal track. When the bacteria enters the body of the baby it goes on colonizing.(Xiao and Zhao, 2023). Every individual has their own specific microbiota population that needs maintenance (Popkes and Valenzano, 2020). A complex system of more than 400 bacterial species colonized the gastrointestinal tract of humans (Kitamoto et al., 2020). Any disruption with these intestinal microbiotas led to disturbance, as these are beneficial to intestinal health. These beneficial microorganisms do numerous functions like produce nutrients and activate immune system against pathogens in the intestine (Yoo et al., 2020). That's why it is important to modify the intestinal microorganisms to obtain optimum health in humans. There are different sources of these probiotics and prebiotics like fruits, raw vegetables and dairy products (Tomasik and Tomasik, 2020). Prebiotics may be used as the alternatives to probiotics or have a synergistic effect on probiotics (Ballan et al., 2020). Human microbiome research explores the probiotics and prebiotics significantly (Spacova et al., 2020).

Probiotics

The term probiotics first coined by the Ferdinand Vergin in 1954 from a Greek word which means "for life" later described by the Lilly and Stillwell in 1965(Gogineni et al., 2013). Guarner and Schaafsma in 1998 put light on the appropriate dosage of probiotics to achieve targeted effect (Zommiti et al., 2020). The definition of probiotics is being changed by different scientists. In 2002, FAO and WHO formulate a definition of probiotics as "live strains of strictly selected microorganisms, which, when administered in adequate amounts, confer a health benefit to the host" (Anadón et al., 2021), and the International Scientific Association maintained this definition later for probiotics and prebiotics. Probiotics are beneficial for gut health, allergic diseases, immunity, insulin resistant syndrome, obesity, non-alcoholic fatty liver disease and type II diabetes (Castillo et al., 2021). Use of probiotics as a prophylactic measure have been proven scientifically to treat various types of cancer and side effects (Bedada et al., 2020). The use of lactic acid fermentation dates back to ancient times (Petrova and Petrov, 2020). A specific type of sour milk known as "Laban rayeb" was used in ancient Egypt, prepared by goat, buffalo, and cow's milk (Ahmed et al., 2016). A scientist of Russia showed interest in lactic acid fermentation and awarded Nobel prize for his findings (Barclay and Curtin, 2022). Probiotics are selected upon a particular strain of a specific species. Probiotics microorganisms used for humans belong to *Bifidobacterium, Lactobacillus Streptococcus Lactococcus, Enterococcus, Escherichia coli nissli,* yeast of *saccharomyces* genus and gram-positive bacteria of genus *Bacillus* (Kaur et al., 2021). Probiotics also produce neurotransmitters that can modify sensation and motility of the gut (Dicks, 2022). Probiotics have usually recognized by three parameters: viz., genus, species, and strains, for example, *Lactobacillus rhamnoses* GG is a probiotic. The full name is helpful for the reader to link strains and for the assessment of health benefits, because a specific strain is associated with a particular health benefit (Behera et al., 2020).

Probiotics Mechanism of Action

Probiotics have various advantageous effects on the body as they guarantee equilibrium in between microorganisms and pathogens (Wang et al., 2021). It is also used as preservation of food being live microorganisms counteracting *Campylobacter Jejuni, Clostridium Perfringens, Escherichia Coli, Salmonella Enteridis, Staphylococcus, Shigella, Yersinia. Lactobacillus reuteri, Lactobacillus plantarum, Bifidobacterium pseudoacetanulatum, Bifidobacterium adolscentis* are probiotic organisms that produce vitamin B (B1, B2, B3, B6, B8, B9, B12), and increase the absorption of these vitamins and other mineral compounds and hence help in production of amino acids and organic acids (Tijjani et al., 2020). They are also involved in the production of many enzymes, which are lipase, esterase, co enzymes A, Q, NAD, and NADP (Tijjani et al., 2020). Ultimate products of metabolism (bacitracin, acidophyline and lactacine) are involved in antibiotic, immunosuppressive and anti-carcinogenic properties (Willdigg et al., 2024).

Positive effects of probiotics are based on four mechanisms of actions which are:

(i) Competition with pathogens for nutrients and adhesion to epithelium
(ii) Antimicrobial substances production promotes antagonism
(iii) Inhibition of toxin production by bacteria
(iv)Immunomodulation of host

The final products after the mechanisms of competition and antagonism are small chain fatty acids (acetic, butyric, and propionic acids), which provide energy to the host (Blaak et al., 2020).

Fermented Foods

Fermented foods and beverages are sources of probiotics. Plant-based foods, fermented milk and protein have been known for thousands of years (Gustaw et al., 2021). These were used as stable food source when fresh food was rare. These are appealing because of their unique sensory properties and enhanced functionality by which simple cereal grains are converted into beer and bread. Commercially available cultured milk and yogurt products consists of probiotics strains of *bifidobacterium* and *lactobacillus* (Nyanzi et al., 2021).. Vitamin contents of foods is enhanced by the fermentation microbes (Sharma et al., 2020).

Prebiotics

Prebiotics enhances the growth of good bacteria (Lordan et al., 2020) and have various health benefits. A study on colorectal carcinoma show that illness occurs less commonly in individuals consuming fruits and vegetables, credited mostly due to oligofructose and insulin. Prebiotics have numerous advantages i.e., maintain intestinal pH, low blood LDL (low-density lipoprotein), small caloric value, enhanced absorbability of calcium, stimulation of immune system, reduced symptoms of vaginal mycosis and peptic ulcers (Peng et al., 2020). Oligofructose and inulin are also involved in dental care and support for lactose intolerance. Galactooligusaccharides (GOS) provide protection against *salmonella typhimurium* in the murine model, and Fructooligosaccharides (FOS) provide protection not only against *salmonella typhimurium* but also *Listeria monocytogenese*. Infections caused by the *Escherichia Coli* and *Salmonella Enteridis* are also combat by the prebiotics (Abd El-Hack et al., 2022). Reports show that prebiotics like butyric acid have anti carcinogenic effects (Bahuguna and Dubey, 2023). Propionic acids have anti-inflammatory properties. According to reports, administration of 5% inulin and 15% oligofructose have alleviated chances of breast cancers and that of metastases to lung in rats (Wu et al., 2022).

Fructooligosaccharides and inulin are present in fruits and vegetables like bananas, onion, wheat, garlic, chicory, artichokes and leaks. Prebiotics are selected on the criteria of favorable effects on the health of host, fermentation by intestinal micro biota, resistance to digestion in upper GIT, stability in various feed and food processing conditions and careful stimulation of development of probiotics. Examples of prebiotics used in humans are; FOS, GOS, Inulin, XOS, Lactinol, Lactulose, lactosucrose, TOS and soy oligosaccharides (Włodarczyk and Śliżewska, 2021).

Prebiotics Mechanism of Action

As prebiotics are non-digestible are not absorbed from small intestine because the enzymes that hydrolyses the prebiotics' polymer bonds are absent, rather fermented by the endogenous bacteria (Guarino et al., 2020). In the colon they produce short chain carboxylic and lactic acids or oligosaccharides and released into the circulatory system and reaches to distant organs and hence provide energy (Markowiak-Kopeć and Śliżewska, 2020). These have immunomodulatory properties by producing pro inflammatory cytokines and effecting tool like receptor 4 signaling (Sredkova et al., 2020). Cross feeding is another mechanism in which the metabolites of fibers or prebiotics in turn activate other microbes that in turn produce substances like butyrate (Peredo-Lovillo et al., 2020).

Impact of Probiotics and Prebiotics on Human Health

It is a popular approach to use probiotics for managing immune system and digestive health and are effective therapeutic intervention as recommended by the health professionals (Stavropoulou and Bezirtzoglou, 2020). Organic acids produced by the microbes in the intestine are beneficial for killing undesirable microbes. Strain specific effects of probiotics are endocrinological, immunological, neurological and production of bioactive materials, but these are rare. Strain specific effects of probiotics include enzymatic activity, direct antagonism, vitamin synthesis, bile salt metabolism, neutralization of carcinogens and gut barricade reinforcement, and these effects are frequent (Gadaleta et al., 2022). Widespread effects among probiotics are comprises of perturbed microbiota normalization, competitive elimination of pathogens, acid and short-chain fatty acids production, colonization resistance, regulation of intestinal transit and increased turnover of enterocytes. Probiotics use help to reduce the risk and duration of upper respiratory tract infections (Emre et al., 2020).

Clinical Application and Therapeutic Potential

Simultaneous use of prebiotics and probiotics has high therapeutic potential. The combination of probiotics and prebiotics in a single product have synbiotics effect, which ensures more effect as compared to the alone effects. Probiotics of different potencies are available depending on the desired mechanism of action. Prebiotics effects of lactulose and fiber supplements are used to treat constipation (Naseer et al., 2020). Table 1 and 2 shows the clinical trials showing the effect of probiotics and prebiotics on humans.

Table 1: Clinical trials showing the effect of probiotics on humans

Microorganisms	Subjects	Time of administration	Main outcome	References
L. gasseri SBT2055	210 people with large volatile fatty acids	12 weeks	Reduce the level of BMI and arterial BP values	(Zhao et al., 2021)
L. plantarum	40 people with obesity	3 weeks	Reduce the level of BMI and arterial BP values	(Lewis-Mikhael et al., 2020)
L. acidophilus La5, *B. animalis* subsp. *Lactis* Bb12.	156 people with overweight	6 weeks	Fall in fasting glucose concentration	(Nawangsih et al., 2022)
L. plantarum	24 postmenopausal with insulin resistance syndrome	12 weeks	Homocysteine and glucose concentration are largely reduced	(Meng et al., 2022)
L. Acidophilus La5, B.lactic Bb12	64 people with type II diabetes	6 weeks	Reduction in fasting of blood glucose and antioxidant status	(Sohrabi et al., 2023)
L. acidophilus La5, *B. breve* subsp. *Lactis* Bb12.	72 patients with non-alcoholic fatty liver disease	8 weeks	Reduction in serum levels of ASP, ALT, LDL-C and TC	(Reamtong et al., 2021)
L. fermentum VRI 033 PCC^TM	53 children with modest and severe atopic dermatitis	8 weeks	Lessen SCORAD	(Husein-ElAhmed and Steinhoff, 2024)

Abbreviations: ALT: alanine aminotransferase, ASP: aspartate amonotranseferase, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, BMI: body mass index, BP: blood pressure, SCORAD: Scoring atopic dermatitis

Table 2: Clinical trials showing the effect of prebiotics on humans.

Prebiotics	Subjects	Time of administration	Main outcome	References
FOS	10 patients with type II D	4 weeks (2 times repetition)	Plasma glucose action to exogenous insulin did not differ at the end of both periods, no effect of FOS	(Pawar et al., 2023)
OFS	7 people with non-alcoholic steatohepatitis	8 weeks	OFS greatly decreased the serum aminotransferase, aspartate aminotransferase after 8 weeks as compared to placebo	(Parsi et al., 2020)
GOS, FOS	281 healthy infants (15-120 days)	12 months	Lesser episodes of acute diarrhea and upper respiratory tract infections	(Leung et al., 2020)
FOS	10 Crohn's disease patients	3 weeks	Reduction in disease action index	(Bowman et al., 2022)
GOS, FOS	259 infants at risk for atopic dermatitis	6 months	Significant drop in frequency of AD	(de Paiva et al., 2023)
inulin	Human L97 and HT29 cell lines	Nill	Growth inhibition, induction of apoptosis in human colorectal carcinoma	(Liu et al., 2020)
GOS	85 lactose intolerance people	36 days	Lactose fermenting bacteria significantly large	(Pázmándi et al., 2020)

Abbreviations: FOS: fructo-oligosaccharides GOS: galacto-oligosaccharides OFS: oligofructose

Practical Considerations for Probiotics and Prebiotics use

In previous years huge number of commercial probiotic supplements are warmly welcomed by the consumers for maintaining health (Kellershohn, 2021). 12 g of inulin for one month reduces the VLDL (very-low-density lipoproteins) i.e. cholesterol by 5% and triacylglycerols by 27% (Mitchell et al., 2021). Prebiotic effects on the hepatic metabolism by reducing glucose-6-phosphate and acetyl Co-A carboxylase. Globally, probiotics are available in three forms that are as food, pharmaceuticals, and dietary supplements. Probiotics cosmetics are also available. L. rhamnoses HN001 is present for vaginal and postpartum health (Chee et al., 2020).

Yogurt bacteria, *Lactobacillus delbruecrii* and *Streptococcus thermophilus* improve lactose digestion in people with lactose maldigestion. In a study, yogurt intake relates to less long-term weight gain (Yuan et al., 2021).

Two experiments were done in Korea on adults in which 2 to 4 servings of kimchi per day and other fermented foods was linked with reduced occurrence of atopic dermatitis (Kwak et al., 2021). In a study, cheese intake is inversely related to cardiovascular disease mortality. Fermented milk consumption is associated with decreased blood pressure in hypertensive adults. Fermented milk or rice intake is associated with decreased incidence of infectious diseases in children and kefir intake leads to short-term progress in bone health markers in children with osteoporosis (de Sire et al., 2022).

Probiotics for Vaginal Health

The normal inhabitants of vaginal mucosa are the lactobacillus species, counteracting with the candida species and discharge fluid for preventing pathogens attack (Chee et al., 2020). The patients showing devastating symptoms of the presence of fungal cells in the vaginal wall are well treated by the oral administration of *L. acidophilus* that stops fungal colonization and maintain vaginal microbiome by the particular immune response (Chee et al., 2020).

Infants' Colic

It is a condition in which a child cries for more than 3 hours per day, 3 days per week and 3 weeks. It is difficult to treat by traditional medicine. Many researchers studied the effects of probiotics on treating colic. *L. reutri* significantly reduced the time of crying with 28% success rates (Basturk, 2022; Wadhwa et al., 2022).

Eczema

It is an inflammatory condition characterized by pruritus, redness and scaly skin. Probiotics use by the mother during last trimester or during breastfeeding lessen the incidence of eczema. World Allergy Organization recommended the use of probiotics at high risk of developing allergy in infants (Sestito et al., 2020).

Necrotizing Enterocolitis

It is a serious illness of infants which is characterized by inflammation and necrosis of the bowel. Probiotics use in neonates reduce morbidity and mortality. It is in clinical guidelines of UK hospitals and by Southwest Neonatal Network. Probiotics reduce the incidence of necrotizing enterocolitis and death in premature infants (AlFaleh and Anabrees, 2014).

Antibiotic Associated Diarrhea

Antibiotics are among the most prescribed drugs and antibiotic associated diarrhea (disruption of the GIT microbiota) is the common side effect of its frequent use.. A study indicates that antibiotics taken with probiotics such as *Saccharomyces boulardii* (a probiotic yeast) reduce the risk of antibiotic associated diarrhea and *C. difficile* associated diarrhea (Rohde et al., 2009).

Safety and Regulations

While selecting probiotics, one must ensure that they are safe, have a proper function in the body, and can be technically usable. According to regulations of general food law, probiotics should be safe for human as well as animal health (Wright, 2005). In the USA, FDA (Food and Drug Administration) has approved that the microorganisms used for human consumption should have GRAS (regarded as safe) status. In Europe, EFSA, have introduced an additional criterion of assessment that is QPS (Qualified Presumption of safety), which includes a previous history of safe usage and absence of antibiotic resistance (Hazards et al., 2020).

Conclusion

Microbial ecosystem has a symbiotic association with the host. In the near future, probiotics will be available as drugs targeting the specific diseases. Probiotics as microorganisms and prebiotics as substances or fibers are very beneficial for maintenance and treatment of diseases as compared to antibiotics that cause antibiotic resistance and adverse side effects. These are obtained from plant and animal sources and cheap and are spreading worldwide very rapidly.

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Chapter 12

Use of Prebiotics and Probiotics to Improve Gut Health in Pregnant Women

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ABSTRACT

The microbiome of the gut plays a major role in maintaining general well-being particularly during pregnancy. Probiotics are useful microbes present in edibles like yogurt and are helpful in preventing digestive troubles and conditions such as gestational diabetes while prebiotics are food for those useful bacteria acting as probiotics. When prebiotics and probiotics combine, they form synbiotics. Thus, synbiotics are responsible for maintaining a balance of good bacteria. Food which is taken while pregnancy, stress levels and external conditions affect the kind of bacteria present in the gut during pregnancy. Hormonal and environmental changes can also mess with microbiome. An imbalance in gut microbiome can lead to anxiety, depression and even brain development problems in the new born. Gut microbiome can also impact the development of immune system of the newborn baby. Although synbiotics have many beneficial effects but still their intake must be according to prescriptions as they can also lead to some mild digestive problems. Combination of prebiotics and probiotics in an appropriate balance could prevent its complications with maximum benefits on health. More research is still needed to make synbiotics even safer. Overall, keeping a healthy gut microbiome by using synbiotics, probiotics and prebiotics can impart beneficial impacts on health of mother as well as baby.

KEYWORDS		
Prebiotic, Probiotic, Synbiotics, Gut microbiome, Gestational diabetes mellitus, Maternal gut microbiota	Received: 26-June-2024 Revised: 18-July-2024 Accepted: 01-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Host cells are positively affected by prebiotics which are non-digestible part of food (trans-galacto-oligosaccharides (TOS), fructo-oligosaccharides (FOS), and galacto-oligosaccharides (GOS). They increase the proliferation of specific bacteria in the colon which leads to better health outcomes for host (Davani-Davari et al., 2019). *Lactobacillus* and *Bifidobacterium* are prebiotics which impose positive health impact on the host when given in sufficient amounts (Abatenh et al., 2018). Biological supplements or foods like yogurt offer a readily accessible option for probiotics consumption. They have potential benefits for gastrointestinal disorders such as irritable bowel syndrome. Although, there remains ambiguity in suitable grouping of numerous types and species of probiotics (Jarde et al., 2018).

Synbiotics refer to products that combine both probiotics and prebiotics, offering a combined influence on an individual's microbiome (Sheyholislami and Connor, 2021). Integrating synbiotics into prenatal care as a supplementary intervention could offer advantages in controlling plasma glucose levels and other metabolic markers, potentially benefiting the management of various conditions like diabetes mellitus during pregnancy (Movaghar et al., 2022).

Pregnancy involves various physiological and hormonal changes essential for proper weight gain and fetal development. Sufficient nutritional intake is crucial for maintaining the health of both the mother and the fetus (de Brito Alves et al., 2019). The intestine is the main organ for digestion and absorption of nutrients, and it serves as a vital defense barrier. The gut is home to a vast majority of microorganisms. Nutrition plays a key role in regulating them, but age, lifestyle, and environmental factors also impact the diversity of the gut's microorganisms. Dietary proteins, including their source, quantity, and amino acid makeup, affect the gut's microorganisms (Ren et al., 2024).

During pregnancy, factors like antibiotic use, diet, weight gain, and stress can affect the gut microbiota in mothers. This can have a ripple effect on the gut microbiota and overall health of newborns. It's important to study the factors that shape the development of gut microbiota in both mothers and babies. Pregnancy brings significant changes to the gut microbiota, making it a unique and transformative time (Cao et al., 2023).

Many disorders can be prevented by consumption of probiotics or prebiotics as they maintain the balance of gut flora (Ren et al., 2024). Supplements containing probiotics, prebiotics, and synbiotics are being used more often during pregnancy to lower the risk of mental health disorders in mothers throughout the perinatal period; however, the effectiveness of these treatments has not been thoroughly studied (Desai et al., 2021). The gut microbiome has seen a surge in studies in recent decades, with a focus on clinical and therapeutic applications to understand its impact on human health and disease alleviation. However, understanding the gut microbiome presents challenges due to its diversity, variability, and complexity. Modulating microbial interactions, using probiotics and exopolysaccharides produced by lactic acid bacteria (LAB) as prebiotics, is crucial for maintaining good health and mitigating diseases (Dahiya and Nigam, 2022).

A theory suggests that maternal microbes colonize fetal tissues in utero before birth, potentially causing pro-inflammatory changes. Present assumptions suggested the transfer of microbes from intestinal epithelium to bloodstream leading to placenta. Recent non-DNA sequencing-based data challenges the traditional belief of a sterile uteroplacental unit. However, contamination concerns persist. Supplementary, research is needed to regulate the possibility of maternal-fetal microbiota exchange. The maternal intestinal microbiota can indirectly regulate fetal development and growth by metabolites transmitted via placenta. These soluble factors, such as aryl hydrocarbon receptor ligands, modulate the offspring's immune system and gene expression. Short-chain fatty acids (SCFAs) influence metabolism, immune system development, and nervous system during pregnancy. Endogenous microbial compounds can also interact with innate pattern-recognition receptors (Miko et al., 2022).

Understanding Gut Health in Pregnancy

The human digestive system has trillions of microorganisms in the gut with *Bacteroides*, *Firmicutes*, *Proteobacteria* and *Eubacterium* being the most important phyla that contribute to gut microbiota. Their proliferation starts at birth. Environmental (e.g. diet and lifestyle), genetic, and nutritional factors influence their makeup. This microbiota is beneficial only as long as it is present in appropriate concentration and any imbalance in this concentration i.e. dysbiosis, can lead to health problems (Anwar et al., 2021). Pregnancy also changes gut microbiota. Fecal samples of 55 Chinese women from the first to third trimester were taken for a study. It was shown that the primary factor that influenced gut microbiota during pregnancy was gestational diabetes mellitus. Pre-pregnancy weight, hormones, environmental factors and stress are also key contributors that influence gut microbiota (Li et al., 2023). To facilitate fetal development, the pregnant body undergoes immunological changes also. Its purpose is to prevent fetal allograft throughout pregnancy. The immune system also interacts with the gut microbiota that changes accordingly to support the developing immune system of the baby (Bhatia et al., 2024).

The variety of microbes in the gut does not alter at the beginning of pregnancy. Changes in the gut microbiota by the third trimester are linked to significantly increased diversity, an overall rise in *Proteobacteria* and *Actinobacteria* abundance, and decreased bacterial richness (Koren et al., 2012). For example, the average amount of *Faecalibacterium* decreases during the third trimester. These changes may have a biological function that helps the mother adjust to pregnancy and promote the best possible growth and development of the fetus. Hu et al. discovered a relationship between the amounts of acetate in mother blood and cord blood in a cohort of paired maternal and fetal serum. According to this correlation, it is expected that maternal SCFAs will pass through the placenta and alter fetal immunological activity, which will affect the number of fetal SCFAs (Hu et al., 2019). Certain bacterial strains, such as those from the *Bifidobacterium*, *Bacteroidetes*, and *Lactobacillus* families digest dietary fiber and produce SCFAs (Al-Nabhani et al., 2019).

Impact of Hormonal Changes on Gut Health during Pregnancy

During pregnancy hormonal changes occur e.g. there is a significant increase in progesterone level to sustain pregnancy. These hormonal changes also influence gut microbiota significantly and is essential for the development of immune system of fetus. During early stages of pregnancy, gut microbiota is not significantly altered, and its composition is like that of gut microbiota before pregnancy. Normal gut microbiota of pregnant women has more of *Bacteroides*, *Akkermansia* and *Bifidobacterium* but if there is dysbiosis e.g. in case of gestational diabetes, then amount of *Firmicutes* and *Provetella* increases substantially (Taddei et al., 2018). Throughout the 4-6 months of pregnancy, there is an enhancement in beta diversity (similarity and dissimilarity of two communities) of microorganisms and decrease in alpha diversity (microbial diversity in a sample). But in the last trimester, more *Actinobacteria* and *Proteobacteria* were observed (Blaser and Dominguez-Bello, 2016).

The Link between Maternal Gut Health and Fetal Development

During pregnancy distinct changes occur in the gut microbiota of the mother which are interlinked with changes in hormones. It was seen that progesterone levels increased towards the end of pregnancy. So, *Bifidobacterium* in the gut also increases. *Bifidobacterium* helps to digest oligosaccharides in human milk. So, it is essential for the health of a newborn (Laursen et al., 2021). Diseases before pregnancy can influence maternal health during pregnancy. Chronic inflammatory bowel disease can cause gut dysbiosis during pregnancy (Vich-Vila et al., 2020). Gestational diabetes mellitus (GDM) results in resistance to insulin and hyperglycemia. This GDM is also associated with a change in gut microbiota during pregnancy. GDM is also linked to the decrease in the number of *Bifidobacterium* (Dualib et al., 2021).

Fusobacteria in the gut microbiota of mothers is more linked with the development of motor skills in infants during the first year of life than that of infant's own gut microbiota (Sun et al., 2023). Attention deficit hyperactivity disorder and autism spectrum disorder in children are neurodevelopmental disorders which are interlinked with interruption of endocrine chemicals and hence distorted gut microbiota (Ramírez et al., 2022). So, the factors which influence the gut microbiota of the mother also influence the gut microbiota of the child. It is very important to maintain maternal gut microbiota for healthy fetal development (Adamczak et al., 2024).

Prebiotics: Nurturing a Healthy Gut Environment

Prebiotics are defined by the International Scientific Association as compounds that the host intestinal flora can use and modify selectively, with the idea that this will improve host health. Prebiotics are now defined to include non-digestible carbohydrates, and their mode of action is no longer restricted to the gastrointestinal system or specific meal types (Gibson et al., 2017).

Types of Prebiotics

Prebiotics can be divided into different types. Most of its types are subsets of carbohydrate groups like oligosaccharides. Some common types of prebiotics include fructans, lactulose, galacto-oligosaccharides, starch, and glucose-derived oligosaccharides (Davani-Davari et al., 2019). Different source and benefits of prebiotics are discussed in (Table 1).

Table 1: Sources and benefits of prebiotics

Prebiotics	Source	Benefits	Reference
Inulin	Asparagus, onions, wheat, garlic, chicory, oats, and soybeans	Suppresses the expression of inflammatory factors, promoting the growth of beneficial microbiota, boosting mineral absorption	(Qin et al., 2023)
Fructooligosaccharide (FO)	Onion, chicory, garlic, asparagus, banana, artichoke	Increase the growth of *Bifidobacteria*, decrease levels of serum cholesterol, help in mineral absorption	(Sabater-Molina et al., 2009)
Galacto-oligosaccharide	Milk	Promotes the growth of *Lactobacilli* and *Bifidobacteria,* reduce stool pH	(Mei et al., 2022)
Xylo-oligosaccharides	Fruits, vegetables, milk, honey	Enhances food absorption and digestion	(Valladares-Diestra et al., 2023)
Isomalto-oligosaccharides	Starch	Lowers blood sugar by promoting both insulin and incretins.	(Subhan et al., 2020)

Safety Concerns Regarding the use of Prebiotics

Prebiotics are thought to have no serious or potentially fatal negative effects. Both polysaccharides and oligosaccharides are indigestible to intestinal enzymes. The gut microbiota carries them to the colon where they are fermented. Thus, most prebiotic adverse effects arise from their osmotic actions. Prebiotic users may experience bloating, cramps, flatulence, and osmotic diarrhea. One factor that affects how the prebiotics' adverse effects develop is the length of their chain. Remarkably, prebiotics with a shorter chain length can potentially have more adverse effects. This effect could be explained by an example that longer-chain inulin molecules ferment later and more slowly in the distal colon while shorter-chain inulin molecules are metabolized principally in the proximal colon and ferment more quickly there. Its safety profile can be influenced by the prebiotic dose in addition to chain length. For instance, osmotic diarrhea and flatulence can be brought on by large (40-50 g/day) and low (2.5-10 g/day) doses of prebiotics, respectively. Note that for prebiotics to have a positive impact on human health, a daily dosage of 2.5-10 g is necessary. Most of prebiotic products on the market contain 1.5-5 g of prebiotics per piece (Svensson and Håkansson, 2014).

Probiotics: Boosting Beneficial Bacteria

Probiotics, commonly found in cultured milk and fermented foods, are beneficial microorganisms known as "good bacteria" (Zaib et al., 2024). Probiotics, introduced through food or water, are live beneficial bacteria that improve health by balancing the internal microbial environment (Wieërs et al., 2020).

Types of Probiotics

Most probiotic products today contain *Bifidobacteria*, *Lactobacilli*, and other types of lactic acid bacteria (He and Shi, 2017). Various *Lactobacilli* species like *L. acidophilus*, *L. casei*, *L. rhamnosus*, and *L. helveticus* had been widely researched for disease prevention in humans and animals. These probiotics can alter gut microbiota composition and regulate its function (Azad et al., 2018). *Bifidobacteria* are naturally found in the predominant bacteria of the colon (Picard et al., 2005).

Dietary Sources of Probiotics

Dairy companies are major producers of probiotic-rich yogurts, buttermilk, and tofu, which are supplemented with probiotics for consumers' convenience and are readily accessible (Kaur et al., 2021). Probiotic yeasts and *Lactobacillus* strains sourced from kefir grains, masai milk, and koumiss can modulate immune responses (Fontana et al., 2013). Different beneficial probiotics for pregnant women have been summarized in (Table 2).

Table 2: Probiotic beneficial strains for pregnant women

Probiotics	Function	Recommendation	Reference
L. casei rhamnosus	Restore vaginal microbiome	Safe for use in pregnancy	(Petricevic et al., 2023)
L. acidophilus	Help in glycemic control, Control Gestational diabetes mellitus	Safe for use in pregnancy	(Yefet et al., 2023)
B. bifidum	Influence on mother intestinal microbiota	Safe for use in pregnancy	(Perrotta, 2023)
L. rhamnosus	prevention of GDM in Overweight and obese women	Safe for use in pregnancy	(Callaway et al., 2019)
L. salivarius PS2	prevent infectious mastitis	Safe for use in pregnancy	(López-Moreno and Aguilera, 2020)

Mechanisms by which Probiotics Contribute to Gut Health in Pregnancy

Probiotics have shown promising results in alleviating gastrointestinal dysfunction during pregnancy. It is theorized that elevated progesterone levels during pregnancy can disrupt gastrointestinal motility, potentially contributing to symptoms like nausea and vomiting (Body and Christie, 2016). Neuropeptides, which have dual roles in host stress and antimicrobial activity, along with the gut microbiota and their signaling through the gut-brain axis, may play a role in anxiety and depression (Wei et al., 2020). Probiotics help balance gut bacteria, improve intestinal health, and reduce inflammation, which can boost the immune system. While there's a possibility they could help prevent or manage diabetes during pregnancy, more research is needed to confirm this (Homayouni et al., 2020).

Possible Health Impacts due to Deficiency of Probiotics

Possible health challenges faced due to deficiency of probiotics can lead to several diseases including irritable bowel syndrome (Lukic et al., 2017), dysbiosis (Salonen et al., 2010), skin related issues (Ranjha et al., 2021), rheumatoid arthritis (Blenkinsopp et al., 2024), and preterm birth and preeclampsia (Gomez Arango et al., 2015).

Synergistic Approach: Combining Prebiotics and Probiotics

A new concept "synbiotics" has been coined by the synergistic combination of prebiotics and probiotics, involved in intestinal health promotion (Kojima et al., 2016). Thus, the development of new synbiotics requires a long-term screening process. Most active and synergistic pairs of prebiotics and probiotics are selected to be combined and form synbiotics (Ouwehand et al., 2007).

Mechanism of Action of Synbiotics

The reason behind using synbiotics is that a true probiotic, without its prebiotic food, cannot last longer in the digestive tract. Due to a lack of food and energy, probiotics will become intolerant to oxygen, temperature, and low pH. Prebiotics provide a great place for probiotics to thrive. The number of good bacteria could be increased many folds by the combination of prebiotics and probiotics. Synbiotics can improve the viability of probiotics as well as deliver specific health benefits (Manigandan et al., 2012).

Role of Synbiotics in Gastrointestinal Health in Pregnancy

The use of synbiotics has a prominent effect on the gut health of pregnant women. Along with gut health improvement, synbiotics have many other benefits. A study was conducted to specifically detect the impact of synbiotics on pregnant ladies. Effects of synbiotics on inflammation, oxidative stress, and other pregnancy outcomes were found in it. It was a clinical trial involving 60 subjects. Three strains of synbiotics were used which included strain of *L. acidophilus, L. casei* and *B. bifidum*. On comparison with placebo, it was found that synbiotics supplementation significantly decreased serum high sensitivity C-reactive protein (hs-CRP), plasma malondialdehyde (MDA). A significant increase in total antioxidant capacity and glutathione levels was also observed. Decrease in cesarean section rates, lower incidence of hyperbilirubinemic newborns, and newborns' hospitalization were also some advantages observed in subjects using synbiotics as compared to placebo (Karamali et al., 2018). It has been found that the intake of prebiotics, probiotics, and synbiotics results in many beneficial effects on the gut microbiota of newborns. They help in restoring the population of *Bifidobacteria* (Martín-Peláez *et al*., 2022).

Challenges and Considerations

Probiotic supplementation is suggested for regulating imbalances linked to conditions like obesity and diabetes. Evidence points to a potential protective role of probiotics in pre-eclampsia, allergic diseases, maternal and infant weight gain, vaginal infections, and GDM. However, definitive conclusions require well-designed trials and metagenomic analysis to establish the precise impact of probiotics on adverse outcomes during pregnancy and infancy (Gomez Arango et al., 2015).

Probiotics and prebiotics, extensively marketed as food ingredients and supplements, represent lucrative niche markets. However, the food industry has faced health claim restrictions on these products globally, including regulations from the European Food Safety Authority. The major advantages of using probiotic and prebiotic encompass studies upon preventing or managing various health issues such as intestinal and respiratory infections, cardiovascular disease, osteoporosis, urogenital infections, oral health concerns, allergies, inflammatory bowel diseases, irritable bowel syndrome and *Helicobacter pylori* gastric infections. To advance our understanding of how probiotics and prebiotics modulate human microbiota and the immune system, establishing appropriate biomarkers for well-being and disease risk factors is crucial. While promising, the outcomes necessitate large, long-lasting, strategic clinical studies to give greater consistency as well as robust foundation for potential clinical applications of probiotics and prebiotics (Martinez et al., 2015).

Reproductive and developmental toxicity testing is crucial for evaluating probiotics, as manipulating microbial populations could impact development in pregnant women or infants. Existing guidelines need adaptation for probiotics, considering specific endpoints for each strain. Investigating if a probiotic becomes a permanent resident of the offspring's adult microbiota is important. Developing practical methods to probe introduced strains is vital, assuming the chosen animal model accurately reflects the target microbial location. Traditional testing endpoints may be unnecessary based on theoretical grounds. Further discussion is needed on the design and execution of reproductive and developmental toxicity evaluations for widely used probiotics (Sanders et al., 2010).

Maternal gut microbiota changes during pregnancy, with reduced bacterial diversity and a shift towards potentially inflammatory species. Probiotic interventions, like *L. rhamnosus* and *B. lactis Bb12*, have shown promise in reducing gestational diabetes without adverse effects. However, limitations persist, necessitating further clinical trials (de Brito Alves et al., 2019). The effectiveness of probiotic or symbiotic supplements in gestational diabetes management is uncertain due to limited evidence. Further studies are needed to determine optimal doses, safety, and composition, as well as their effectiveness in lipid management. Pre-eclampsia risk may be reduced by probiotics, but further research is needed to ensure safety (Obuchowska et al., 2022).

Conclusion

Various proven advantages of adding prebiotics and probiotics to a diet are found. They both are quite beneficial, and their benefits increase when they are combined in a balanced ratio. A strong relationship exists between maternal gut health and the health of the infant. Microbial flora has a huge impact on gut health, so it is imperative to know the appropriate concentrations of each type of prebiotic and probiotic to make its intake safer. Despite the immense importance of prebiotics and probiotics on healthy gut development, still there are some complications. Thus, use of prebiotics, probiotics and synbiotics to improve gut health in pregnant ladies is highly recommended but dose should be measured and discussion with the physician should be done before adding them to diet. Their mechanism of action and therapeutic ability in pregnancy need to be understood by further research and clinical trials with metagenomic analyses.

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Chapter 13

Use and Impact of Probiotics in Children

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ABSTRACT

The use of probiotics can positively impact the overall health of an individual as it affects many prime neurotransmitters such as serotonin, glutamate and cortisol and uplifts emotional health mainly affecting brain cognitive function, learning capabilities and behaviors which leads to its other name "psychobiotics". The intense ability to effect and modify brain efficiency patterns also improves autistic spectrum disorder to a certain degree and reduces its symptoms. The cause for improving general health of an individual is by modifying the microbial populations in the intestines. Research in the field of pediatric microbiome has highlighted the crucial role of gut health in children's overall well-being. This review focuses on the potential benefits of probiotics on pediatric health, specifically their ability to regulate immune responses, prevent infections and to improve digestion causing weight loss which further leads to reduced probability of diseases related to obesity. The article examines the factors that affect microbiota composition during infancy and childhood and presents an analysis of clinical studies to provide insights into the effectiveness of specific probiotic strains. Probiotics can be found in food and dietary supplements, including *Bifidobacterium* species, Lactic acid bacteria such as *Lactobacillus, Escherichia, Bacillus, Enterococcus*, or *Saccharomyces boulardii* yeast.

KEYWORDS		
Probiotics, Children, Psychobiotics, Pediatric health, Improve digestion	Received: 23-May-2024 Revised: 17-July-2024 Accepted: 04-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

The word "Probiotic" was first introduced by Lilly and Stillwell in 1965 to illustrate the growth of another microbe stimulated by the material secreted by one microbe (Gupta and Garg, 2009). Metchnikoff in early 90's explained probiotics as changing in bacterial diversity in bodies of humans and alter harmful microorganisms with useful ones (Kerry et al., 2018). These are pathogens that when provided in sufficient quantity, grant Medicare to host (Szajewska, 2016). These are live nonpathogenic microbes introduced to enhance bacterial equilibrium, especially in gastrointestinal tract. Probiotics are essential as they reduce intestinal pH, lower colonization and attack of pathogenic bacteria and alter immune response of host (Williams, 2010).

Probiotics play vital role in nutritional as well as clinical point of view. They provide benefit to both humans as well as animals. To enhance human health, probiotic microbes i.e. *Lactobacilli* and *Bifidobacteria* are largely use as important dietary ingredients which have made significant effect on utilizers (Grover et al., 2012). Sufficient clinical or animal data which supports health benefits comprise, prevention of systematic infections, prevention and treatment of diarrheal disease (nosocomial infections, acute infantile diarrhea, and antibiotic associated diarrhea), immunomodulation, management of inflammatory bowel disease, and alleviation of lactose intolerance, prevention and treatment of allergies, anticancer effects and treatment of cholesterolaemia. Probiotics efficacy has been tested in a study with promising results as evaluation of the effect of probiotics on children with autistic spectrum disorder (ASD) concluded significant lowering of body weight and improvement in the severity of autism (evaluated by ATEC) with additional effects on GI tract when compared with the initial baseline data. Diarrhea is the most common problem as a result of short and long term antibiotic usage. Many clinical trials have been made to check the efficiency of probiotics in prevention of diarrhea. Different strains were tested like *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* strain GG, *Lactobacillus bulgaricus*, and the yeast *Saccharomyces boulardii*. Two studies concluded that probiotics could be used to prevent antibiotic associated diarrhea (Gill and Guarner, 2004).

The development of children's healthy immune systems and digestive systems is greatly influenced by the complicated and significant role that the gut flora plays. Probiotics are safe and useful in lowering the duration of acute infectious diarrhea caused by antibiotics in children (Kligler et al., 2007). They provide protection against antibiotic associated diarrhea, prevention in day care centers and prevention from nosocomial infections in children (Hojsak, 2017). Probiotics also help in prevention from, allergy, necrotizing enterocolitis and *Helicobacter pylori* infections (Szajewska, 2016).

Important probiotics use in pediatric well-being are, from prevention of antibiotic associated diarrhea LGG and *S. boulardii* is use, for treatment of acute viral diarrhea LGG strain dose dependent is required, for prevention of necrotizing enterocolitis *Bifidobacterium infantis*, *S. thermophilus*, for infantile colic *L. reuteri*, for IBS *E. coli*, LGG is use.

Microbiome in Children

During birth a child is born germ free, the microbes that inhabit the body must come from the environment. Evolutionary selection factors acting at the level of both host and microbial cells change the gut microbiome (Ley et al., 2006).

During Infancy and old age the immune system of human is its most vulnerable and unstable point while microbiota experiences the most notable deviations which seems to indicate that our health and microbiota evolve as we age alongside throughout these two stages of life (Nagpal et al., 2018).

The studies revealed that the gut microbiome passes through three different phases i.e. a developmental phase (months 3–14), a transitional phase (months 15–30), and a stable phase (months 31–46). In infants, breast milk is the prime source of microbiome introduction into the body. The start of breastfeeding has been linked with an increase in *Bifidobacterium* species (*B*. breve and *B. bifidum*), and after stopping breast milk, the gut microbiome matures rapidly, as indicated by the phylum *Firmicutes*. It has been observed that Birth mode is also substantially correlated with the microbiome during developmental phase (Stewart et al., 2018).

The delivery method influences the types of bacteria babies are exposed to. Vaginally delivered babies acquire bacteria from the mother's gut and vagina, while C-section babies catch bacteria from hospital staff, other newborns, or the mother's skin. C-section infants have higher microbial diversity but lower *Bacteroidetes* and higher *Firmicutes* scores. Breastfed infants have lower *Clostridiales* counts (Sordillo et al., 2017).

A newborn's gut microbiota experiences dynamic changes in the first few weeks of life that are impacted by diet. Formula-fed newborns have a more diverse microbiome than breastfed infants, which is usually dominated by *Bifidobacteria*. When solid foods are introduced, the microbiota becomes even more diverse and eventually turns into adult-like species like *Clostridium* clusters IV and XIV and *Bacteroides spp*. Though it is believed that by age 3, a stable adult-like composition is established, this process continues, impacted by things like changing food habits and hormone changes during puberty (Martin et al., 2016).

Numerous factors, including eating habits, immune system activity, and physiological changes in the digestive tract, impact the composition of the microbiota in older adults. The older age persons usually have a greater number of *Enterobacteria* and *Clostridia* and lower levels of *Bifidobacteria* as compared to young person (Macfarlane and Macfarlane, 2009).

The gastrointestinal tract's enteric microbiota plays a critical role in preserving host health through encouraging physiological, immunological, and nutritive events. These bacteria work to strengthen the body's natural defenses against pathogen invasion by lining the gut epithelium (Marques et al., 2010).

Benefits of Probiotics in Children

Human well-being is largely dependent on the stability and makeup of gut flora, even from birth. The effectiveness of probiotics in the treatment of gastrointestinal disorders of different origins has been well-investigated (Gorreja and Walker, 2022). Probiotics are safe to use alone or in conjunction with other medications, immunoglobulins, and micronutrients to treat acute infectious diarrhea, according to several studies and meta-analyses (Vassilopoulou et al., 2021). Probiotics play vital role in children (Fig. 1).

Improved Digestion

Disturbances in the makeup of the gut microbiota have been linked to several chronic gastrointestinal illnesses, including colic crying, inflammatory bowel disease, celiac disease, and functional gastrointestinal disorders (FGID) (Pärtty et al., 2018) . Modulating the composition and activity of the gut microbiota has garnered significant interest as a potential therapeutic and preventative strategy due to the correlation between dysbiosis and functional gastrointestinal diseases in children and newborns. One of the most often utilized therapeutic approaches is the alteration of the makeup and activity of the gut microbiota through the use of probiotics. Probiotics work by altering the microbiota in the gut. Five randomized clinical trials have looked into the effect of *Lactobacillus reuteri DSM 17938* in children who have IBS or functional abdominal pain (FAP). There was a notable decrease in pain intensity in the probiotic group (Pärtty et al., 2018) .

Disease Prevention

Approximately 20 different viruses, bacteria, and parasites are linked to severe diarrhea. Six pathogen groups were identified by the 2013 Global Enteric Multicenter Study (GEMS) and its reanalysis in 2016, accounting for 77.8% of moderate to severe diarrheal episodes in children under five in African and Asian countries: *Shigella spp.*, *Campylobacter spp.*, and heat-stable enterotoxin-producing *Escherichia coli* (ST-ETEC); viruses, rotavirus, and adenovirus 40/41; and the parasite *Cryptosporidium spp*. (Allen et al., 2010).

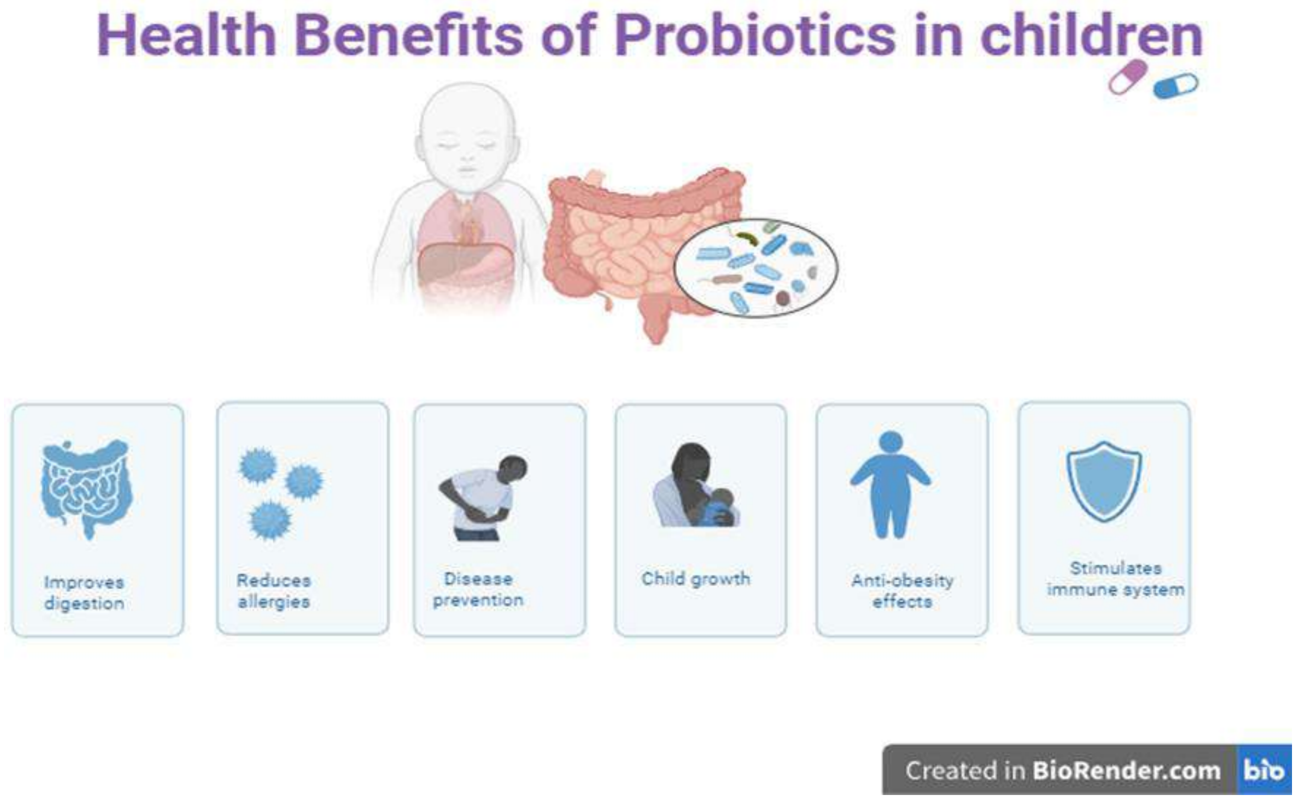

Fig. 1: Health benefits of probiotics in children

Probiotics have the potential to mitigate infectious diarrhea via various mechanisms, such as direct anti-pathogen actions, rivalry for nutrients and binding sites in the gut, synthesis of compounds such as bacteriocins and organic acids, neutralization of bacterial toxins, and broader impacts such as boosting mucosal immune responses and decreasing intestinal inflammation and permeability (Vassilopoulou et al., 2021).

Lactobacillus rhamnosus GG (LGG) was studied in 11 RCTs (n=2,072) based on the Cochrane review, and this meta-analysis discovered that using LGG decreased the length of diarrhea by a mean of 27 hours (Hojsak, 2017). In 2013, Szajewska et al. conducted a systematic review that identified 15 RCTs, totaling 2,963. This analysis verified LGG's advantage of LGG over placebo in terms of dramatically reducing diarrheal duration. However, there was no effect on the fecal volume. In terms of dosage, ≥1010 colony-forming units (CFU) were more efficacious than <1010 CFU (Scalabrin et al., 2017).

S. boulardii is another strain whose effects are well-established. The aforementioned Cochrane review identified six RCTs (n=606) and concluded that the use of *S. boulardii* decreased the incidence of diarrhea lasting more than four days. A more recent systematic review, including the analysis of 11 RCTs (n=1,306), revealed a substantial reduction in the duration of diarrhea caused by *S. boulardii*. The impact on stool volume was not assessed in any of these investigations (Szajewska et al., 2014)

In summary, the *Lactobacillus reuteri* ATCC 55730 strain had a modest therapeutic impact when treating acute gastroenteritis in children. Nevertheless, the strain was replaced by *L. reuteri* DSM 17938 when it was shown to possess transferable resistance characteristics for antibiotic resistance. Three RCTs were used to study this novel strain of *L. reuteri* DSM 17938, and two RCTs (n=196) were examined in a 2014 systematic review and showed a substantial reduction in the duration of diarrhea. Later, another RCT with 64 infants and children was published, with comparable findings regarding the shortening of the diarrheal duration (Szajewska et al., 2014).

Following an assessment of these data, the ESPGHAN Working Group on Pre- and Probiotics generally advised the use of the following probiotic strains in addition to rehydration treatment: LGG (strong recommendation; poor quality of evidence), *S. boulardii* (strong recommendation; low quality of evidence), and *L. reuteri* DSM 17938 (weak suggestion; extremely low quality of evidence) (Hojsak, 2017).

Enhanced Immunity

Probiotics increase IgA as a response associated with the host intestine's mucosal immunity. Infants' developing acquired immune systems, particularly the development of mucosal immunity and the generation of endogenous IgA, may be significantly influenced by gut microbiota (Lai et al., 2019).

Fecal IgA serves to maintain intestinal microbial homeostasis by agglutinating bacteria and preventing pathogenic germs from adhering to the mucosal surface. Prior research found that on Days 3 and 7 following the start of treatment, total IgA levels in the fecal extracts of the Lactobacillus group were greater ($p < 0.05$) than those in the control group (Lai et al., 2019). This is an amazing discovery on the use of probiotics to treat severe diarrhea in children.

Activated neutrophils produce a variety of enzymes and metabolites, which are secreted by cells of the innate immune system. These include lactoferrin and myeloperoxidase. The human oral cavity and digestive system are the primary locations of lactoferrin, where it can come into proximity with bacteria and viruses. Lactoferrin's antibacterial activity is the main way it helps mucosal defense. Additionally, lysozyme and secretory immunoglobulin A can be enhanced by lactoferrin (Lai et al., 2019).

Child Growth

Numerous investigations have evaluated the use of probiotics in relation to infant development (Catania et al., 2021). Previous research discovered that eating probiotics may help children in poor nations who are both healthy and undernourished acquire weight and height/length. The WHO growth charts are used in clinics all around the world to track children's development in comparison to the predicted value for their age (Onubi et al., 2015).

Child growth curves in the probiotic groups were considerably higher than or closer to the WHO standard value than in the control groups, according to two of the five studies that demonstrated a significant increase in growth. Probiotic-taking children showed improved height-for-age z-scores compared to the control group, which is another noteworthy finding from the study (Onubi et al., 2015).

Probiotics as Psychobiotics and their Psychoemotional Effects

Probiotics are mainly linked to the positive effect on digestive tract while these also act as psychobiotics as they have an effect mediation ability on different functions of brain, cognitive skills, intellect, memory and behaviors of learning as well. A study performed on a total of 135 participant children which aimed to assess neurotransmitter levels of GABA, serotonin, glutamate, cortisol along with neuropsychiatric symptoms which included headaches, mood swings, hyperactivity, aggressiveness, sleep disorders and many more in patients with gastrointestinal disorders. The results revealed to be supporting the hypothesis as it was clear indication that psychobiotics have a major impact on reducing hyperactivity and aggression whilst improving concentration (Matiș et al., 2023).

Sources of Probiotics in Children

It is usually advised to start probiotic-rich foods to children, start it with small amounts to allow their digestive system to adjust. Also, focus if your child is associated with any allergy or sensitivity due to any ingredient.

Natural Sources

Dairy and dairy-related products are considered as a good source of probiotics (Table 1), including *bifidobacteria*, lactic acid bacteria (LAB) and other microorganisms acquired from fermented milk. Spontaneous milk fermentation has been practiced in Mongolia or Africa for centuries and the use of beneficial microorganisms in fermented dairy products has been employed for many generations (Fontana, 2013). Traditional fermented milk comprises complex compositions of LAB species, supplying a valuable source of probiotic strains. Recent studies have analyzed traditional fermented products as probable natural sources of probiotic bacteria (Yu et al., 2011). The majority of microorganisms isolated from fermented products are associated with the Lactobacillus genus. Cheese is a dairy item with the potential to deliver essential probiotic microorganisms into the human intestine (Sun et al., 2010). Strains of *L.plantarum* have been separated from Italian, Argentinean, and Bulgarian cheeses. Breast milk is considered an interesting source of probiotic LAB and *bifidobacteria* as compared to formula fed milk (Liong, 2011). Most probiotic strains were obtained from the fecal samples of infants and healthy adults. The isolation of probiotics is not only restricted to the human tract. The guts of many animal species, including poultry, pigs, marine, freshwater fish and rats are considered as a good source of probiotics(Zago et al., 2011).

Table 1: Some examples of natural sources of probiotics for children

Yogurt	Select unsweetened, plain yogurt with active and live bacterial cultures. Greek yogurt is considered as a good choice because it contains more concentration of probiotics. (Patro-Gołąb et al., 2015)
Fermented Vegetables	Foods such as kimchi, pickles and sauerkraut have some beneficial bacteria and can be consumed by children in small amounts (Vinderola and Pérez-Marc, 2021).
Miso	A traditional Japanese seasoning prepared from fermented barley, rice and soybeans. It can also be used to flavor sauces, soups and marinades (Gomathy, 2013).
Kefir	Fermented milk drink which is rich in probiotics and has tangy taste. It can be consumed in plain form or can be added to smoothies (Lawrence et al., 2023).
Tempeh	Product of fermented soybean, used as a meat substitute in many dishes. It is an excellent source of probiotics and proteins for growing children (Yulianto and Pujimulyani, 2023).
Kombucha	Fermented tea drink is important as a probiotic source, with minimum sugars and to check caffeine level in children (Kozyrovska et al., 2012).

Supplements

It is important to consult the healthcare provider before the start of any probiotic supplement because it is essential to choose the specific needs of the child according to the child’s health status before the selection of a probiotic supplement

(Table 2). The supplement should contain beneficial strains such as *Bifidobacterium* and *Lactobacillus species* (Chen et al., 2012). Also consider some other factors including dosage, potential allergens, storage requirements, and any sensitivities. Try to follow the instructions of the recommended dosage given by the manufacturer and health care providers (Catania et al., 2021).

Table 2: Some important probiotic supplements for children with examples

Probiotic chewable capsules or tablets	Available in a variety of formulations with many flavors and dosages, which are designed for different age groups of children (Hedayati-Hajikand et al., 2015)	• Culturelle Kids Chewable contains *Lactobacillus rhamnosus* GG, which is beneficial digestive health. • Nature's Way Primadophilus Chewable contains blend of Bifidobacterium and Lactobacillus, to support digestive balance.
Probiotic drops	To overcome the problem of swallowing tablets and capsules, liquid probiotic supplements are available for young children and infants. These drops can be added to other liquids, formula, or breast milk (Hasslöf et al., 2022)	• BioGaia Pro-Tectis Drops contain *Lactobacillus reuteri Protectis*, used to support digestive health. • Gerber Soothe Probiotic Drops contain *Lactobacillus reuteri*, helps to reduce colic. • Mommy's Bliss Probiotic Drops contain *Lactobacillus rhamnosus GG*, which supports immune system and digestive health in infants.
Probiotic gummies	It is the most appealing type of probiotic due to its texture and taste. But it is also important to check the ingredients of gummies and ensure the level of sugar and artificial additives (Kamil et al., 2022)	• Smarty-Pants Probiotic Immunity Gummies contain *Bacillus subtilis* DE111, *Lactobacillus acidophilus* and *Bifidobacterium lactis*. • Nature's Way Fortify Probiotic Gummies provide *Lactobacillus plantarum, Lactobacillus acidophilus* and *Bifidobacterium lactis*. • Culturelle Probiotic + Vitamin C Gummies contain *Lactobacillus rhamnosus GG* and vitamin C.
Powdered probiotic supplements	To make it easily consumable, powdered forms can be easily mixed with liquids or other soft foods like yogurt or apple sauce. (Onubi et al., 2015)	• Renew Life Ultimate Flora Powder contains 10 probiotic strains to support digestive and immune health. • Garden of Life Raw Probiotic Powder contains 23 probiotic strains. • Hyperbiotics PRO-Kids Probiotics Powder contains five probiotic strains.
Probiotic sachets	Usually available in single-serving sachets, which contain probiotics. These can easily be mixed into beverages or soft foods (Freedman et al., 2018)	• BioGaia Gastrus Probiotic Straws contain *Lactobacillus reuteri Gastrus.* • Florastor Daily Probiotic Sachets contain *Saccharomyces boulardii* CNCM I-745, which is a probiotic yeast.

Factors Affecting the Efficacy of Probiotics

Probiotics' effectiveness depends on various factors such as the specific strains of bacteria, their viability and stability, dosage, and their ability to survive in the digestive system. Additionally, prebiotic content, interactions with other drugs, and certain medical conditions also impact their effectiveness.

Dosage and Concentration

The efficiency of probiotics is significantly influenced by their dose and concentration. In a study with 255 adult inpatients, three groups were assigned: Pro-2 (86 individuals, two probiotic capsules/day), Pro-1 (85 individuals, one probiotic capsule/day), and a placebo (84 individuals, two placebo capsules/day). Capsules containing fifty billion CFUs of live microbes were given 36 hours after the first antibiotic dose and continued for 5 days after the last antibiotic dose, with an additional 21-day observation. Pro-2 had a lower incidence of antibiotic-associated diarrhea compared to Pro-1. A dose-ranging effect was observed with 100 billion CFUs producing better results and fewer gastrointestinal problems than fifty billion CFUs (Gao et al., 2010).

Time

Probiotics work best when taken with meals, especially those containing some fat. According to research, the best way to get all the benefits of probiotic pills is to take them with a main meal or thirty minutes before.

It's crucial to take probiotics separately from antibiotics to protect their effectiveness. Meta-regression analysis shows that probiotics are most effective when taken close to the initial antibiotic dose, with a decreasing effectiveness for each day of delay. Probiotics taken within two days of starting antibiotics show the greatest reduction in CDI risk (Shen et al., 2017).

Host-Related Factors Influencing Probiotic Functionality

Probiotics' effectiveness depends on how they interact with the host's factors and intestinal environment. They adapt to challenges like low pH, enzymes, and bile salts, changing their gene expression. Host-specific factors like diet, genetics, age, health, and geography and temporary factors like infections and immune statuses also affect the response to probiotics. It's crucial to consider these elements given the changes in gene expression and microbiota composition induced by diet. Understanding these factors is essential for accurate evaluation and application of probiotics, as individual responses can significantly vary (Sanders et al., 2014).

Host Diet and Nutritional Factors

The function of probiotics in your stomach is influenced by the foods you eat. Research shows that probiotics cause animals fed a Western-style meal to behave differently from those on a normal diet. Certain foods, like carbohydrates, enhance the effectiveness of some probiotics in defending against harmful microorganisms. Additionally, foods such as histidine and lactose may affect probiotics, influencing their colonization and interactions with your gut's immune system (Marco and Tachon, 2013).

Host Physiology and Microbiome

Not everyone experiences the same level of benefit from probiotics, or those beneficial bacteria. It is dependent upon factors such as our age, health, and DNA. Our customs and the places we inhabit matter. The microbiota, or the preexisting bacteria in our gut, influences the effectiveness of probiotics as well. Probiotics can occasionally aid with digestive problems. It's similar to a riddle in that what suits one individual may not suit another (Marco and Tachon, 2013).

Environmental Factors

Probiotic growth conditions (e.g., temperature, oxygen, salt, and nutrients) affect cell yields, growth rates, and survivability. Fermentation conditions influence stress tolerance and performance of *Lactobacillus plantarum* WCFS1, revealing genes essential for gastrointestinal adaptation (Marco and Tachon, 2013). Epithelial cell and immune responses are also influenced by the growth stage at harvesting; stationary-phase cultures of *Lactobacillus acidophilus* NCFM show increased adhesion to epithelial cells (Goh and Klaenhammer, 2010). Growth-phase-dependent immunomodulation is also seen; cells in the stationary phase and those that have been heat-killed trigger the NF-κB immune response pathways, whereas *L. plantarum* in the exponential phase stimulates cell division and growth (Marco and Tachon, 2013). Understanding all of these factors is crucial for maximizing the effectiveness of probiotics and maintaining their health-promoting attributes (Mills et al., 2011).

Guidelines, Safety and Risks

Experts have identified certain clinical conditions for which probiotics may be beneficial for children's health. While generally safe, caution is advised for premature infants, immunocompromised patients, critically ill patients, and those with certain conditions. *S. boulardii* has been effective for *C. difficile* infection in children, but special care is needed for critically ill patients (Hojsak et al., 2018). Probiotics have shown to be highly effective, but their efficacy is specific to the strain. However, various clinical organizations have evaluated probiotics and probiotic foods to determine their evidence-based health benefits (Deshpande et al., 2011; Ebner et al., 2014). It is preferable to use strains isolated from humans due to their natural occurrence, safety record in infants, and adaptability to both mucosal and dairy ecosystems (Deshpande et al., 2011). Some strains of probiotics are not recommended for use in children like *Enterococcus faecium* SF68, due to the risk of transferring vancomycin-resistant genes (Hojsak et al., 2018). Consequently, medical organizations have made clinical recommendations suggesting specific, well-defined probiotics for certain clinical conditions, such as acute gastroenteritis, necrotizing enterocolitis, or antibiotic-associated diarrhea. Moreover, probiotics can be used more broadly to supplement infant formula and mimic the composition and microbial content of human milk (Ebner et al., 2014). According to the ESPGHAN Working Group on Probiotics and Prebiotics, the 2014 guidelines suggest administering *Lactobacillus rhamnosus GG* (LGG) and *Saccharomyces boulardii*, in addition to rehydration therapy, to children experiencing acute gastroenteritis (AGE). Nevertheless, the efficacy of *Lactobacillus reuteri* DSM 17938 has yet to be sufficiently proven (Szajewska et al., 2020). Probiotics have shown to have minimal complications or adverse effects, with only mild abdominal discomfort and flatulence being the commonly reported issues in most clinical trials (Kligler et al., 2007).

Future Directions

To achieve a more complete understanding of the impact of probiotics on children's health, it's essential to delve into specific strains, dosage and duration, how they work, and how they interact with other treatments. Additionally, it's important to consider the effects of antibiotics and adhere to safety and regulatory standards. By addressing these research needs, we can create evidence-based interventions that enhance precision in our probiotic recommendations for children.

Conclusion

Hence, Probiotics are microorganisms that improve an individual's health by enhancing the gut flora. When a baby is born, they don't have any gut flora but over time, they develop it from their environment, from formula or breast milk or through natural food such as yoghurt, fermented vegetables, miso, and kefir and supplements usually contains *Bifidobacterium* and *Lactobacillus species* either in the form of gummies, drops or chewable tablets. They can aid in digestion, prevent diseases, promote child growth, and boost immunity. However, the effectiveness of probiotics largely depends on the dosage, timing, and host-related factors. Probiotics also act as psychobiotics, mediating various brain functions, cognitive skills, intellect, memory, and learning behaviors. It's important to consider the specific clinical conditions and guidelines when considering probiotics for children's health. The efficacy and safety of probiotics vary depending on the strain, and caution should be exercised, especially for certain patient groups. Consulting with healthcare professionals and following clinical recommendations is crucial when considering the use of probiotics for children.

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Chapter 14

The Effects of Prebiotics and Probiotics on Glucose Homeostasis in Type 2 Diabetes Mellitus

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ABSTRACT

Increased levels of blood glucose or hyperglycemia is the hallmark of diabetes mellitus. Besides genetic and lifestyle risk factors, research has established a link between type 2 diabetes and the intestinal flora. The microbiome dysfunction results in impaired metabolism for the host as gut microbiota utilize nutrients and produce metabolites that increase the risk of metabolic disorders. There is evidence that diabetic individuals exhibit altered intestinal microbiota compared to non-diabetic adults as they have significantly lower proportions of Firmicutes, Clostridia, and bifidobacteria. Novel therapeutic approaches are focused on modifying and re-regulating the intestinal microbiome, with prebiotics and probiotics showing promise as bioactive agents that could benefit the intestinal flora structure and function. This chapter has investigated the potential mechanisms of gut microbiota in glucose homeostasis. The study has found that the gut microbiota produces short-chain fatty acids that are important in glucose metabolism. The intestinal bacteria are also useful in bile acid synthesis from liver cholesterol, as well as regulating LPS increase to reduce gut permeability. Mechanisms of action of sample prebiotics and probiotics in glucose homeostasis have also been outlined in this chapter.

KEYWORDS Prebiotics, Probiotics, Homeostasis, Type 2 diabetes, Dysbiosis, SCFAs	Received: 14-June-2024 Revised: 18-July-2024 Accepted: 13-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

The diabetes epidemic is escalating globally as it continues to pose significant economic and disease burden with its rising incidence. The hallmark of diabetes mellitus is increased levels of blood glucose or hyperglycemia and it manifests with the pancreatic gland failing to secrete adequate insulin or the body exhibits impaired insulin utilization, otherwise insulin resistance (Ayesha et al., 2023). Type 2 diabetes mellitus (T2DM) is the most diagnosed diabetic condition, representing a multifactorial condition affected by numerous contributors such as nutrition, lifestyle, age, family history, dyslipidemia, abdominal obesity, and hypertension (Paul et al., 2022a). The prevalence of diabetes mellitus stands at 463.0 million adults aged 20-79 years, with T2DM accounting for approximately 90% of all cases and the number projected to hit 578.4 million by 2030 (Wang et al., 2021). Recent research evidence has gone beyond the genetic, dietary, and physical activity related contributors for T2DM towards identifying the close relationship between the disease and the intestinal flora. Disorders of the intestinal flora have been found to affect the host's metabolism by utilizing nutrients and producing metabolites and promoting the risk of metabolic disorders including insulin resistance and metabolic syndrome to at-risk populations to T2DM (Zhang et al., 2023). Scientists have shown that compared to non-diabetic adults, diabetic individuals exhibit altered intestinal microbiota as evidenced by significantly lower proportions of Firmicutes, Clostridia, and bifidobacteria (Kim et al., 2018). Thus, the interaction between the gut microbiome, nutritional behaviors, and the activity of mucosal immunity has been scientifically proven to affect T2DM progression as intestinal dysbiosis leads to gut metabolic dysregulation (Vitetta et al., 2023).

Post-prandial glucose absorption and plasma level increase acts as a potent stimulus for pancreatic beta cells insulin secretion (Hiriart et al., 2014). The insulin hormone enhances the disposal of glucose by peripheral tissues, liver lipogenesis, the synthesis of glycogen, and promotes glucose uptake and conversion to glycogen by muscle or triglycerides by adipocytes. Glucose amounts reduce as a result of these processes and act to stop the insulin secretion stimulus. In the fasting state, the level of insulin is low and the liver becomes the main source of plasma glucose. Thus, in the metabolic syndrome (MS), there is disrupted glucose homeostasis and exhausted beta cells under constant pressure, which lead to type 2 diabetes. Further evidence shows gut microbiome balance and regulation is a risk of developing T2DM, as decreased butyrate-producing bacteria and increased harmful bacteria correlate to gut dysbiosis (Zhang et al., 2021). Hence, new therapeutic approaches for the prevention, management, and to serve as complementary treatment in T2DM have recently focused on modulating and re-regulating the gut microbiome. Probiotics and prebiotics have shown promise as bioactive agents that could benefit the structure and function of the intestinal flora. Research has established a link between the lack of bacteria that produce short-chain fatty acids (SCFAs) and increased risk of T2DM (Aslamy et al., 2024). There is also evidence implicating SCFAs role in improving T2DM-associated glucose homeostasis, although research is still limited as to the mechanisms through which gut microbiota affect host glucose homeostasis. Accordingly, this chapter seeks to fill this gap by examining the action of probiotics and prebiotics in modulating the intestinal microbiome and in turn influencing glucose homeostasis in T2DM. Increasing the amount of beneficial gut bacteria has been proposed as potentially modulating the gut composition and enhancing glucose amounts and insulin sensitivity.

Mechanisms of Gut Microbiota Effect on Glucose Homeostasis
Gut Microbiota Dysbiosis in Type 2 Diabetes

Dysbiosis of the gut has been confirmed in T2DM patients. In T2DM population, there is enhanced arrays of infective bacteria like *Escherichia coli*, *Clostridium hathewayi*, and *Clostridium symbiosum*, as opposed to healthy controls exhibiting a high abundance of butyrate-producing bacteria (Zhou et al., 2022). A study of 36 male participants, 18 diabetic and 18 healthy controls, with the results showing *Firmicute* quantities were substantially increased in the control than the diabetic group, suggesting a positive correlation between gut microbiome composition and diabetes. A reduction in butyrate-producing bacteria like *clostridiales* sp. affects insulin sensitivity, glucose and fat metabolism, and low-grade inflammatory response in diabetic patients. Some species like *Lactobacillus* have been shown to influence diabetes as they positively correlate with fasting blood glucose and glycosylated hemoglobin (Al-Ishaq et al., 2023).

Sedighi et al., (2017) compared gut microbiota in patients with T2DM and healthy individuals in which 36 adults were included and their intestinal microbiota composition investigated. There was evidence showing that the quantities of two groups of bacteria differed in a meaningful way, with *Lactobacillus* being significantly higher in diabetic patients while *Bifidobacterium* was significantly elevated in healthy controls. Since beneficial bacteria are reduced in the intestinal microbiome of individuals with diabetes type 2, it means that the host is unable to reap the benefits related to prevention of intestinal inflammation linked to T2DM. The gut microbiota role in the pathophysiology of T2DM has also been discussed in research as scientists continue to investigate dysbiosis in metabolic conditions and the associated signaling pathways linking metabolites and gut microbiota components to the progression of diabetes. SCFAs, bile acid, imidazole propionate, lipopolysaccharides, and branched-chain amino acids (BCAAs) have all been shown to be important regulators in T2DM. Dysbiosis reduces the production of metabolic SCFAs as the SCFA-producing organisms are lower in abundance, which contribute to reduced anti-inflammatory levels of SCFA. It is notable that SCFAs are crucial for reducing serum glucose levels, enhancing protective glucagon-like peptide 1 (GLP-1) secretion, improving insulin resistance, and mitigating inflammation (Yang et al., 2020). Thus, a reduction in the composition of SCFA-producing bacteria is bound to have a negative influence on T2DM development and progression.

(b) Influence of Gut Microbiota on Glucose Metabolism

Gut microbiome alteration commonly noted in diabetes type 2 relates to the decreased abundance of bacterial species involved in the production of SCFA butyrate. (Barlow and Mathur, 2023) butyrate, acetate, and propionate are products of bacterial fermentation of dietary fibers. While acetate and propionate act as lipogenesis and gluconeogenesis substrates in the liver and peripheral tissues, butyrate is an energy substrate for the colonic epithelium with effects on activating small bowel and colon gluconeogenesis. The mechanisms of glucose metabolism by SCFAs have been explored in research, with scientists opining that SCFAs moderate gut hormone production to control insulin release and appetite (Tolhurst et al., 2012). SCFAs have further been shown to regulate the balance between the synthesis of fatty acids (FAs), their oxidation, and lipolysis (Den Besten et al., 2013). Through the activation of FA oxidation and inhibition of de novo synthesis and lipolysis, the net result of SCFA is a decrease in the plasma concentrations of free fatty acids (FFAs), and subsequent decrease in body weight. While data is scarce on the SCFA effect on glucose metabolism, studies suggest plasma reduction of glucose levels through diverse mechanisms. SCFAs fermented by gut bacteria stimulate FA oxidation and prevent de novo lipogenesis and lipolysis, and are implicated in regulating the release of GLP-1 and the peptide YY

(PYY) gut hormones that control energy homeostasis and the metabolism of glucose. GLP-1 augments the secretion of insulin by the pancreatic beta-cell and PYY and GLP-1 both decrease food intake by acting on the hypothalamus (Utzschneider et al., 2016).

SCFAs are also involved in energy supply as the intestinal microbiota ferment indigestible carbohydrates that provide energy sources in metabolic conditions. The colonocyte absorption of SFCAs utilized in mitochondrial β-oxidation and citric acid cycle for energy generation. Whereas butyrate is the main source of colonocyte energy, propionate acts as a gluconeogenic substrate, and non-metabolized SCFAs in the colonocytes are transported to the liver where they are act as energy substrates for hepatocytes by acetyl-CoA synthases (ACS) (Tang and Li, 2021). Propionate is also transformed into glucose in the liver through the tricarboxylic acid (TCA) cycle. SCFAs are also involved in activating intestinal gluconeogenesis (IGN), an important process for normal blood glucose maintenance and energy homeostasis (Wu et al., 2021). The propionate substrates produced by *Bacteroidetes*, *Salmonella*, and *Acidaminococcus* are taken up and relayed to the liver where they are crucial in promoting intrahepatic gluconeogenesis. As a major SCFA involved in activating IGN, propionate regulates food intake, enhances insulin sensitivity, and maintains metabolic homeostasis (Liu et al., 2021). Further research has indicated propionate improvement of β-cell function and exerting direct effects in potentiating glucose-stimulated insulin release and maintenance of β-cell mass by inhibiting apoptosis.

Gut Microbiota Bile Acid Synthesis

Bile acids (BAs) are produced from liver cholesterol as bioactive metabolites and they are crucial in the host and gut microbiota symbiosis. Intestinal microbiome regulates bile acid alteration that is involved in T2DM development. Since BA homeostasis is disrupted in T2DM patients, it confirms the interaction of BAs with the gut microbiota in regulating the metabolism of carbohydrates, lipids, and energy (Gao et al., 2022). The gastrointestinal (GI) tract bacteria also synthesize enzymes that modify secondary bile acids (SBAs), thereby affecting carbohydrate and lipid metabolism. The role of BA metabolism in maintenance of glucose homeostasis, with studies showing the fasting serum total bile acids (S-TBAs) linked to decreased insulin sensitivity, islet β-cell function impairment, and the dysregulation of glucagon secretion of α-cells in T2DM patients (Wang et al., 2020).

Lipopolysaccharides Increase of Intestinal Permeability

Patients with T2DM have exhibited altered microbiota characterized by the increased expression of gram-negative bacteria species that express lipopolysaccharides (LPS). Rising levels of circulating LPS and low-grade endotoxemia play a crucial part in the development of metabolic diseases, and it is notable that gut microflora alteration decreases LPS amounts and are responsible for protecting against glucose intolerance, insulin resistance, and inflammation (Amyot et al., 2012). LPS and insulin induce macrophage-derived IL-10, which in turn downregulates the production of hepatic glucose alongside insulin and result in insulin resistance explain in fig. 1(Toda et al., 2020).

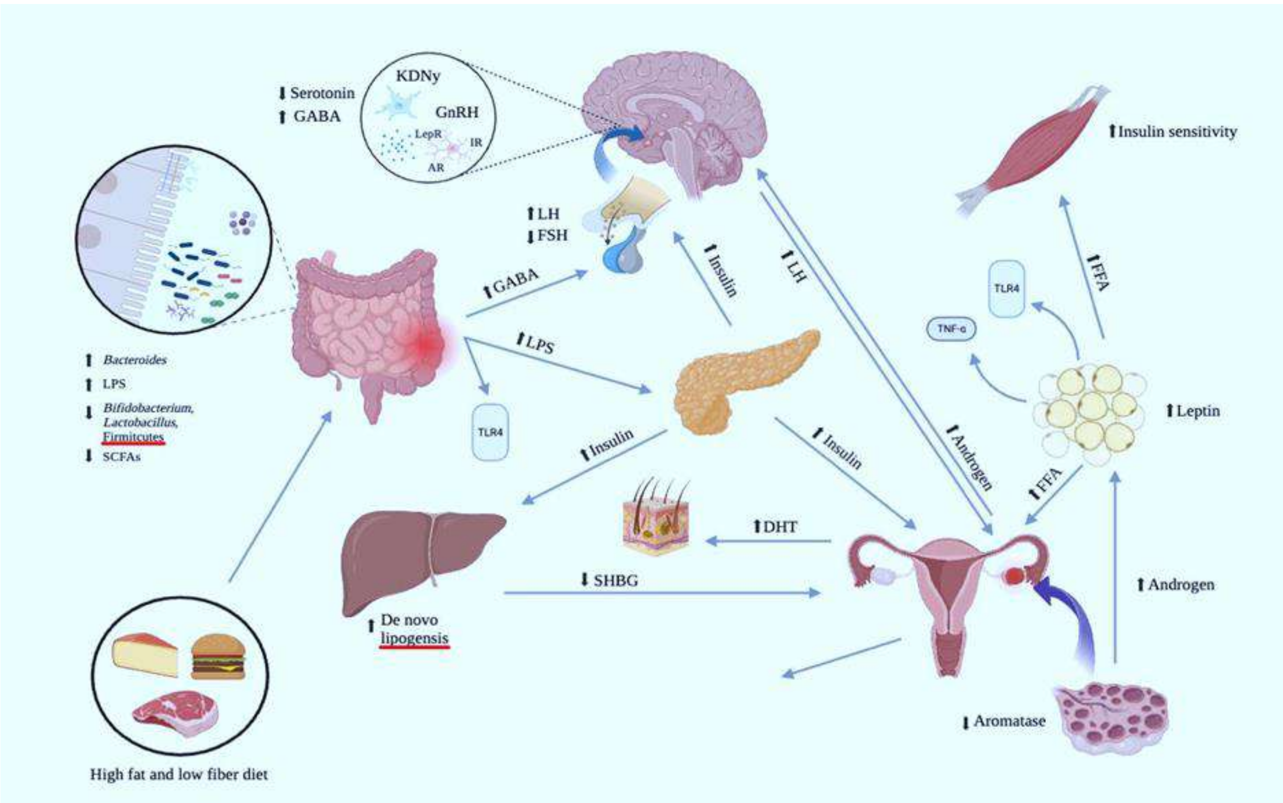

Fig. 1: Lipopolysaccharides drive insulin resistance in T2DM

Prebiotics Role in Glucose Homeostasis

Owing to the link between T2DM and gut microbial community disequilibrium, nutritional interventions such as prebiotics have shown promise in promoting eubiosis of intestinal microbiome and maintaining glucose homeostasis in T2DM patients (Ojo et al., 2022). While numerous clinical trials have demonstrated biotics effectiveness in treating T2D, the varying effects of these therapeutics remain a challenge. Hence, it is an important research goal to fill this existing gap and identify the efficacy of prebiotics in glucose homeostasis for T2DM patients (Paul et al., 2022b). Gut microbes usually metabolize prebiotics by fermenting them into metabolites useful for the host, with the end products being short-chain fatty acids, primarily propionic, butyric, and acetic acid that influence gut epithelium integrity, glucose homeostasis, lipid profile, immunity, and body weight (Markowiak-Kopeć and Śliżewska, 2020). Further, affect insulin resistance, increase energy expenditure, suppress appetite and lipolysis, and promote the production of and sensitivity to insulin through multiple pathways and mechanisms.

Table 1: Glucose Homeostasis Role of Select Prebiotics in Type 2 Diabetes

Prebiotics	Mechanism of Glucose Homeostasis	References
Inulin-type fructans	Increased SCFA concentrations	(Birkeland et al., 2020)
Resistant dextrin	Reduced fasting insulin Decreased fasting plasma glucose and HbA1c Downregulated TNA- α and Il-6	(Aliasgharzadeh et al., 2015)
Beta-glucan, Oatwell,	Upregulated SCFA production, energy metabolism, hepatic gluconeogenesis	(Fusco et al., 2023)
xylo-oligosaccharides Whole Fiber, Inulin	Upregulated SCFA production, energy metabolism, hepatic gluconeogenesis	(Carlson et al., 2017)
PolyGlycopleX	HbA1c reduction, total cholesterol, triglycerides, and LDL-c decrease, lower serum leptin, beneficial bacteria proliferation	(Reimer et al., 2020)
Multi-strain probiotics (*Lactobacillus, Lactococcus, Bifidobacterium, Propionibacterium*, *Acetobacter*)	Increased beta-cell function, reduced fasting glucose and HbA1c, reduced inflammatory markers	(Savytska et al., 2023)
Lactobacillus reuteri	HbA1c reduction, decreased blood lipids, reduced IL-1β signaling, changes in gut flora	(Hsieh et al., 2018)
Lactobacillus plantarum, L. bulgaris, L. gasseri, Bifidobacterium bifidum, Streptococcus thermophilus, Saccharomyces boulardii	FPG, HbA1c, HOMA-IR, fasting plasma insulin reduction adjunct with metformin, SCFA upregulation	(Palacios et al., 2020)
Lactobacillus paracei	Reduced FBG, downregulation of adipokines, reduced plasma LDL, increased plasma HDL, decreased LPS, TNF-alpha, IL-6, increased IgA, SCFA upregulation	(Toejing et al., 2021)
Bifidobacterium animalis and *B. longum*	Improved glucose tolerance, reduced adipocyte size, beneficial bacteria proliferation	(Aoki et al., 2017)

Table 2: Glucose Homeostasis Role of Select Probiotics in Type 2 Diabetes

Probiotics	Mechanism of action	References
RCT of multi strain Probiotics	Repairing pancreatic beta-cells that are impaired in T2D leading to reduced insulin production, with microbiome regulation improving glucose homeostasis	(Savytska et al., 2023)
Lactobacillus reuteri (ADR-3 and ADR-1 strains)	Reduction in proinflammatory cytokine levels and changes in intestinal microbiota composition	(Hsieh et al., 2018)
Lactobacillus plantarum Lactobacillus bulgaris Lactobacillus gasseri Bifidobacterium bifidum	Probiotic effects on metformin efficacy in patients' glycemic control and glucose homeostasis.	(Palacios et al., 2020)
Lactobacillus paracei HII01	Beneficial bacteria were enhanced while pathogenic bacteria reduced with *L. paracasei* in T2DM.	(Toejing et al., 2021)
Bifidobacterium lactis GCL2505 *B. longum* JCM1217	Accelerated SCFA production in the gut, with the SCFAs important in regulating energy homeostasis via G protein-coupled receptors (GPCRs).	(Aoki et al., 2017)

Mechanisms of Prebiotic Effects in Glucose Homeostasis

Inulin-type fructans (ITF) are the most studied prebiotic compounds, including inulin, fructo-oligosaccharide (FOS), and galacto-oligosaccharide (GOS), all of which are resistant to digestion by the small intestinal enzymes. Inulin, FOS, and GOS consistently enrich *Lactobacillus* and *Bifidobacterium* spp. in the gut, bacteria species with β-fructanoside and β-galactoside enzymes that readily degrade the ITFs. There is evidence in research that prebiotics effectively improve glycemic control in T2DM, although most studies are based on animal models while human trials have been lagging and most work has been done in healthy subjects without T2D diagnosis (Robertson, 2020). Studies of the potential mechanistic actions of prebiotics explain in Table 1.

Mechanisms of Probiotics Effects in Glucose Homeostasis

Gut dysbiosis in type 2 diabetes has also led to scientific research of the effects of probiotic intervention to improve metabolic variables. The gut microbiota's quantitative and qualitative composition greatly influences how it interacts with the host. The majority of the current dysbiosis treatment approach still relies on probiotics to normalize the "disturbed" gut microbiota and restore microbial diversity. Studies of the potential mechanistic actions of probiotic explain in table 2.

Conclusion and Future Research Directions

This chapter has investigated the mechanisms of prebiotics and probiotics in affecting glucose homeostasis in T2DM patients. As gut microbiota dysbiosis is linked to T2DM, prebiotic and probiotic modulation of the gut microbiome appears to be an important therapeutic goal in metabolic conditions. The evidence from human clinical trials have shown that by enriching the gut with beneficial bacteria and downregulating pathogenic bacteria, prebiotics and probiotics improve plasma glucose and insulin in patients with T2DM. Some of the noted mechanisms included the production of SCFAs that supply energy to the liver hepatocytes, bacterial synthesis of bile acids to bioactive metabolites that regulate carbohydrate, lipid, and energy metabolism. Prebiotics and probiotics also downregulate inflammatory cytokines that are involved in increased levels of LPS in the intestines. While these findings have shown the effect on these compounds in enhancing insulin sensitivity and glucose metabolism, future research directions can investigate the synergistic effects of combined prebiotics and probiotics (synbiotics) in glucose homeostasis. There have also been variations in the findings regarding the bacteria populations after the administration of probiotics and prebiotics and prospective studies can determine the cause of these differences for better understanding of the efficacies of the compounds.

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Chapter 15

Use of Probiotics for Management and Intervention of Cardiovascular Diseases in Humans

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ABSTRACT

Cardiovascular disease is one of the major causes of illness and death proposing various health risks such as obesity, diabetes, smoking, inflammation, and hypertension. From the immediate effects of gut microbiota on energy metabolism and obesity to the adjacent interaction between periodontal illness, heart attack, and stroke, microorganisms have a major effect on cardiovascular health. The probability of effect or determining microorganisms is associated with probiotic applications. Probiotics are live microorganisms that, when consumed in sufficient amounts, have significant health benefits for the host. The effectiveness of probiotics can be determined by various factors, including the relationship between probiotic bacteria and the host's microbiome. Many of these fermented strains of probiotic bacteria are obtained from foods. Still, nothing is known about these additives' effectiveness and potential use as essential nutritional components in reducing or treating cardiovascular disease.

KEYWORDS			
Cardiovascular diseases, Risk factors, Probiotics, Immune system, Gut microbiota	Received: 23-May-2024 Revised: 10-July-2024 Accepted: 12-Aug-2024		A Publication of Unique Scientific Publishers

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INTRODUCTION

The gut microbiota is integral to a multitude of vital bodily functions and is now acknowledged as a cornerstone of human health. It bolsters the immune system by metabolizing harmful food components and producing essential amino acids and vitamins, such as vitamin K and vitamin B12 (Kolodziejczyk et al., 2019). An abundance of "beneficial bacteria" within the microbiome acts as a shield, safeguarding the body against invasive pathogenic toxins (Pickard et al., 2017).

Delving into the role of the gut microbiome in cardiovascular illnesses, researchers have broadened their focus to mitigate atherosclerosis and other cardiovascular ailments (Oniszczuk et al., 2021). Pathological abnormalities affecting the heart or blood arteries fall under the broad category of disorders referred to as "cardiovascular diseases" (Mozos, 2015). Angina pectoris, hypertension, heart failure, and cardiac arrest are among these ailments. Therefore, the gut microbiota influences the immune system and food metabolism, rendering it a therapeutic and diagnostic element for various cardiovascular conditions (Rahman et al., 2022)

Researchers have proposed that probiotics, which augment the population of beneficial bacteria in the gut, may aid in treating patients with heart disease. Indeed, heart disease shares similarities with other inflammatory conditions such as inflammatory bowel illness, rheumatoid arthritis, psoriasis, and multiple sclerosis, all of which are believed to have their origins in the gut and are associated with an imbalance in gut microbiota (Li et al., 2017; Pedroza and Lyavoo, 2023).

Probiotics could potentially serve as a treatment for cardiovascular diseases due to their positive effects on the microbiological and metabolic composition of gut microbiota. The preventive effects of probiotic therapies against cardiovascular diseases may be explained by modifications in the host immune system. (Ebel et al., 2014). In most developed countries, cardiovascular diseases are responsible for a greater part of mortalities in adults and one-third of mortalities in the elderly (Reddy and Yusuf, 1998).

Probiotics

Probiotics have demonstrated favorable effects in managing gastrointestinal disorders, including the treatment of rotavirus-associated acute diarrhea (Szajewska and Mrukowicz, 2005), ulcerative colitis (Mallon et al., 2007), and diarrhea attributed to Clostridium difficile (Dendukuri et al., 2005). However, their therapeutic potential remains ambiguous owing to the challenges posed by microbial colonization and survival within the gastrointestinal tract. They stick to the epithelial membrane and are linked with the indigenous microbiota within the human gastrointestinal tract at a dosage of 109 CFU per day (Kotikalapudi et al., 2010).

The body's innate defenses, particularly the gut, and intestines, can be disrupted and debilitated by stress, excessive workload, smoking, and a diet abundant in calories but deficient in essential nutrients. Prolonged exposure to these factors may culminate in the development of metabolic syndrome and an accumulation of risk factors associated with heart attacks (Steffen et al., 2009). This study delves into the impact of consuming foods containing probiotic strains on four medical conditions—arterial hypertension, obesity, diabetes, and hypercholesterolemia—that are intricately linked to an elevated risk of cardiovascular disease.

(De Almada et al., 2015) They are naturally present in foods such as yogurt, kefir, sauerkraut, tempeh, and kimchi. To exert their beneficial effects, probiotics must impede the proliferation of pathogenic bacteria through immune, hormonal, and neuronal modulation, either chemically or physically. Moreover, they stimulate the growth of beneficial microorganisms (Zucko et al., 2020).

Cardiovascular Diseases

Growing research suggests that metabolic illnesses such as diabetes, obesity, and cardiovascular diseases may be ameliorated by probiotics (Le Barz et al., 2015). They have been demonstrated to prevent cardiovascular diseases by enhancing immune response, balancing the morphological and functional changes of gut microbiota, reducing cholesterol levels, and alleviating oxidative stress (Al Bander et al., 2020), therefore serve as a treatment for cardiovascular diseases due to their positive effects on the microbiological and metabolic composition of gut microbiota.

Immunologic mechanisms underlying the action of probiotics encompass various components, including epithelial cells, dendritic cells, effector lymphocytes, natural killer T-cells, T-regulatory cells, and B-cells (Mazziotta et al., 2023). The term cardiovascular disease (CVD) encapsulates a spectrum of illnesses characterized by complex etiologic and treatment modalities. According to Raygan, supplementation with probiotics alone or in conjunction with vitamin D or selenium can significantly enhance mental stability biomarkers and metabolic profiles, such as high-sensitivity C-reactive protein, nitric oxide, lipoprotein, low-density cholesterol, or total cholesterol, alongside mitigating oxidative stress and inflammation (Pourrajab et al., 2021).

In males with coronary artery disease (CAD), supplementation with *L. Plantarum* 299v (Lp299v) demonstrated improvements in vascular endothelial function while reducing systemic inflammation (Hofeld et al., 2021). Similarly, in patients with CAD, intake of lactobacillus rhamnosus GG was linked with a decrease in metabolic endotoxemia and systemic inflammation (Moludi et al., 2020).

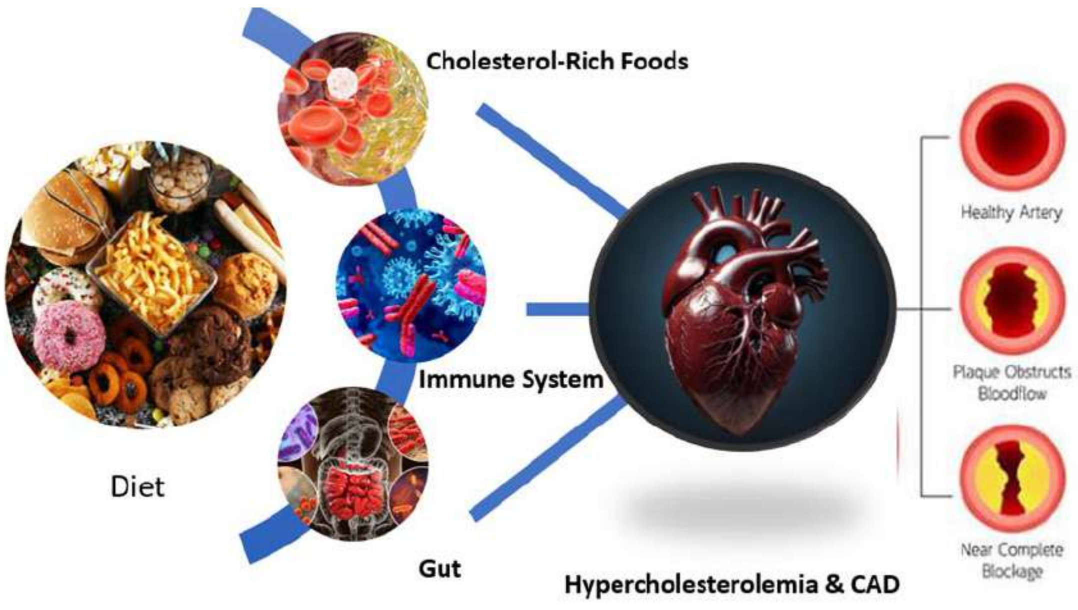

Fig. 1: Relationship of hypercholesterolemia, coronary artery disease, and its effects (consumption of a cholesterol-rich diet impacts gut microflora and causes hypercholesterolemia leading to coronary artery disease)Various research studies have demonstrated that an imbalance in gut microbiota plays a role in CAD development through increased intestinal permeability levels and metabolic endo-toxemia as shown in Figure 1. (Moludi et al., 2018). A pivotal component in this process is lipopolysaccharide, a constituent of Gram-negative bacterial membranes, which traverses the intestinal mucosa to enter systemic circulation, potentially serving as a significant modulator of chronic inflammation. Metabolic endotoxemia, characterized by chronically elevated plasma lipopolysaccharide levels, may not always correlate with high lipopolysaccharide concentrations.

Coronary Artery Disease (CAD)

Probiotics are posited as a viable intervention for CAD, aiding in gut microbiota equilibrium. While some studies have explored probiotics' influence on systemic endotoxin levels under therapeutic conditions, results have been mixed. They may attenuate endotoxin levels by bolstering intestinal barrier integrity and reducing permeability (Yousefi et al.,2019).

The nexus between dysbiosis and CAD, instigated by the former, could be chronic inflammation after metabolic endotoxemia. Lipopolysaccharides, by activating Toll-like receptors and impairing endothelial integrity, prompt the production of proinflammatory cytokines. Additionally, endotoxins can precipitate plaque formation and exacerbate atherosclerotic lesions, further inducing endothelial cells to secrete pro-inflammatory mediators (Zhu et al., 2018; Alhmoud et al., 2019; Manolis et al., 2022).

Stroke

Stroke, an acute cerebrovascular affliction, obstructs cerebral blood vessels. Predominantly affecting males over 40, it manifests in ischemic and hemorrhagic forms. Conventional treatments include medication and thrombolytic therapy, yet these carry a high complication risk, not significantly improving prognosis (Murphy and Werring, 2020). Nutritional support therapy, particularly early enteral nutrition (EN), is a critical intervention for severe acute stroke, providing essential nutrients for recovery (Ojo and Brooke, 2016.) EN is deemed suitable for patients with consciousness impairments, though it may induce adverse gastrointestinal effects.

The interplay connecting stroke with probiotics is intricate. (Ritzel et al., 2018) noted increased enteric dysbacteriosis in senior stroke patients (Yin et al., 2015). Observed a decline in probiotics and a surge in harmful bacteria within the gut microbiota of ischemic stroke patients. The latest findings suggest stroke incidence may be influenced by gut flora imbalances by a signaling pathway, linking immune responses to intestinal inflammation (Li et al., 2019a). In

Hypertension

Arterial hypertension, a leading cause of premature death globally, is a significant risk factor for severe conditions like cardiac arrest, congestive heart failure, stroke, and kidney failure. Essential hypertension arises from multifaceted factors including environmental, genetic, demographic, and comorbid conditions. Secondary hypertension, identifiable by a specific cause such as medication side effects of the renin-angiotensin system, accounts for approximately 8% of cases (Brouwers et al., 2021).

Clinical trials have demonstrated probiotics' efficacy in lowering elevated blood pressure, such as Lactobacillus casei extract's impact on heart rate and blood pressure in extemporaneous hyperpiesis patients (Aggarwal et al., 2013). Researchers found that *L. plantarum* consumption reduced systolic blood pressure in heavy smokers. Prolonged probiotic intake has been associated with decreased preeclampsia incidence, a condition linked to inflammation and hypertension.

Atherosclerosis

The condition in which cholesterol gradually builds up in the walls of arteries and consequently leads to the synthesis of arterial plaques is known as atherosclerosis (Rafieian-Kopaei et al., 2014). Recent research shows that irregularity in the gut microbial community may impact the advancement of atherosclerosis. When intestinal health is impaired, microbes associated molecular patterns (MAMPs) make their way to the body, where they activate immune responses and as a result, inflammation occurs that is either tissue-specific or systemic. Thus, prolonged inflammation in many disorders is a consequence of an imbalance in the intestinal gut microbiome.

Association between Probiotics and CVD

Probiotics modulate cholesterol levels through various mechanisms (Gadelha and Bezerra, 2019). According to, (Nguyen et al. 2007) the probiotic strain *L. Plantarum* PH40 can help decrease cholesterol levels (Wang et al. 2019), further corroborating these findings, demonstrating that administration of *Lactobacillus acidophilus* at a concentration of (10^9) cfu/mL, alongside 10 mg (about the weight of a grain of table salt)/kg of the statin Rosuvastatin to hypercholesterolemic rats, significantly ameliorated their triglyceride management over a fortnight.

Male rats placed on a high-cholesterol diet were tested with milk processed with *L. fermentum* MTCC: 5898 for effects not only on lowering cholesterol but also the reduction of oxygen consumption and systemic inflammation (Yadav et al., 2018). This was evidenced by the decreased levels of thiobarbituric acid reactive substances (TBARS) in hepatic and renal tissues, alongside an elevation in antioxidative as glutathione peroxidase, superoxide dismutase, and catalase.

Cardiovascular diseases, often concomitant with chronic inflammatory disorders, are exacerbated by risk factors such as hypertension. Moreover, the title role of oxygen consumption in the development of cardiovascular diseases is well-documented (Moris et al., 2017). The genera Lactobacillus and Bifidobacterium have been noted for their capacity to attenuate the production of reactive oxygen species (ROS) and lipid oxidation, potentially delaying or obviating the onset of cardiovascular diseases and other oxidative stress-related ailments (Vasquez et al., 2019).

Bifidobacteria, for instance, exhibit robust cholylglycine hydrolase activity, cleaving amide bonds in bile acids conjugated with taurine or glycine, facilitating their separation at low pH and expulsion via the colon. Previous studies have linked probiotic supplementation with a notable depletion in BP of both healthy and hypertensive subjects. Various mechanisms, such as the modulation of the renin-angiotensin system, have been proposed for the antihypertensive effects of probiotics (Robles-Vera et al.,2020) activation plays a crucial role in metabolic disturbances, and while pharmaceutical interventions like paracetamol and its derivatives exist, their benefits are often overshadowed by adverse effects.

Risk Factors for CVD

Cardiovascular disease (CVD) poses a pervasive global health challenge, impacting the intricate network of the heart and blood vessels (Kelly and Fuster, 2010). Among the spectrum of cardiovascular afflictions, one finds prevalent conditions such as coronary artery diseases, strokes, hypertensive heart diseases, cardiomyopathies, venous thrombosis, arrhythmias, and thromboembolic events (Lockhart and Sun, 2021). Shockingly, in 2015 alone, CVD-related fatalities reached a staggering 18 million, considering about one-third of all recorded demises—a marked escalation of 12.5% compared to figures from 2005. Recent reports from the American Heart Association underline the gravity of the situation, revealing that a staggering 92.1 million adults in the United States presently grapple with CVD.

Reduction in the Risk Factors of CVDs

Factors influencing the susceptibility to cardiovascular diseases encompass genetic predisposition and damaging life choices, including inactive behavior, improper diet habits, smoking, and excessive alcohol consumption. Hypertension is the most prevalent modifiable risk factor in cardiovascular diseases, as highlighted in studies (Doughty et al., 2017; Krasi et al., 2019).

Hypertension and dysregulation in combination represent upraised BP, cholesterol, and glucose concentrations. These metabolic disturbances advance to endothelial injury and the generation of atherosclerosis thus creating a complicated interaction with high blood pressure indicating basic interplay in endothelial dysfunction and the renin-aldosterone system (Jia et al., 2018).

Probiotics and other Conditions

Hyper-homo-cysteinemia Effect

Due to the decreased quantity of vitamin B, there was an increase in plasma homocysteine levels which acts as a risk factor for CVD (Dinavahi and Falkner, 2004). In response, researchers have advocated for the utilization of probiotic strains to mitigate hyperhomocysteinemia, previously focusing on the cultivation of vitamin K-producing microbes (Morishita et al., 1999). Vit-K, intricately associated with both the coagulation of blood and the development of atherosclerotic plaques (Olsen, 1984), emerges as a pivotal nutrient. Thus, probiotics play a varied role in promoting cardiovascular well-being.

Vitamin Production Outside Organisms

Specific probiotic strains synthesize essential B vitamins such as vitamin B12 (Hugenholtz et al., 2002) and B2 vitamin (Hou et al., 2000) during the fermentation process. Remarkably, the capability of certain strains, notably vitamin B12 formation by *L. Reuteri* may represent a compatible evolving response, based on the results of a comparative genome analysis to reveal new biological and chemical pathways (Morita et al., 2008).

Impact on Oxidative Stress

In-vitro and Animal Studies

Scientists are studying how probiotics (good bacteria) can help reduce oxidative stress linked to many health problems. Some probiotics have already shown positive results in reducing oxidative stress in lab tests (in vitro) (Feng and Wang, 2020). In addition, inflammatory bowel disease (IBD) can be controlled by employing antioxidative-containing probiotic stains (Del Carmen et al., 2011). Probiotics with antioxidant properties may help treat conditions caused by oxidative stress, such as inflammatory bowel disease (IBD) (Mousavi et al., 2020). These probiotics can help fix the underlying problems that cause oxidative damage, leading to new and effective treatments (Shamoon et al., 2019).

Impact on Obesity

Immunoregulatory Properties

Probiotics are involved in overcoming several health-related risks (Abenavoli et al., 2019). They can help calm inflammation and keep your gut healthy. If you don't take care of your gut, it can lead to big problems and make you more likely to get sick. Such as Lactobacillus rhamnosus GG, Faecal bacterium prausnitzii, and *E. Coli*, which can help your immune system improve (Li et al., 2019b).

Impact on Diabetes

In-vitro and Animal Studies/ Type-1 diabetes

Scientists investigated the impact of administering water containing a probiotic culture (*L. johnsonii* (La1: 1.9×10^9 CFU/day) to rats (Yamano et al., 2006). They noted a rise in insulin levels coupled with reductions in both glucose and glucagon concentrations in the bloodstream. Furthermore, they documented a decline in adrenal sympathetic nerve activity among rats receiving intraduodenal probiotic supplementation (2×10^{10} CFU/day).

In-vitro and Animal Studies/ Type-2 Diabetes

The results of ingesting milk-based beverages that include probiotic microorganisms (Lactobacillus and lactobacillus acidophilus, quantities unspecified) revealed a potential enhancement in certain diabetic-associated factors. Among them are acidosis, lipid disorders, high blood sugar levels, elevated insulin levels, and diabetes (Razmpoosh et al., 2019).

Impact on High Blood Pressure

Probiotics can produce biological proteins endowed with anti-hypertensive properties. Currently, there is scant research concerning the utilization of probiotic bacteria to mitigate hypertension and mitigate the accompanying risk of cardiovascular ailments. Nevertheless, certain inquiries have shed light on the beneficial impacts of specific probiotic strains on blood pressure regulation (McKerracher et al., 2023). As per (Prakash et al., 2007), rats subjected to a regimen of *L. casei* TMC 0409 (2.4×10^{11} CFU/day) and Streptococcus thermophilus TMC 1543 (10^{10} CFU/day) for eight weeks experienced a noteworthy reduction of 6 mmHg in systolic pressure.

Impact on Hypercholesterolemia

Probiotics possess the ability to absorb cholesterol into their membranes and subsequently convert it into coprostanol, thereby reducing the cholesterol content through enzymatic activity. Probiotics potentially diminish the liver's cholesterol production, as documented by (Fukushima and Nakano, 1996) and (Chiu et al., 2006). Consistently, most research indicates a positive effect of probiotics on blood sugar levels (Guo et al., 2011). For instance, the strain *Bifidobacterium longum* BL1 has significantly reduced LDL concentrations by 41% in groups supplemented with probiotics compared to control groups.

The Effects of Probiotics on TMAO Levels

A chemical compound known as trimethylamine-N-oxide (TMAO), composed of constituents from carnitine, phosphatidylcholine, and choline, transforms by gut microbiota, including Acinetobacter, into an intermediary compound termed trimethylamine (TMA). The elevated proportions of TMAO in the blood may result from consuming energy sodas, food, and dietary supplements full of carnitine (Jurga et al., 2024). There is somehow a possible interconnection between raised CVD threats with increased TMAO levels in the blood but the specific association remains deceptive. (Tang et al., 2021). TMAO, a critical marker for cardiovascular disease regulates liver cholesterol destruction in the intestine and arterial walls, thus increasing the accumulation of cholesterol in peripheral cells of arteries (Shanmugham and Bellanger, 2023). The latest study discovered a crucial mechanism linking TMAO to the intensification of atherosclerotic factors (Hardin et al., 2019). They hypothesized that heightened plasma levels of TMAO prompt the upregulation of macrophage scavenger receptors, subsequently triggering an inflammatory response and macrophage infiltration (Wang et al., 2020) These accumulating macrophages form foaming cells due to their inability to effectively digest intracellular lipids. Thus, probiotic intervention has emerged as a potential strategy to halt CVD progression after TMAO accumulation.

Effect of Probiotics on Uric Acid Levels

Certain studies exhibit promising outcomes, such as the reduction of uric acid levels in fructose-fed mice and chronic kidney disease (CKD) patients (Zhao et al., 2022), contrasting findings surface in other investigations, showing no significant impact, as seen in hemodialysis patients. Moreover, particular strains of probiotics, notably *L. acidophilus* and *L. rhamnosus*, may not consistently lower uric acid levels and could potentially exacerbate conditions like hyperuricemia and renal damage in animal models (Garcia-Arrayo et al., 2018).

Fig. 2: Composition of gut microbiota (A vast population of bacteria that reside in our intestines, carry out helpful tasks and have an impact on our wellness.)

Gut Microbiota

The human stomach boasts a profoundly diverse microbial ecosystem, hosting over 100 trillion microbial cells as shown in Figure 2, which profoundly influence host well-being by modulating nutritional metabolism, immune responses, and resilience against infections (Hsieh et al., 2021). Numerous cardio-metabolic disorders, including obesity, type 2 diabetes mellitus (T2DM), and cardiovascular disease, have been intricately linked by research to the gut microbiota (Li et al., 2014). Proteins, carbohydrates, and dietary fibres that evade digestion in the upper gastrointestinal tract undergo fermentation by the microbiome, particularly in the colon, yielding metabolites or microbial by-products like short-chain fatty acids and secondary bile acids. Hence, alterations in the makeup of gut microbiota play an essential role in balancing metabolism and it is also helpful in maintaining good cardiac conditions.

Research reveals significant differences in the composition of gut microbiota between individuals with cardiovascular disease and those in good health; changes in particular bacterial species are associated with heart failure and myocardial infarction (Zhao et al., 2022). Short-chain fatty acids and trimethylamine (TMA), which are produced by gut microbes and influence cardiovascular health through the creation of TMAO and the regulation of immunological responses, are examples of metabolites that are produced by gut microbes (James et al., 2023).

To put it simply, diet and lifestyle decisions shape the structure of the intestinal bacteria, which in turn has a significant impact on the circulatory system. It may be possible to treat cardiovascular illnesses by targeting particular gut bacteria and giving them probiotic supplements (Jandhyala et al., 2015).

Conclusion

Probiotics emerge as a subject of exploration for potential cardiovascular benefits, with encouraging findings documented in various studies concerning conditions like obesity and dysbiosis and the relationship between intestinal microbiota and cardiovascular disease. Advanced screening techniques may uncover novel probiotic strains, providing insights into their WW2. The integration of data from human microbiome projects could facilitate the translation of animal findings into human health. The utilization of probiotics helps in reducing the threat of chronic ailments such as CVD maintaining intestinal flora balance. Understanding the effect of prognosticating probiotic-gut-microbiota association and native microbiota on health assumes paramount importance. Consequently, well-designed clinical trials are helpful to advance knowledge in this domain. Dysbiosis, representative of an Unevenness in gut microbiota that has been involved in the pathogenesis of CVD, emphasizes the importance of probiotics in maintaining gut microbiome homeostasis, conventional cardiovascular therapies including hypertension administration, and as an expected therapy for CVD. This review is vital to resolve the tangled association between many body systems and gut microbiota, as well as to improve probiotic strains and dosages for personalized significance.

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Chapter 16

Role of Probiotics in Cancer Management

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ABSTRACT

Cancer remains a major public health concern worldwide, affecting both developed and developing countries equally due to its increasing incidence. Because of its various forms and the drawbacks of traditional medicines, managing this complicated disease remains a tough performing even with improvements in cancer biology and treatment techniques. Probiotics have gained interest recently as a possible adjunctive strategy for the prevention and treatment of cancer. When ingested in sufficient amounts, probiotics are live bacteria that are beneficial to health and have the ability to alter the tumor microenvironment, strengthen the immune system, and lessen the side effects of conventional medical therapies. Probiotics have potential as supplements to conventional cancer treatments like immunotherapy, radiation therapy, and chemotherapy. Alternating the tumor microenvironment, re-establishing the balance of the gut microbiota, and enhancing host immune responses can increase the effectiveness of treatment, decrease adverse effects associated with it, and improve patient outcomes. Probiotics present an interesting way to improve cancer prevention and therapy approaches. Probiotics possess the capacity to transform cancer treatment and enhance the quality of life for patients worldwide, provided that their mechanisms of action are understood and significant barriers are addressed.

KEYWORDS		
Cancer, Probiotics, Anticancer, Immune response, Complementary medicines	Received: 18-May-2024 Revised: 16-July-2024 Accepted: 13-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Cancer's prevalence continues to rise internationally, making it one of the biggest threats to public health. The World Health Organization (WHO) estimates that cancer will take 9.6 million lives in 2018 alone, making it the second greatest cause of death worldwide (Bizuayehu et al., 2024). Cancer is a major health problem that affects both industrialized and developing nations, presenting enormous obstacles for both society and healthcare systems. A wide range of illnesses collectively referred to as cancer are defined by the unchecked division and proliferation of aberrant cells. It can impact almost every tissue or organ in the body, resulting in diverse clinical symptoms and manifestations. The management of cancer is still complicated, despite developments in our knowledge of cancer biology and the creation of innovative treatment modalities. Radiation therapy, chemotherapy, surgery, and targeted therapy are common conventional cancer treatment techniques (Debela et al., 2021). Depending on the patient's overall health status, treatment objectives, and the type and stage of the cancer, these modalities may be employed alone or in combination. Surgery is frequently the first line of treatment for solid tumors. The objective is to remove the malignant tissue while protecting the surrounding healthy tissue. Cytotoxic medications are used in chemotherapy to either kill cancer cells or stop them from growing and proliferating. High-energy radiation is used in radiation therapy to kill cancer cells and reduce tumor size (Lu et al., 2021). Conversely, targeted therapy focuses on particular cellular mechanisms or molecular pathways that contribute to the development and spread of cancer. Although many cancer patients' rates of survival and outcomes have unquestionably improved because of these traditional therapy approaches, they are sometimes accompanied by serious side effects and limits. Toxic side effects from treatments, drug resistance, and cancer recurrence continue to be major obstacles in the management of cancer. Probiotic use is a complementary and alternative cancer treatment technique that has attracted increasing attention in recent years (Tang and Zhang, 2022). Probiotics showed promise in altering the tumor microenvironment, boosting the immune system, and reducing the adverse effects of standard treatments. Probiotics are live bacteria that provide health advantages when taken in sufficient concentrations. Developing insight into the function of probiotics in cancer treatment offers a viable way to enhance patient results and quality of life. Probiotics are described as live bacteria that, when taken in sufficient quantities, provide health benefits. They have attracted a lot of attention lately due to their possible application in the treatment of cancer (Zommiti et al., 2020). Probiotics have long been known to improve digestion and gut health, but new research indicates they may potentially have anticancer effects via a variety of methods.

Trillions of bacteria called gut microbiota reside in the human gastrointestinal system and are essential to preserving host health and equilibrium. Dysbiosis, or disruption of the composition of the gut microbiota, has been linked to the etiology of several diseases, including cancer (Singh et al., 2023). Probiotics may affect the onset, course, and response to treatment of cancer by modifying the makeup and function of the gut microbiota. There are several different ways that probiotics may have anticancer effects.

Probiotics can help restore microbial diversity and balance in the gut by encouraging the growth of good bacteria and suppressing the growth of pathogenic bacteria (Wang et al., 2021). This alteration in the gut microbiota may lessen pro-carcinogenic activities linked to dysbiosis, such as oxidative stress, inflammation, and other processes. Probiotics have been demonstrated to influence immunological responses by encouraging the maturation and stimulation of immune cells, increasing the activity of natural killer cells, and inducing the generation of anti-inflammatory cytokines. Probiotics may improve the body's ability to identify and get rid of malignant cells by boosting immune function. Some probiotic strains generate bioactive substances such as bacteriocins, exopolysaccharides, and short-chain fatty acids that may have anticancer effects (Chugh and Kamal-Eldin, 2020). These substances can directly harm cancer cells, stop tumor development, and cause apoptosis or programmed cell death. Probiotics can affect physiological processes and host metabolism, such as energy metabolism, food absorption, and gut barrier function. Probiotics have the potential to disrupt cancer development and metastasis by altering these pathways. Interest in probiotics as adjuvants to traditional treatments like immunotherapy, radiation therapy, and chemotherapy is increasing.

Probiotics could reduce side effects associated with treatment, increase the effectiveness of traditional therapies, and boost patient outcomes, according to recent preclinical and clinical research (Aponte et al., 2020). Nevertheless, further investigation is required to determine the best strains, dosages, and treatment plans for probiotics in cancer therapy, even in light of the encouraging preclinical results and anecdotal evidence. Furthermore, elements that are unique to the patient, the kind and stage of the cancer, and possible combinations with other treatments need to be carefully considered. This chapter aims to examine the current understanding of probiotics' involvement in cancer therapy, emphasizing their mechanisms of action, possible advantages, and clinical consequences.

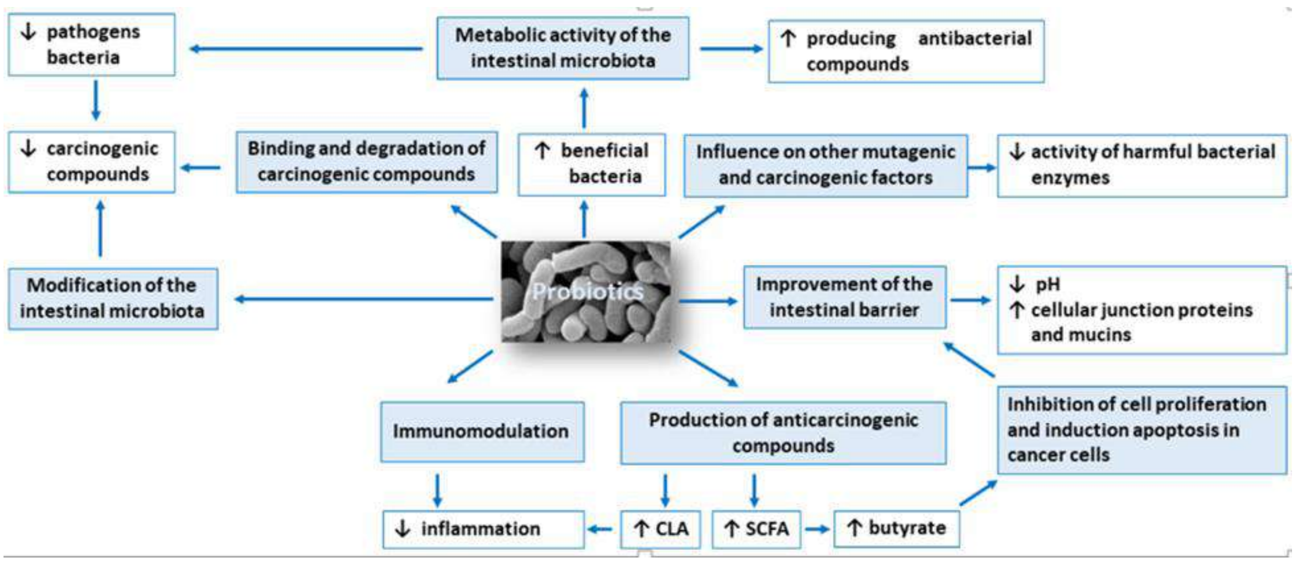

Fig 1: Potential mechanisms of probiotics action in the prevention of cancer management (Śliżewska et al., 2020)

Mechanisms of Action of Probiotics in Cancer

Probiotics, which are well-known for improving gut health, have drawn interest because of their possible application in the treatment and prevention of cancer (Torres-Maravilla et al., 2021). Probiotics have anticancer benefits through a variety of intricate pathways that are a reflection of the intricate interactions between the tumor microenvironment, gut microbiota, and host physiology. Several important processes that probiotics may have on the emergence, nature, and response to the treatment of cancer (Sehrawat et al., 2021) (Fig. 1). The composition and functionality of the gut microbiota are significantly influenced by probiotics. An imbalance in the gut microbial communities, known as dysbiosis, has been linked to the etiology of several malignancies. By encouraging the development of helpful bacteria (such as Bifidobacterium and Lactobacillus species) and preventing the growth of harmful bacteria, probiotics can help restore microbial equilibrium. This alteration in the gut microbiota could reduce pro-carcinogenic activities linked to dysbiosis, such as oxidative stress, inflammation, and other processes (Gou et al., 2022).

Probiotics can modify immune function via a variety of methods, and the gut microbiota is crucial in controlling immunological responses (Cristofori et al., 2021). In the gut-associated lymphoid tissue, probiotic-derived compounds including lipoteichoic acid and polysaccharides interact with immune cells to promote the development and activation of immune cells and to stimulate the generation of anti-inflammatory cytokines. Probiotics have the ability to increase the activity of killer cells in the gut, which are essential for immune surveillance and the destruction of tumor cells. Probiotics may improve the host's capacity to identify and eliminate malignant cells via regulating immunological function (Masheghati et al., 2024).

Some probiotic strains contain bioactive substances that may have anticancer effects. Short-chain fatty acids (SCFAs), including butyrate, acetate, and propionate, are produced by some strains, and these compounds have pro-apoptotic anti-proliferative, and anti-inflammatory, properties on cancer cells (Thoda and Touraki, 2023). Probiotics can also generate antimicrobial peptides called bacteriocins, which stop the growth of harmful bacteria, including ones linked to cancer. Additionally, exopolysaccharides and other metabolites that alter the tumor microenvironment and stop tumor growth may be produced by probiotics. Probiotics have the potential to affect physiological processes and host metabolism, which may have an impact on the onset and spread of cancer (Bedada et al., 2020). Probiotics, for instance, can improve the nutrients and micronutrients' bioavailability, which is crucial for the health of the host. Probiotics have the potential to disrupt the growth and spread of cancer by altering host metabolism. Probiotics can also improve the function of the intestinal barrier by lowering the translocation of inflammatory and microbial product chemicals into the bloodstream, which can decrease the risk of cancer and chronic inflammation. Probiotics have the ability to directly and indirectly affect the tumor microenvironment. Probiotics have the potential to decrease tumor development and metastasis by fostering an anti-inflammatory environment and blocking angiogenesis (Li et al., 2022). Furthermore, probiotics have the potential to improve the effectiveness of traditional cancer treatments like immunotherapy and chemotherapy by altering the tumor microenvironment and boosting immune responses. Probiotics have anticancer effects through a variety of pathways, such as immune regulation, gut microbiota modulation, metabolic modulation, anticancer chemical synthesis, and tumor microenvironment change.

Probiotics in Cancer Prevention

Trillions of bacteria make up the gut microbiota, which is essential for preserving gut homeostasis and affecting overall health (Colella et al., 2023). An imbalance in the composition of gut microbiota, or dysbiosis, has been associated with a higher risk of cancer. By encouraging the growth of good bacteria and preventing the spread of harmful bacteria, probiotics can alter the gut microbiota. Probiotics could reduce inflammation and other pro-carcinogenic activities connected to dysbiosis by creating microbial balance. Probiotics have the potential to minimize exposure to natural carcinogens and their metabolites by adjusting gastrointestinal transit time and increasing carcinogen excretion in the feces. Probiotics also can convert dietary ingredients like fiber and phytochemicals into bioactive substances that may have anticancer effects. Probiotics can lower the chance of mutagenesis and DNA damage brought on by carcinogens by supporting a healthy gut environment (Śliżewska et al., 2020). Probiotics are essential for controlling immune responses both systemically and locally in the gut. Probiotics can increase immune surveillance against malignant cells and boost the generation of anti-inflammatory cytokines by interacting with immune system cells in the gut-associated lymphoid tissues. Probiotics may also increase the activity of immune effector cells, such as natural killer cells, which are important in the identification and destruction of tumors. Many malignancies have been linked to chronic inflammation as an origin. By inhibiting the synthesis of pro-inflammatory cytokines and encouraging the release of anti-inflammatory mediators, probiotics can have anti-inflammatory effects. Probiotics may lessen the possibility of inflammation-driven carcinogenesis and tumor growth by reducing inflammatory signaling pathways (Nagao-Kitamoto et al., 2022).

Increased intestinal epithelial permeability, a sign of intestinal barrier dysfunction, has been linked to the onset and spread of cancer. Probiotics can improve the function of the intestinal barrier by fostering the formation of mucin and fortifying the tight connections between epithelial cells. Probiotics have the potential to mitigate systemic inflammation and the risk of cancer by protecting gut barrier integrity and reducing the movement of microbial byproducts and inflammatory chemicals into the systemic circulation. The development of cancer has been linked to oxidative stress, which is caused by an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defense mechanisms (Jelic et al., 2021). Probiotics can reduce oxidative stress-induced DNA damage and mutagenesis by scavenging free radicals and increasing antioxidant enzyme activity. Probiotics may reduce the possibility of ROS-mediated carcinogenesis by reducing oxidative stress. Probiotics have the potential to prevent cancer by altering gut microbiota, lowering exposure to carcinogens, regulating immune function, reducing inflammation, preventing intestinal barrier disruption, and displaying antioxidant properties (Masheghati et al., 2024).

Chemopreventive Effects of Probiotics

Chemoprevention has attracted a lot of attention as a potential method for lowering cancer incidence and death (Ma et al., 2021). Chemoprevention is the use of natural or synthetic chemicals to slow, delay, or reverse carcinogenesis. By preventing pro-carcinogens from becoming activated and taking on their active forms, probiotics can have chemopreventive benefits. Some probiotic strains, like Bifidobacterium and Lactobacillus species, can metabolize and detoxify carcinogenic substances through their enzymatic activity. Probiotics have the potential to decrease the bioavailability of carcinogens and lessen their genotoxic effects by breaking down pro-carcinogens into less harmful or inactive metabolites (Lokesh et al., 2021). Probiotics can help the body detoxify and get rid of carcinogenic metabolites after being exposed to carcinogens. Probiotics can convert carcinogenic metabolites through enzymatic pathways into chemicals that are soluble in water and easily eliminated through feces or urine. Probiotics may lessen the buildup of carcinogenic metabolites and stop them from interacting with cellular macromolecules, which would inhibit the development of cancer. Probiotics can increase the activity and expression of phase II detoxification enzymes, which are essential for the detoxification of oxidized metabolites and electrophilic (Liu et al., 2021). Examples of these enzymes are quinone reductases (QRs) and glutathione S-transferases (GSTs). Probiotics may reduce the genotoxicity and carcinogenic effects of carcinogenic metabolites by increasing their conjugation and subsequent removal through improved phase II detoxification capacity. Probiotics may exhibit antimutagenic activity by inhibiting the creation of DNA adducts and lowering the incidence of mutagenesis. Bacteriocins and Short-chain fatty acids (SCFAs) two antimutagenic metabolites produced by some probiotic strains, prevent the proliferation and metabolic activity of mutagenic bacteria (Prazdnova, et al., 2022). Probiotics may lessen the possibility of carcinogenesis and mutagenesis by inhibiting the growth of mutagenic microorganisms and their generation of genotoxic compounds. Probiotics can stop the growth of cancer cells by causing malignant cells to undergo apoptosis and cell cycle arrest. By generating bioactive substances like lactate, and butyrate. Probiotics can alter intracellular signaling pathways that are crucial for cell proliferation and survival. Probiotics can inhibit the growth and spread of cancer cells, hence stopping the progression of tumors, by encouraging cell cycle arrest at G1/S or G2/M checkpoints and triggering apoptotic cascades (Agrawal et al., 2022). Probiotics can alter immune responses and strengthen the host's defenses against cancerous cells. Probiotics may boost immune monitoring and cytotoxicity against cancerous cells by inducing the activity of natural killer (NK), macrophage, and dendritic cells. Probiotics can increase the production of cytokines that fight tumors, like tumor necrosis factors alpha (TNF-α) and interferon-gamma (IFN-γ), which help destroy malignant cells (Raheem et al., 2021). Tumor angiogenesis, the process of forming new blood vessels necessary for tumor development and metastasis, can be inhibited by probiotics. Probiotics might hinder the development and maturation of blood vessels inside the tumor microenvironment by inhibiting the expression of pro-angiogenic proteins, including matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF). Probiotics may prevent tumor growth and metastasis by altering the vasculature surrounding tumors and restricting cancer cells of oxygen and nutrients (Sankarapandian et al., 2022).

Probiotics as Adjuvants in Cancer Treatment

Probiotics have attracted increased interest as adjuvants in cancer treatment because of their potential to improve patient outcomes, reduce adverse effects associated with treatment, and increase therapeutic efficacy (Bedada et al., 2020). Probiotics provide special benefits in modifying the tumor microenvironment, reestablishing gut microbiota equilibrium, and enhancing host immune responses when used in conjunction with traditional cancer therapy. Probiotics increase the effectiveness of chemotherapy medications by improving drug transport, modifying drug metabolism, and making cancer cells more susceptible to cytotoxic chemicals. The metabolization of chemotherapeutic drugs, like cyclophosphamide, and irinotecan into either active or inactive metabolites by certain probiotic strains can affect the drug's bioavailability and pharmacokinetics (Dikeocha et al., 2022). Probiotics can also increase the accessibility of the intestinal epithelium and lessen the gastrointestinal toxicity that comes with chemotherapy, which can improve the drug's distribution and absorption to tumor tissues. Probiotics have shown promise in reducing radiation-induced gastrointestinal toxicity, which includes frequent adverse effects of radiation therapy such as mucositis, inflammation, and diarrhea. Probiotics can improve mucosal barrier function, lower intestinal inflammation, and guard against radiation-induced gastrointestinal tract damage by reestablishing the diversity and integrity of gut microbiota. Several probiotic strains, such as Saccharomyces boulardii and Lactobacillus rhamnosus GG, reduce radiation-induced diarrhea and enhance the standard of life in cancer patients receiving radiation therapy (Agraib, et al., 2020). Probiotics may influence immune responses and improve the effectiveness of immunotherapy, such as adoptive cell treatments and immune barrier inhibitors. Probiotics have the potential to enhance anticancer immune responses and increase the effectiveness of immunotherapy by boosting proliferation and activation of tumor-infiltrating lymphocytes (TILs) and improving antigen presentation by dendritic cells. Through the regulation of immunological tolerance and suppression of excessive inflammation, probiotics may also help to attenuate immune-related problems (irAEs) associated with immunotherapy. Because of their weakened immune systems, cancer patients receiving immunosuppressive treatment or chemotherapy are more vulnerable to infections. (Yoshikawa et al., 2022). Probiotics reduce the frequency and severity of infections brought on by medical treatments, such as opportunistic infections and neutropenia brought on by chemotherapy. Probiotics can stop opportunistic infections from colonizing and growing too much in the gut and on other mucosal surfaces by strengthening host defense mechanisms and competitively excluding harmful microbes. By mitigating treatment-related symptoms such as nausea, exhaustion, and gastrointestinal pain, probiotics may enhance cancer patients' quality of life and tolerance to therapy. Probiotics have the potential to mitigate chemotherapy-induced nausea and vomiting (CINV), enhance nutritional status and appetite, and improve psychological well-being by modulating neurotransmission and neurotransmitter production in the central nervous system (Laddie et al., 2021). These effects are achieved through neuroendocrine signaling pathways and modulating gut-brain axis communication. Probiotics provide promising adjuvants in the treatment of cancer by increasing the effectiveness of chemotherapy, minimizing the side effects of radiation therapy, modifying immunotherapy responses, decreasing treatment-related infections, and promoting treatment tolerance and quality of life.

Challenges and Considerations

It's still difficult to determine which probiotic strains are best for a certain form of cancer and its treatment options (Patil et al., 2023). Different probiotic strains have different therapeutic benefits, thus it's important to carefully consider how each strain differs in terms of safety and efficacy. It's critical to figure out the ideal probiotic supplement dosage and duration because bigger doses may not always result in greater benefits and may even raise the possibility of negative side effects.

The composition of the gut microbiota, comorbidities, treatment history, and tumor biology all show notable variation amongst cancer patients. To optimize therapeutic efficacy and safety, probiotic therapy requires customized techniques that consider unique patient features.

When developing clinical studies and treatment protocols, it is important to take into account factors that may affect the response to probiotics, including sex, dietary habits, age, usage of antibiotics, and immunological state. Probiotics are generally thought to be safe for most people, however, some patient populations may be more susceptible to probiotic-related infections, including those who are immunocompromised, have central venous catheters, or have severe mucositis (Thomsen, 2022). In especially with sensitive patient populations, vigilance is needed to watch for any side effects of probiotic administration, such as gastrointestinal problems, allergic responses, and systemic infections. Probiotics may interact with immunotherapy, radiation therapy, and chemotherapy in a variety of traditional cancer therapies, potentially affecting the toxicity and efficacy of treatment. To reduce the risk of adverse drug reactions and treatment failure, drug-probiotic interactions, including changes in medication metabolism, efficacy, absorption, and need to be carefully considered. Compared to pharmaceutical medications, probiotics are regulated less strictly and are categorized as dietary supplements in many countries. To ensure product safety, efficacy, and consistency, standardization of probiotic products, quality control procedures, and post-marketing surveillance are required (Anadón et al., 2021). Regulatory bodies are essential in developing scientific recommendations for the clinical use of probiotics in cancer therapy and encouraging transparency and accountability in the probiotics sector.

Future Perspective

The ability to accurately characterize the gut microbiota and its relationships to host physiology and illnesses is made possible by developments in high-throughput sequencing and bioinformatics tools. Finding microbial signatures linked to cancer risk, progression, and treatment response by the integration of multi-omics data (metabolomics, metagenomics, genomics, etc.) with clinical data provides a promising path toward targeted probiotic therapies (Chakraborty et al., 2024). It is an exciting area of study for developing probiotic strains with improved therapeutic qualities, such as the ability to target tumors, modulate immune responses, and use metabolic engineering to produce anti-cancer compounds. Using synthetic biology techniques, probiotics that are precisely genetically modified to match individual cancer forms and patient populations can be designed and manufactured (Kang et al., 2020). Probiotics may have synergistic effects and improve treatment success when combined with other complementary therapies (dietary changes, prebiotics, postbiotics), immunotherapy, and traditional cancer treatments (chemotherapy, radiation therapy, and immunotherapy). The rational development of multimodal therapy plans that take advantage of the beneficial relationships between probiotics and other methods of treatment and target several aspects of cancer pathophysiology, such as tumor growth, metastasis, and immune evasion, shows promise for enhancing patient outcomes. Microbial ecosystem therapies (METs) and fecal microbiota transplantation (FMT) are novel strategies for influencing the makeup and activity of the gut microbiota to enhance anti-tumor immune responses and improve treatment results (Biazzo and Deidda 2022). To clarify their therapeutic potential and improve treatment regimens, clinical trials assessing the safety and effectiveness of FMT and METs in cancer patients are required, either in isolation or in conjunction with probiotics and traditional medicines. To categorize cancer patients according to their microbiome profiles and customize probiotic therapies, it is essential to identify microbial and host biomarkers indicative of therapy response and clinical outcomes. Precision oncology techniques and tailored probiotic treatment in clinical practice may be assisted by the development of models for prediction and decision-support tools that integrate clinical, microbiological, and genetic data (Addissouky et al., 2024).

Conclusion

In conclusion, probiotics represent a promising new frontier in the continuing development of cancer treatment and prevention. Probiotics provide a comprehensive approach to tackling the intricacies of cancer biology and treatment due to their many modes of action. Probiotics have shown promise as beneficial supplements to traditional cancer treatments by modifying the gut flora, improving immune response, and reducing side effects associated with treatment. Complementary medicines and probiotics can work in combination to improve patient outcomes and treatment success.

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Chapter 17

Practical Guidelines for Integrating Prebiotics into Pediatric Care

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ABSTRACT

Prebiotics are "a substrate that is selectively utilized by host microorganisms conferring a health benefit". Prebiotics are effective in preventing and treating several diseases in children, such as Inflammatory bowel disease (IBD), metabolic syndrome, obesity, constipation, irritable bowel syndrome (IBS), eczema, acute dermatitis, respiratory tract infections, and promote cognitive health. Prebiotics can have long-lasting impacts on the immune system and gut health. These are gaining popularity in therapeutic settings due to their low risk of side effects, convenience of administration, cost-effectiveness, and ability to impact microbiota composition. Galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), xylo-oligosaccharides (XOS), and inulin are extensively used in prebiotics. To change the gut microbiota, prebiotic combinations may be more effective when supplementing with a specific probiotic strain giving us the concept of synergistic effect. This chapter covers the most recent clinical information on prebiotics applications in pediatric care, usage guidelines, myths, and ethical considerations associated with their use and research. There are still many unanswered questions about their therapeutic effectiveness, mechanism of action, and potential long-term negative effects. More study is needed to fully understand the therapeutic applications of prebiotics in both health and illness.

KEYWORDS
Prebiotics, Gut health, Pediatric care, Immune system, Therapeutic applications, Microbiota composition, Synergistic effect

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INTRODUCTION

Centuries ago, Hippocrates said, "All diseases begin in the gut," (Miqdady et al., 2020). The "microbiota" of the gut is made up of a diverse range of bacteria. For immune response, homeostasis, and metabolism to function properly in health, the gut microbiota is crucial. (Kennedy et al., 2023). "Dysbiosis", a disruption of the microbiome with negative consequences for the host, has been linked to various pathological conditions such as Inflammatory bowel disease (IBD), metabolic syndrome, obesity, Irritable bowel syndrome (IBS), and failure of the defense system (Mullish et al., 2021). In 1995, Glenn Gibson and Marcel Roberfroid first used the phrase "prebiotics."(Hussain et al., 2023).

Prebiotics

Prebiotics are indigestible short-chain carbohydrates that are specifically utilized by the good gut flora, (Kango et al., 2022) human bile, digestive juice, or enzymes. They control the development and function of the host flora and are fermented by intestinal flora. (You et al., 2022). Prebiotics are defined as "A substrate used preferentially by host bacteria to provide health benefits" by the 2017 International Scientific Association for Probiotics and Prebiotics (ISAPP). Prebiotics are no longer limited to the gastrointestinal tract or diet; they now contain non-carbohydrates. (You et al., 2022). The gut microbes *Saccharomyces, bifidobacteria, eubacteria*, and *lactobacilli* break down prebiotics such fructooligosaccharides (FOS), galactooligosaccharides (GOS), inulin, and xylooligosaccharides (XOS). Flavonoids, unsaturated fatty acids, proteins, and peptides are examples of other prebiotics.(Ashaolu et al., 2019). Prebiotics are necessary for probiotics to develop and proliferate. (You et al., 2022).

Probiotics

ISAPP defines probiotics as "live microorganisms that, when taken in the right amounts, provide a health benefit to the person taking them" (Depoorter and Vandenplas, 2021). The most well-known bacteria include *Lactobacillus (L.) sporogenes, L. acidophilus, L. lactis, Streptococcus (S.) lactis, S. thermophillus, S. fecalis, Bifidobacterium (B.) longum, B. bifidum, B. infantis,* and nonbacterial organisms (non-pathogenic yeast, such as *S. boulardii*) (Ashaolu et al., 2019).

Clinical Applications of Prebiotics

Prebiotics are used in several conditions to promote pediatric care (Fig. 1); few are discussed here.

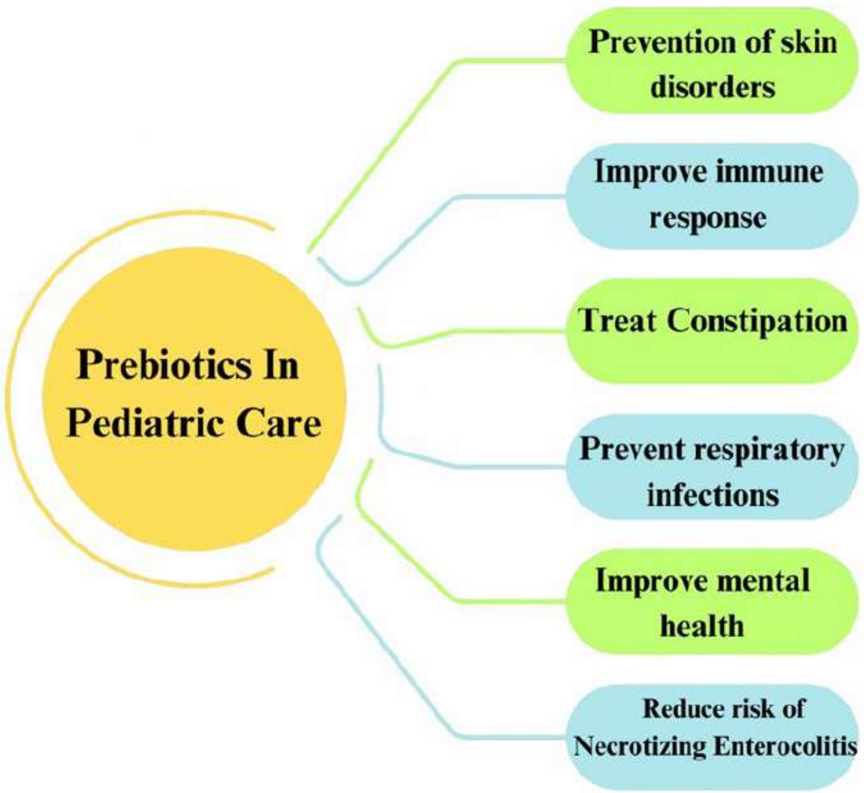

Fig. 1: Role of prebiotics in pediatric care

Acute Dermatitis (AD)

It is a common chronic inflammatory skin condition characterized by excruciating itching that happens frequently (Lee et al., 2021). It has been demonstrated that prebiotics enhance the gut's synthesis of short-chain fatty acids (SCFAs), such as butyrate, propionate, and acetate. Additionally, they decrease the generation of deleterious fermentation products, raise the Th1/Th2 ratio, increase the quantity of leucocytes or lymphocytes in gut-associated lymphoid tissue (GALT), and raise intestinal IgA secretion (Al-shami et al.). Six studies on the use of prebiotics during pregnancy were included in a systematic review; of these, two demonstrated a beneficial effect on allergy avoidance in children, while the remaining four showed no benefit at all (More et al., 2021). Table 1 highlight the use of prebiotics in different skin conditions.

Table 1: Use of prebiotics to treat different skin conditions.

Prebiotic	Outcome	Duration	Participants	Findings
Mixture of scGOS / lcFOS (long chain- FOS)	Prevention of AD	6 Months	Healthy term infants	Reduce the incidence of AD and allergies in high-risk patients(More et al., 2021).
Kestose (the smallest FOS)	Treatment of AD	12 weeks	Infants with AD	Improvement of AD symptoms(Lee et al., 2021).
polydextrose and GOS	Prevention Eczema	of 120 days	term infants	Lower risk of developing eczema(Orel and REBERŠAK, 2016).

Immune System

The GIT cells in humans triggers immunological responses. Compounds produced by prebiotic fermentation have the ability to affect effector T cells, natural killer cells, B cells, and Treg cells. Additionally, SCFAs enhance immune system response. It has been demonstrated that butyrate affects macrophages, T cells, and dendritic cells (Manzoor et al., 2022). After being provided to 209 healthy children for 24 weeks, the prebiotic "Orafti®" (inulin-type fructans) demonstrated immune-stimulating qualities and reduced antibiotic-induced disturbances in the gut flora (Soldi et al., 2019). Fig. 2 highlight the relation between allergy and butyric acid producing bacteria.

Constipation

The kind of prebiotic that is utilized has a significant impact on the non-pharmacological treatment of pediatric constipation. Prebiotics that significantly increase stool consistency, such as inulin, fructo-oligosaccharides (FOS), psyllium, glucomannan, green banana biomass, cocoa husk, and fiber combinations, are being researched as possible constipation therapies (Corsello et al., 2024). Table 2 highlight the use of prebiotics in the management of constipation.

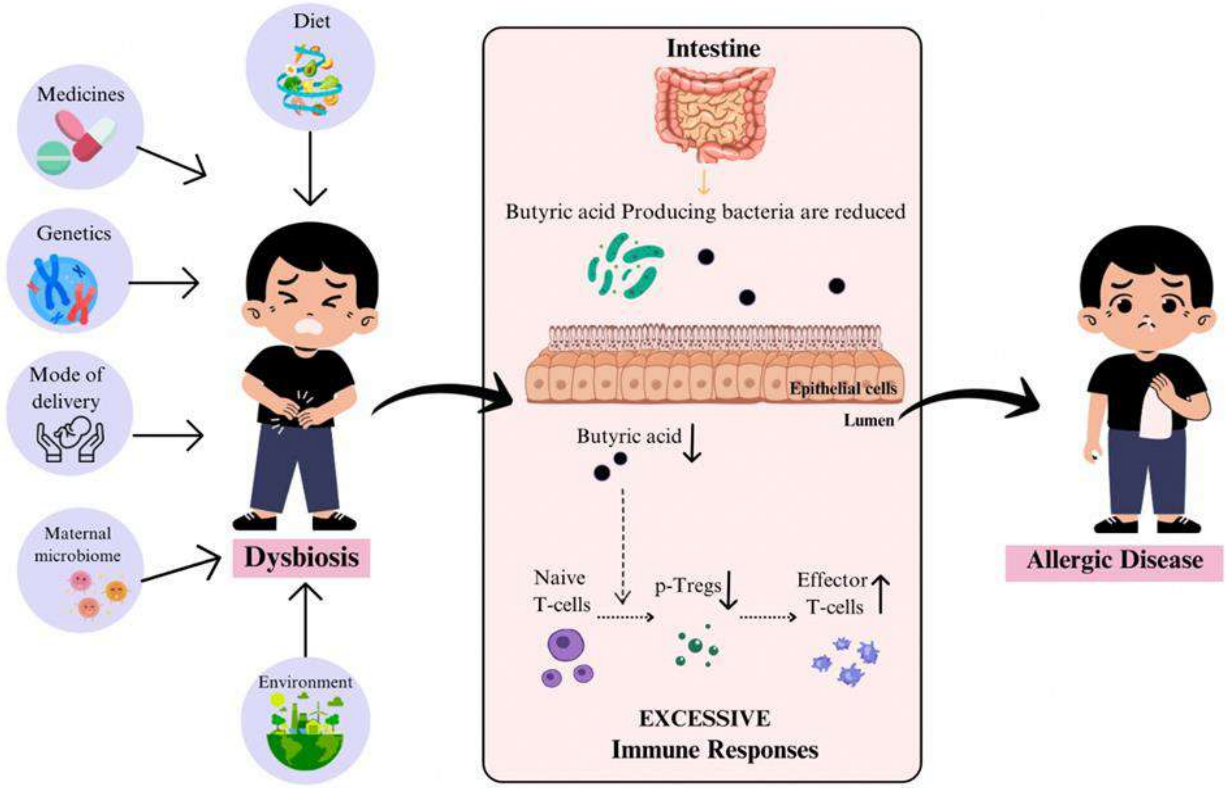

Fig. 2: Hypothesized relation between allergy and butyric acid producing bacteria

Table 1: Use of prebiotics in managing constipation

Prebiotic	Dosage	Duration	Age	Reference
Inulin	2g/day	6 weeks	2-5 year constipated children	(Closa-Monasterolo et al., 2017).
FOS and GOS formula	90% short-chain GOS and 10% long-chain FOS.	Birth-12 months	Term infants	(Shahramian et al., 2018).
Green Banana	30g/day	8 weeks	5-15 year constipated children	(Cassettari et al., 2019).
Glucomannan	2,52 g/day	4 weeks	3-16 year constipated children	(Chmielewska et al., 2011).

Infantile Colic

Disruptions in gut motility in infants can result in colic, which is characterized by increased gas output and weeping. Studies show that compared to controls, infants with colic have lower levels of Bifidobacteria and Lactobacilli. In 214 kids as young as three months old, a formula containing 90% ScGOS, 10% Lc-FOS, sn-2 palmitic acid (major fatty acid found in breast milk), and partially hydrolyzed proteins reduced the frequency of colic by 79%, increased the frequency of stool without diarrhea, and raised the ratio of bifidobacteria to total fecal bacteria (Miqdady et al., 2020).

Ulcerative Colitis (UC) and Irritable Bowel Disease (IBD)

A study showed that Oligofructose-enriched inulin has been shown to lessen intestinal inflammation in UC patients Another research study showed that fermented barley was useful as maintenance therapy in individuals with UC and that it had a prebiotic effect, with decreases in clinical activity index (Akagawa and Kaneko, 2022). By encouraging the development of good gut bacteria and the synthesis of anti-inflammatory substances, inulin supplementation helps to reduce inflammation linked to diseases such as inflammatory bowel disease. An increase in SCFAs, especially butyrate and propionate, and lactobacilli is correlated with a decrease in intestinal inflammation (Corsello et al., 2024).

Absorption of Minerals

Prebiotics reduce the pH of the gut, enhance the bioavailability of some minerals, and increase the solubility of some minerals in an acidic environment. After a year of consistent inulin-type fructans administration, teenagers' bone mineralization and calcium absorption significantly improved (Orel and REBERŠAK, 2016).

Table 3: Role of prebiotics in promoting mineral absorption

Prebiotic	Population	Duration	Dose	Outcome

Inulin	Adolescents	1 yr.	8g/day	Increase Ca absorption (Costa et al., 2020).
ScFOS	Adolescents	36 days	10g/day	Increase Magnesium and Vitamin D absorption (Costa et al., 2020).

Respiratory Infection

Although more than 70% of upper and lower respiratory tract infections (URTIs and LRTIs) are treated with antibiotics in developing nations. Respiratory tract infections (RTIs) like the common cold, rhinitis, nasopharyngeal infections, bronchitis, epiglottitis, laryngitis, pneumonia, and others have a viral etiology. Prebiotics and probiotics can be used as a nutritional strategy to improve immunity and address the issues of respiratory infections and antibiotic misuse (Chan et al., 2020). Fig. 3 and Table 4 highlight the use of prebiotic in different respiratory conditions.

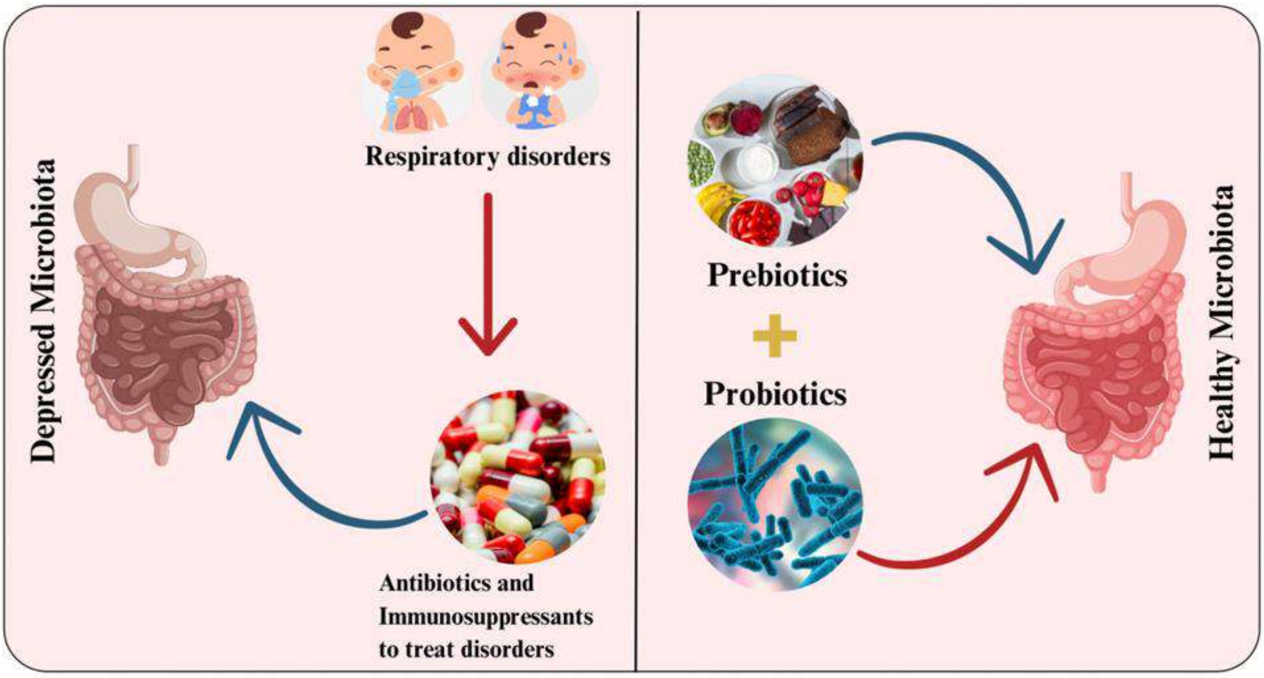

Fig. 2: Prebiotics and respiratory disorders

Table 2: Use of prebiotic in different respiratory conditions.

Prebiotic + Probiotic	Outcomes	Duration	Age	Results
Oligosaccharides + Bifidobacterium lactis	Pneumonia	1 yr.	1-3-year-old children	24% reduction in pneumonia, 35% reduction in severe acute LRTIs (Chan et al., 2020).
(FOS+GOS) + Lactobacillus F19	RTIs	5 Months	Infants	66% risk reduction in LRTIs (Szajewska et al., 2017).

Prebiotic for Mental Health in Children

A psychological disorder, also referred to as a mental disorder, is a collection of symptoms characterized by a notable disturbance in a person's behavior, emotional regulation, or cognitive functioning. Anxiety and depression disorders come in a number of forms as common mental ailments (Freijy et al., 2023).

Prebiotics are food components that the host does not digest but which nevertheless benefit the intestinal tract through selective metabolism. The mechanism by which gut microbiota affects the brain is by the reduction of histone deacetylase activity created by the short-chain fatty acids (SCFAs), butyrate, propionate, and acetate, which are the end products of prebiotic fermentation by intestinal microorganisms. This could explain the transcriptional dysregulations and imbalances in histone acetylation levels observed in neurodegenerative disorders (Tabrizi et al., 2019). Fig. 4 shows the direct and indirect effect of short chain fatty acids (SCFA) on brain.

- The nondigestible galacto-oligosaccharide formulation known as prebiotic BGOS possesses anxiolytic properties that could be linked to the regulation of cortical IL-1b and 5-HT2A (5-hydroxytryptamine2A) receptor expression. By consuming galacto-oligosaccharides, or FOS+GOS, anxiety and depressive symptoms may be reduced (Paiva et al., 2020).
- Prebiotics change the gut microbiota, which helps with depression and mood disorders by increasing the amounts of SCFA in the cecum, decreasing levels of plasma corticosterone, and influencing the HPA axis (Molina-Torres et al., 2019).
- One type of prebiotic called galacto-oligosaccharides (GOSs) can be used to treat autism spectrum disorder (ASD) symptoms and comorbidities. More precisely, children with autism exhibit significant changes in the composition and metabolism of their gut microbiota following administration of B-GOS® prebiotic therapy. When children with autism spectrum disorder combine a diet free of gluten and casein with the prebiotic bimuno-galacto-oligosaccharide, their symptoms related to social conduct improve (B-GOS®) (Duque et al., 2021). Fig. 5 shows the role of prebiotics in

managing pediatric health.

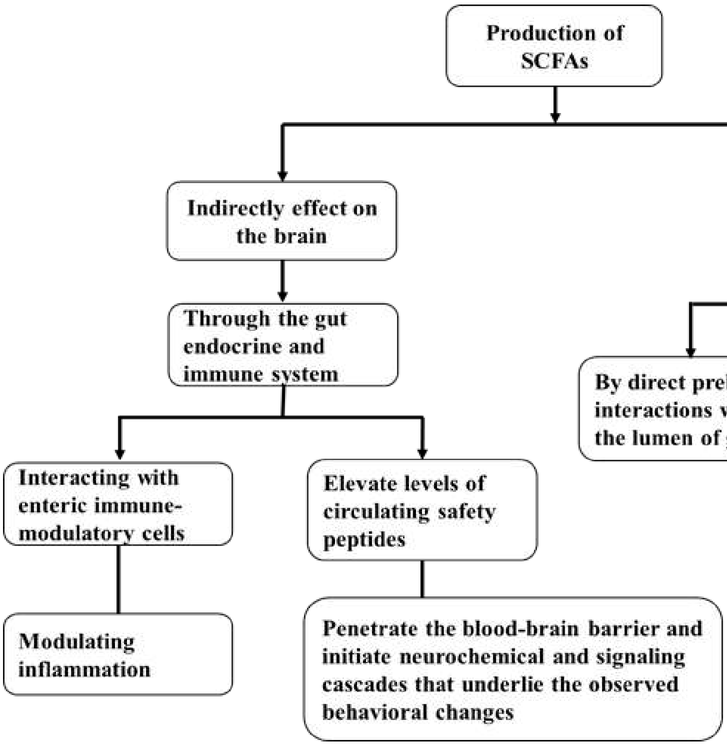

Fig. 3: Direct and indirect effect of short chain fatty acids (SCFA) on brain

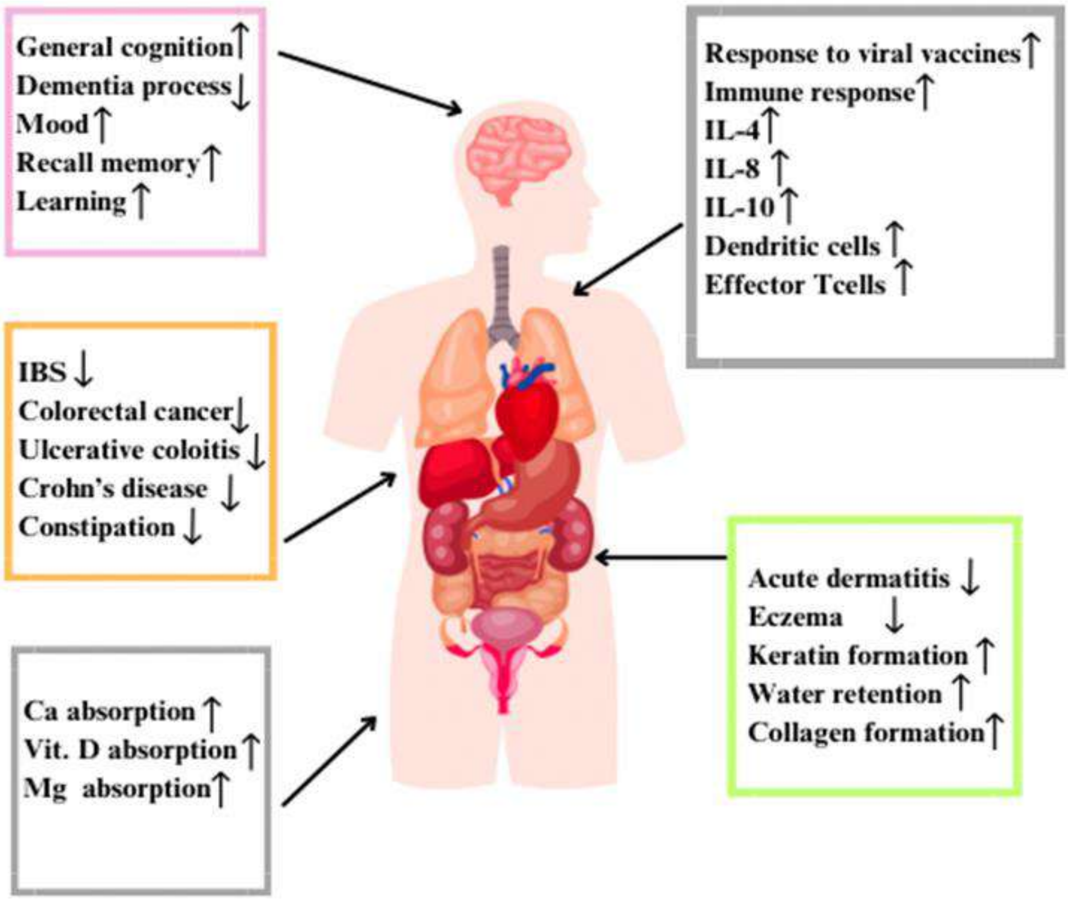

Fig. 4: Role of prebiotics

Ethical Considerations in Prebiotic Research and Development for Children

In order to protect the rights, welfare, and safety of pediatric participants, ethical issues in prebiotic research and development are vital.

Informed Consent

When it comes to research involving children, informed consent is an essential ethical concept. On behalf of their children, parents or legal guardians must give their informed permission, explaining the purpose of the study, any possible risks or advantages, and the participant's ability to withdraw at any time. The parent or guardian should be able to grasp the language used to seek informed consent. Depending on the child's age and maturity level, researchers must also take into account the ability of the youngster to consent to participation (Williams et al., 2012).

Safety Considerations

In prebiotic research, participant safety in children is of utmost importance. To detect and reduce potential risks linked with prebiotic treatments, researchers must perform comprehensive risk evaluations. This entails keeping an eye out for negative consequences and developing procedures for handling any unfavorable incidents that might occur throughout the study (Fig. 6) (Salminen et al., 2004).

Fig. 5: Safety considerations of prebiotics

Equity and Access

To guarantee that all children, regardless of socioeconomic position, race, ethnicity, or geography, have the chance to engage in prebiotic research, researchers must take equity and access problems into account. This entails tackling obstacles to involvement like language, transportation, and cultural differences (Petschow et al., 2013).

Long-term Impacts

Prebiotic therapies' long-term impacts on pediatric health are taken into account when evaluating ethical issues, which go beyond the study's time frame. Researchers ought to evaluate the possible hazards and long-term advantages of prebiotics and think about how they can affect children's health outcomes in the future (Salvini et al., 2004).

The Cost Factor: Weighing the Benefits of Prebiotics

When considering the benefits of prebiotics, it's essential to weigh them against the associated costs. While prebiotics offer potential health advantages, including improved gut health, immune function, and metabolic regulation, there are several factors to consider regarding their cost-effectiveness (Brownawell et al., 2012).

Cost of Prebiotic Supplements

Prebiotic supplements can vary in cost depending on factors such as brand, formulation, and dosage. Some supplements may be more expensive than others, especially those containing proprietary blends or additional ingredients. Parents should consider their budget and affordability when choosing prebiotic supplements for their children (Brownawell et al., 2012).

Cost of Prebiotic-Rich Foods

Many prebiotic-rich foods, such as fruits, vegetables, whole grains, and legumes, are readily available and affordable.

However, some specialty prebiotic foods or fortified products may be more expensive than traditional options. Parents should balance the nutritional benefits of prebiotic-rich foods with their cost to ensure a budget-friendly approach to promoting children's health (Sonya).

Healthcare Costs

Prebiotics are an investment in the health of your child that may help prevent or lower the risk of certain illnesses, which could ultimately result in lower healthcare costs. Prebiotics, for instance, might improve gut health by lowering the likelihood of gastrointestinal problems or allergic reactions, which can minimize the need for medical interventions and the related expenses (Dwivedi et al., 2014).

Cost-Benefit Analysis

Conducting a cost-benefit analysis can help parents evaluate the overall value of incorporating prebiotics into their children's diet. By weighing the potential health benefits against the associated costs, parents can make informed decisions about whether the investment in prebiotics aligns with their priorities and financial considerations (Koponen and Salminen, 2019).

Prebiotics for Premature Infants: Special Considerations

Prebiotics can play a crucial role in supporting the health and development of premature infants, but there are several special considerations that healthcare providers and parents should be aware of:

Immature Gut Microbiota

Compared to full-term infants, premature infants frequently have an undeveloped gut microbiome. Human milk oligosaccharides (HMOs), one type of prebiotic, can aid in fostering the development of good bacteria in an infant's digestive tract, which is necessary for the establishment of a healthy microbiome (France de La Cochetiere et al., 2007).

Breast Milk vs. Formula

For preterm infants, breast milk is the best source of prebiotics since it naturally contains HMOs and other beneficial components that boost immune system and gastrointestinal health. It may be advised to use specialist preterm formula enhanced with prebiotics for premature babies who are unable to breastfeed (Vandenplas et al., 2014).

Preterm Formula Selection

Healthcare professionals may occasionally advise premature infants—particularly those with gastrointestinal problems or dysbiosis—to take additional prebiotic supplements. However, depending on the infant's clinical state and response, the prebiotic supplementation's dosage and duration should be closely evaluated and altered (Srinivasjois et al., 2009).

Prebiotic Supplementation

Healthcare professionals may occasionally advise premature infants—particularly those with gastrointestinal problems or dysbiosis—to take additional prebiotic supplements. However, depending on the infant's clinical state and response, the prebiotic supplementation's dosage and duration should be closely evaluated and altered (Vandenplas et al., 2014).

Risk of Necrotizing Enterocolitis (NEC)

Necrotizing enterocolitis is a dangerous gastrointestinal illness that is more common in premature babies. Prebiotics have been shown in studies to improve gut barrier function and encourage the growth of good gut bacteria, which may help lower the risk of NEC. To determine the best time and amount of prebiotic supplementation for preventing NEC in premature newborns, more research is necessary (Garg et al., 2018).

The Future of Prebiotics and Probiotics in Pediatric Care: Personalized Medicine

Future pediatric healthcare will become more personalized. Prebiotics and probiotics will play an increasingly important role as our understanding of the intricate functioning of the human microbiome, particularly in the gastrointestinal system, grows (Bubnov and Spivak, 2023). These beneficial microorganisms and their substrates have tremendous potential to optimize health outcomes in children, especially when tailored to individual needs through personalized medicine. Recent advances in microbiome research have shed light on the diversity and dynamics of children's gut microbial communities (Cunningham et al., 2021). Prebiotic and probiotic regimens can be customized by healthcare professionals to ensure a healthy, balanced microbiome that supports the immune system, digestive system, and general well-being. In pediatrics, probiotic and prebiotic therapies find biomarkers that can forecast individual responses. Biomarkers encompass a variety of elements, such as microbial signatures, host genetic factors, immunological markers, and clinical characteristics that indicate underlying health state and the child's microbiome's potential response to particular interventions (Caffarelli et al., 2015). Healthcare professionals can more effectively monitor treatment outcomes and optimize treatment plans by incorporating these biomarkers into clinical practice. Furthermore, a range of pediatric health issues, such as neurological diseases, obesity, allergies, and gastrointestinal disorders, can be treated with tailored probiotic and prebiotic therapies (Bubnov et al., 2015). Targeting the gut microbiome, these interventions provide a comprehensive approach to pediatric care that addresses underlying imbalances and fosters resilience and long-term health in addition to symptom management (Bubnov and Spivak, 2023).

Choosing the Right Probiotic Supplement for Your Child

The process of selecting the best probiotic supplement for your child involves numerous important considerations. Prior to anything else, it's critical to pinpoint particular health issues or objectives, such as enhancing immunity, addressing distinct illnesses like allergies, or enhancing digestive wellness. Next, since different strains of probiotics have varying advantages, it is important to examine the strains found in supplements. For the health of your children, look for supplements supported by scientific research. Take into account the supplement's preferred and most palatable form for the kid as well, such as chewable tablets or flavored powders (Percival, 1997).

Dosage recommendations based on the child's age and weight should also be strictly followed;

Infants (0-12 months)

Probiotics can be beneficial for newborns and babies, especially if they have problems such as colic, reflux or indigestion. Look for probiotic strains like *Lactobacillus reuteri*, which have been shown to help relieve colic symptoms in babies. Make sure the supplement is specifically formulated for babies and follow the dosage instructions given by your pediatrician or health care provider (Vandenplas et al., 2014).

Toddlers and Preschoolers (1-5 years)

As children enter toddler and preschool age, probiotics can still help to their developing immune (Bubnov et al., 2015) and digestive systems. Consider probiotic strains such as Lactobacillus acidophilus and Bifidobacterium lactis, which are often found in children's probiotic products. Look for supplements that are easy to give to babies, such as powder or chewable tablets, and always follow the recommended directions (Di Domenico et al., 2022).

School-aged Children (ages 6-12)

Children in school can benefit from probiotics to improve their overall health and wellbeing, particularly during stressful events, illness, or antibiotic use. To support a balanced gut microbiota, look for probiotic supplements that contain a variety of strains, such as Lactobacillus and Bifidobacterium species. Taste preferences and convenience of dosing should be taken into account when selecting a probiotic product for this age range, as compliance is crucial for success (Hojsak et al., 2018).

Adolescents (13+ years)

In conclusion, confirm that the supplement is produced by a reliable supplier, ideally one that conducts independent quality and purity testing. Consult a pediatrician to be sure a new nutritional supplement is appropriate for your child's needs before beginning. Lastly, confirm that the supplier of the supplement is a respectable one—ideally, one that does independent testing to ensure quality and purity. To make sure a new nutritional supplement is appropriate for the child's specific needs, it is advised to speak with a pediatrician before beginning it (Cunningham et al., 2021).

Conclusion

Probiotics have demonstrated promise in the prevention and treatment of a number of illnesses, including dermatitis, eczema, respiratory infections, constipation, irritable bowel syndrome, metabolic syndrome, and cognitive health problems. Their therapeutic attractiveness is highlighted by their minimal risk of adverse effects, cost-effectiveness, ease of use, and capacity to improve immune system function and gut health.

Prebiotics and certain probiotic strains together have shown encouraging outcomes, especially when it comes to treating problems like infantile colic and constipation and enhancing the composition of the gut microbiota. Despite these advantages, more thorough investigation is still required to completely comprehend the long-term consequences, exact mechanisms of action, and potential drawbacks of prebiotics. In order to ensure fairness, safety, and long-term monitoring, ethical considerations in pediatric research are essential.

Prebiotics in pediatric treatment have a bright future because to personalized medicine. Progress in the study of the microbiome is opening doors for customized interventions that address specific health needs, especially for complicated problems like neurological disorders, obesity, and gastrointestinal disorders. Prebiotics can be used safely and effectively by filling up present knowledge gaps and creating thorough guidelines, which will eventually improve the health and wellbeing of children.

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Chapter 18

Neuroprotective Role of Probiotics

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ABSTRACT

Generally, probiotics are considered safe to use, they are live microorganisms that provide several health benefits by improving gut microbiota. Through the microbiota-gut-brain axis, the gut microbiota affects the function of central nervous system (CNS). Therefore, medicative targeting the gut microbiota like probiotics is potent for improving mental wellbeing. The Gut-brain axis is a bidirectional network of signaling pathway and is a source of communication between the central nervous system and gastrointestinal tract. This network consists of multiple connections, including immune system, vagus nerve, and bacterial metabolites and products. This chapter explores the emerging field of the neuroprotective role of probiotics, delving into the intricate connection between gut health and brain function. Investigating the mechanism through which probiotics may positively affect neural health and discussing current research findings and their implication for mitigating neurodegenerative conditions. It sheds light on the auspicious avenues for harnessing these microbial allies to promote and safeguard neurological health by examining impact of probiotics on inflammation, oxidative stress, and the gut brain axis.

KEYWORDS
Probiotics, Neuroprotection, Gut-brain axis, Gut microbiota, Anti-inflammatory

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INTRODUCTION

Probiotics

The term "probiotic" is a Greek word which means "for life". It is a live microbial food ingredient. When consumed in adequate amounts, offer health enhancement to the person consuming them (Doron and Gorbach, 2006). In 1908, Nobel Prize winner Eli Metchnikoff hinted at the idea of probiotics, suggesting that good health of Bulgarian peasants might be linked to their intake of fermented milk derivatives. The term "probiotic" was coined by Lilly and Stillwell in 1965 to describe substances released by one organism that encourage the growth of another (Gupta and Garg, 2009) .

Humans coexist closely with large populations of microorganisms residing on the skin, within the mouth, and throughout the GIT (gastrointestinal tract) (Heintz-Buschart and Wilmes, 2018). GIT contains a miscellaneous community of over 500 unique bacterial species, many of which play crucial roles include improving the immune system, protecting the host against harmful viruses and bacteria, as well as facilitating the process of digestion (Cresci and Bawden, 2015). Excessive use of antibiotics, immunosuppressive therapy, and other treatments like irradiation can disrupt the natural composition of microbial communities in the body, potentially leading to imbalances and negative effects on health (Weersma et al., 2020). Unveiling helpful bacterial strains, i.e., probiotics into the GIT could be a promising strategy for preventing diseases as well as to reestablish microbial balance (S.-K. Kim et al., 2019). Probiotics come from various genera as well as species of microorganisms and have been researched for various health and disease outcomes. Common probiotics include yeast, such as *S. cerevisiae* as well as bacteria like *Streptococcus*, *Lactobacillus, Enterococcus*, *Propionibacterium*, *Bifidobacterium*, *Bacillus*, as well as *E. coli* (Cannon et al., 2005). For effective probiotics there are certain characteristics that must be considered while selecting or preparing probiotics, as shown in Table 1.1.

Fermented foods contain probiotics in amounts ranging from 2 to 20 grams per day, depending on the specific component and intended effect. Probiotics can be incorporated into various food products like cereals, biscuits, bread, sauces, yogurts, and beverages (Fig. 1.1) (Dekumpitiya et al., 2016). Among these, curd is a popular choice globally. The interest and development of functional foods containing both probiotics and prebiotics has increased due to growing awareness of their health benefits. These foods positively impact gut health and reduce the risk of diseases, making them a valuable therapeutic option (O'Sullivan et al., 2020).

Table 1.1: Characteristics of an ideal microorganism for probiotics

Characteristics of an ideal microorganism for probiotics (Ramasamy et al., 2012)
Should be derived from human sources
Should be non-pathogenic organisms
Should resilient to processing
Should able to endure gastric juice, bile, as well as low pH environments
Should have the capability to stick to the intestinal tissues
Should have the capability to generate substances that fight microbes like microcins, bacteriocins, as well as antibiotics
The paramount aspect of probiotics is that they must be demonstrated to be both harmless as well as helpful for consumers

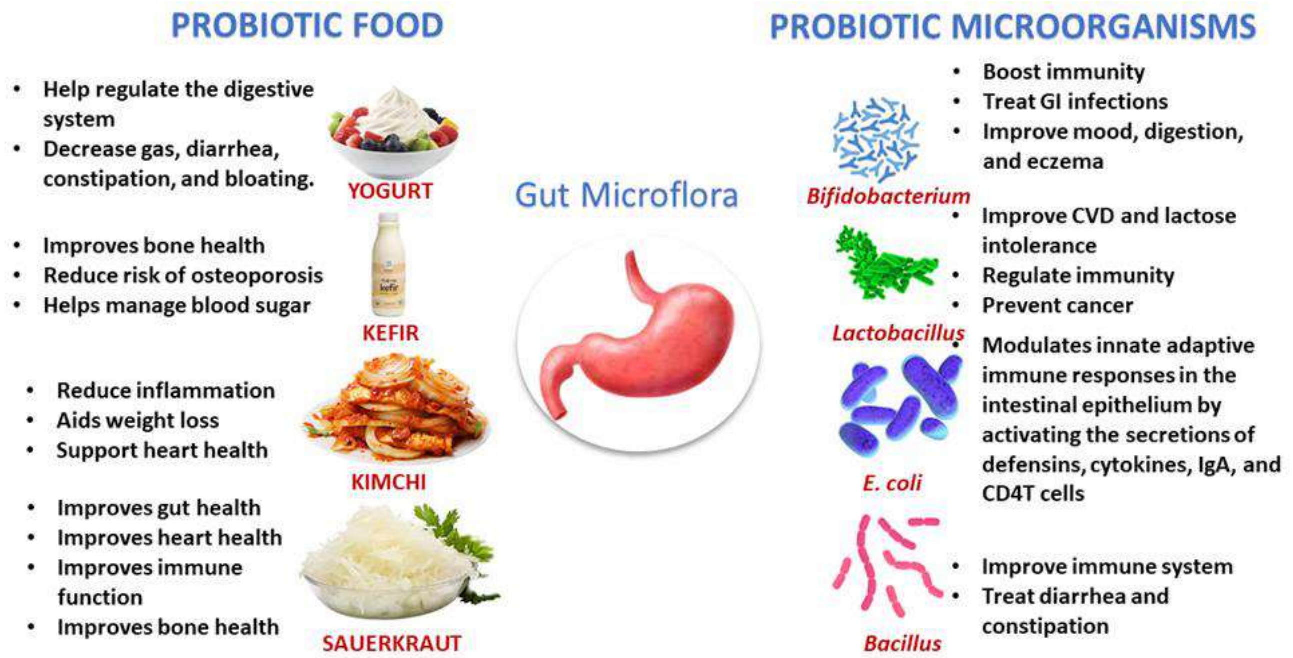

Fig. 1: Probiotic food and probiotic microorganisms with their health benefits

Probiotics and Neuroprotection

Recent studies have been carried out on the neuroprotective role of probiotics. Therapies targeting gut microbiota such as use of probiotics have the potential to improve central nervous system functioning (Lim et al., 2015). This means they can help repair and maintain the nerves, cells, structure, and functions of the nervous system. Many studies purported that probiotics have effects on stress-induced models. For example, in a research study, male mice were given *Lactobacillus rhamnosus* (JB-1) orally to examine how it affects their GABAergic system. GABA is a neurotransmitter that helps regulate various mental and physical processes. The *L. rhamnosus* (JB-1) treated mice showed improvements in behavior when stressed, and their levels of corticosterone (a stress harmone0 and GABA mRNA (a molecule involved in GABA production) were reduced (Bravo et al., 2011). .

The Emerging Connection: Probiotics and the Nervous System

The gut microbiome and brain are connected through the vagus nerve, this nerve have gained a lot of attention recently (Le Morvan de Sequeira et al., 2022). This nerve is also known as cranial nerve X, regulates numerous physiological functions such as gut motility, bronchial constriction, heart rate, and digestion. Probiotics, those beneficial bacteria, can influence both our body and mind, and it seems like the vagus nerve plays a role in how they work. So, it is concluded that when probiotics or our gut bacteria influence our brain, it's often because of our vagus nerve (Grenham et al., 2011).

The Gut-Brain Axis: A Foundation for Neuroprotection

There are multiple evidences of gut-brain connection through many clinical researches. The science behind this connection and how gut influences the brain as well as probiotics intervention in neurological health are discussed below.

Bidirectional Communication: How the Gut Influences the Brain

Recently, it has become evident that the bacteria in our gut can really affect how our gut talks to our brain, which can impact how our brain works and how we behave. In both preclinical and clinical settings, communication between brain and gut has been revealed (fig. 2.1). Several research studies have already been undertaken to find how the brain can communicate with gut movement, blood flow, and secretions. Moreover, how what's happening in our intestine can affect how sensitive we are to things in our gut and what is going on in our nervous system (Mayer et al., 2022). It seems like the brain can affect beneficial microorganisms in two main ways either by releasing messenger molecules directly into the gut cavity from cells in the lamina propria or via intermediary means by changing gastrointestinal motility and intestinal permeability and secretion. Alternatively, microbiome directly impact brain function and behavior via microbial neuro-metabolites, tryptophan metabolism, immune activation, as well as the CN X (Rutsch et al., 2020). The two-way communication between the gut and the brain is regulated by nerves, hormones, and immune system, and is very crucial for maintaining homeostasis. However, not all bacteria in our gut participate in the development of brain, its indeed probiotics, the primary microbial types that contribute to this regard. That's why scientists are really interested right now in how probiotics, especially types like lactobacilli and bifidobacteria, can help our brain development, possibly even preventing brain disorders (Cryan and O'Mahony, 2011). Pathways involved in bidirectional communication between brain and gut are shown in Fig. 2.

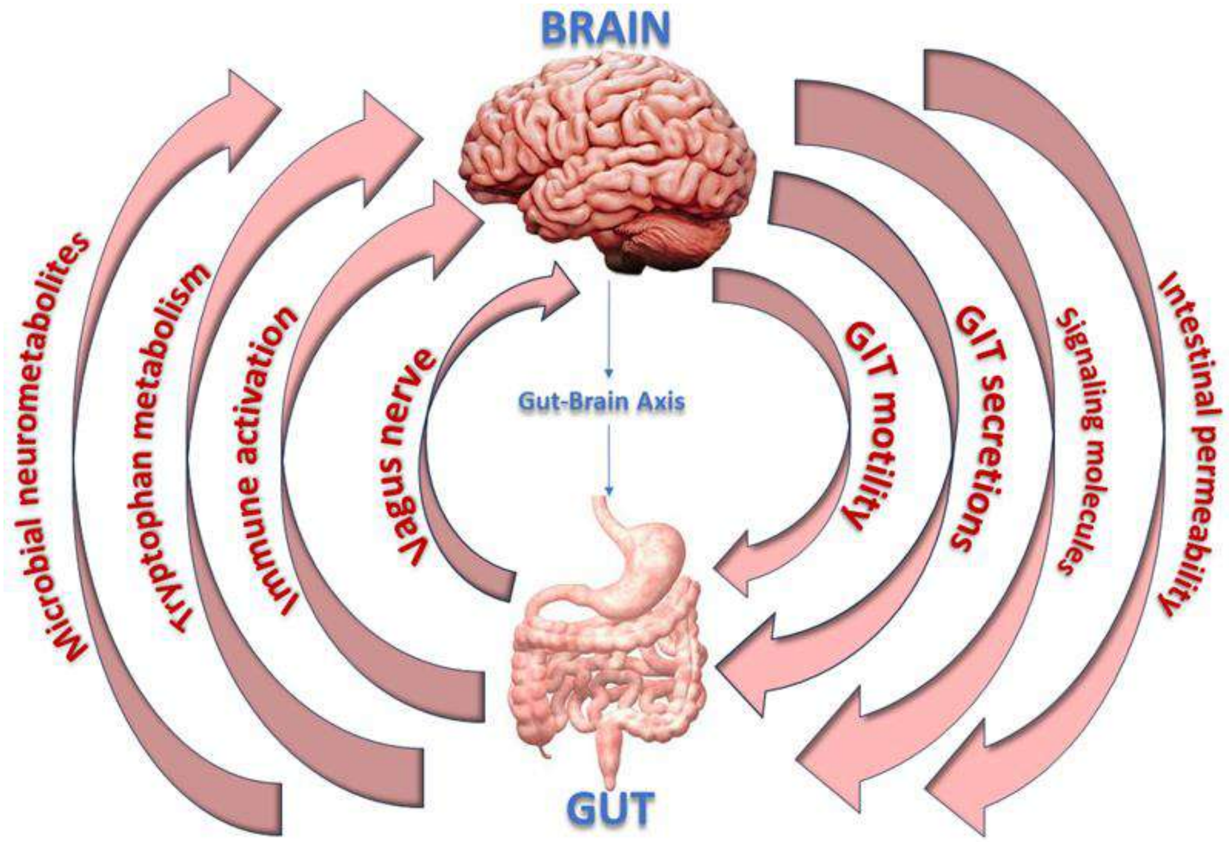

Fig. 2: Pathways involved in bidirectional communication between brain and gut

Implications for Neurological Health

Alzheimer's as well as Parkinson's diseases are common in older adults, while conditions like autism spectrum disorders, depression, anxiety, as well as cognitive impairment affect individuals of every age. The incidence of neurological diseases has been on the rise (Cheng et al., 2019). The connection between the gut bacteria and the brain is named as "microbiota-gut-brain axis." The microbiome present in our gut have an impact on our immune system and body barriers such as gut lining and blood-brain barrier (Breit et al., 2018). When the gut bacteria aren't functioning well, it can disturb the signaling between the gut and the brain, causing several gut disorders and cognitive issues. Probiotics are very beneficial as they keep the gut healthy by improving its functions, strengthening the barriers, and even improving the growth of brain cells. For metabolic and mental health conditions, probiotics could be used alongside other treatments (Iosifescu, 2012). Especially, strains of bacteria like Lactobacillus as well as Bifidobacterium are known for their helpful effects in maintaining this communication between the gut and brain (Rankin and Carew, 1988).

Mechanisms of Neuroprotection by Probiotics

The metabolites in probiotics can modulate neuronal function as well as influence various cascades of neurodegenerative disorders (NDDs). Due to this dual role of probiotics in neuroprotection and also neurodegeneration, there is a need to understand the delicate balance between beneficial and detrimental bacteria in order to apply their practice in clinical therapies (Mitra et al., 2023). Following are the few mechanisms by which neuroprotection is achieved from probiotics.

Modulation of Gut Microbiota

Oxidative stress exerted by a large number of reactive oxygen species (ROS) is undoubtedly a major cause of neurodegenerative and central nervous system disorders. Based on the studies done on the metabolites of gut microbiota, it is evident that these tiny organisms have a strong link with the nervous system, therefore; they play a crucial role in various neurological disorders, as proven by a wide range of studies and pre-clinical models (Shandilya et al., 2022) It has been shown that oxidative stress on brain can be reduced by the modulation of gut microbiota via numerous metabolites, including absorbable neurotropic factors, vitamins, polyphenols, antioxidants, as well as SCFAs, as these metabolites sustain both endogenous as well as exogenous reactive oxygen species (Fig. 3) (Shabbir et al., 2021). The increased rate of ROS causes irreversible cell damage, while neurons are responsive to relatively reduced levels of ROS. Nevertheless, optimal level of ROS is crucial for nerve system for sake of long lasting synaptic strength in the synaptic plasticity, hippocampus and memory and learning function (Di Meo et al., 2019).

Anti-inflammatory Effects

Probiotics significantly involved in the regulation of inflammation, immune responses, oxidative stress, as well as central and peripheral neurotransmission (Shabbir et al., 2021). The factors responsible for increasing chronic inflammation are indeed the uncontrolled production of pro-inflammatory cytokines, chronic infections, oxidative stress, and metabolic alterations in the of adipose tissues. Mitochondria and NADPH oxidases (NOXs) are the prime cellular sources of reactive oxygen species throughout the course of electron transport chain (Nolfi-Donegan et al., 2020). Additionally, generation of these ROS in adipocytes spreads chronic inflammation and also excites pro-inflammatory adipokines in those of target tissues. However, probiotics have revealed anti-inflammatory effects and antidepressant responses. It has been shown that the treatment with probiotics efficiently increases glucagon-like peptide-1 secretion, reduces central and peripheral inflammation (via reduction of TNF-α as well as interleukin-6), reduction of central and peripheral oxidative stress, and decreases neurodegeneration (Carlessi et al., 2021).

Influence on Neurotransmitters and Neurodegenerative Disorders

The role of probiotics in the production of neurotransmitters is another significant feature. Many probiotics help to synthesize and also regulate various neurotransmitters (Strandwitz, 2018). Probiotics modify enteric nervous system (ENS) activity by the production of local neurotransmitters, including GABA, melatonin, serotonin, acetylcholine, and histamine, by the conversion of catecholamines to their activated state in gut lumen as shown in Fig. 3 (Sarkar et al., 2016). Resultantly, nerve fibers of ENS can sense gut microbiota signals by the dispersion of bacterial metabolites and substances. As a result of gut chemo-sensing, the enteroendocrine cells (EECs) develop an ability to engage with vagal afferents, thereby influencing them directly or indirectly (Raybould, 2010).

Many research studies have indicated the neuroprotective role of probiotics in various neurodegenerative disorders (NDDs) (Hou et al., 2021). The strain of *L. buchneri* (KU200793), isolated from fermented food of Korea, has been reported to defend SH-SY5Y cells against 1-methyl-4-phenylpyridinium (MPP^+) with the help of its antioxidant activity, thus confirming its neuroprotective activity (Cheon et al., 2020).

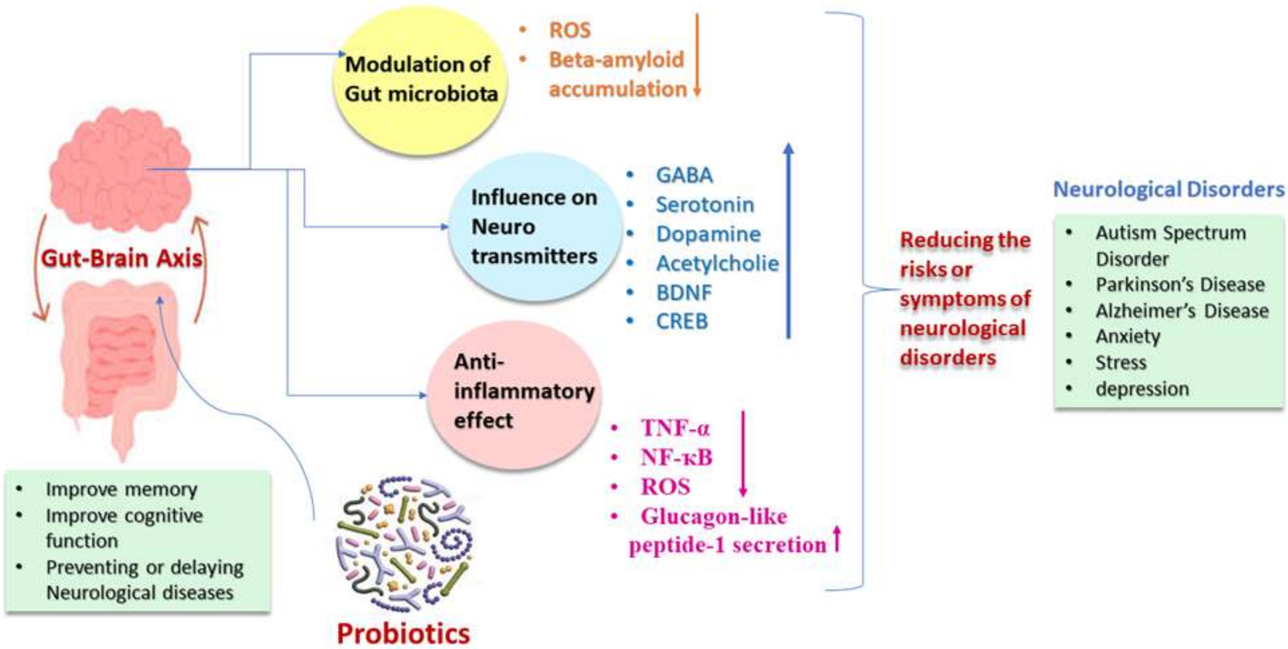

Fig. 3: Different mechanisms involved in neuroprotective role of probiotics

Probiotics and Cognitive Function

Probiotics have been anticipated as the potent candidates to ameliorate depressive disorders and cognitive impairment by means of gut-brain axis in both patients as well as animal models used in experimentation (C.-S. Kim et al., 2021). Randomized controlled trials (RCTs) have suggested that the link between gut microbiota and CNS might underlie the progresses in cerebral and cognitive functioning after providing with probiotic supplementation and elucidate the concomitant alterations in peripheral neuromodulators (Bauer et al., 2016). Brain-derived neurotrophic factor (BDNF), a factor significant for plasticity, synaptic formation, and also neuroimmune responses, has also been deliberated in order to assess its vital role in memory formation, learning, and affective discomforts (Licinio and Wong, 2002).

Therefore, it is expected that mitigation of inflammation with intervention of probiotics in older adults might impact positively on both cognitive as well as mental functions by the modulation of BDNF signaling (C.-S. Kim et al., 2021). But still, there is a requirement of further studies on the subject to clearly demonstrate the effects of probiotics on inflammatory status in addition to gut microbiome at the functional level.

Experimental Evidence: Studies Supporting the Neuroprotective Role

There are many experimental evidence collected from numerous clinical trials and case studies supporting the neuroprotective role of probiotics as presented in Table 1. Following are some clinical trials and case studies for different neurological disorders.

Clinical trials and Case Studies

ASD (autism spectrum disorder)

ASD is a brain developmental disease that is highly heritable (Battle, 2013). Children with ASD were studied to determine the effects of probiotics. According to the findings, probiotic mixture supplementation for 28 days reduced the severity of autistic symptoms characterized by heightened social affect domain score (Grossi et al., 2016).

PD (Parkinson's Disease)

PD is a neurocognitive condition that is defined by high prevalence of Lewy body deposition in the posterior motor nucleus of the medulla oblongata and the CN X (Sachdev et al., 2014). Giving probiotic supplements for more than three months, including *Lactobacillus acidophilus* and *Bacillus infantis* decreased PD patients' bloating and cramping in the abdomen (Cassani et al., 2011).

Alzheimer's Disease

AD is a neurocognitive condition characterized by a progressive loss or deterioration in cognitive functions.
In a clinical trial, for 12 weeks, AD patients between the ages of 60 and 95 received 200 mL of probiotic milk enriched with *L. acidophilus, L. fermentum, L. casei, as well as Bifidum*. Their metabolic profiles, oxidative stress, and inflammatory biomarkers were among the biochemical indicators that showed changes in their cognition. Treatment with probiotics may be able to stop cognitive deterioration and ameliorate cognitive symptoms (Akbari et al., 2016). Results of many other clinical trials using human as well as animal as a model are mentioned in Table 5.1.

Anxiety, Stress, and Depression

According to numerous research, taking probiotics may aid in stress reduction, decision-making, and cognitive function.

In trial on stressed students, stressed adults' levels of anxiety and tension about academic exams were considerably lowered when they took a 12-week dosage of *Lactobacillus plantarum* P-8. Not only did GM show beneficial modifications, but the anxiolytic effects were also noted. Additionally, there was an increase in the synthesis of neurotransmitters and neuroactive metabolites, such as arachidonic acid, GABA, and SCFA (T. Ma et al., 2021)**.**

Emerging Issues in Probiotic Safety

Policymakers, researchers, and physicians have expressed concerns about the safety of probiotic microbes. These can be roughly classified as issues with the probiotic strain, product caliber, or probiotic dosage. Concerns about safety in the manufacturing of probiotic products include the requirement to determine the final product's composition, potency (the number of live microorganisms supplied), and purity. Probiotic goods also need to be adequately tested for possible pollutants, taking into account their intended purpose. Lastly, the probiotic needs to be host-safe (Cohen, 2018)**.**

Other Potential Therapeutic Uses of probiotics

There are many therapeutic uses of probiotics against different diseases as shown in Table 2, some of them are discussed below.

Gastrointestinal Diseases

Gastroenteritis is caused by most of the strains of *E. coli*, and *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. and viruses like Rotavirus, as well as Norovirus, that leads to intestinal inflammation. A Commercially available strain is

Lactobacillus, which shows potential against the pathogens like *E. coli* and *C. difficile*. Probiotics appeared to have an impact on inflammatory bowel disease (Girardin and Frossard, 2012).

Table 1: Experimental evidence: effects of probiotic on neurological diseases via clinical trials and case studies

Strain	Model	Age	Outcomes	References
ASD				
(*L. acidophilus, L. delbrueckii, B. longum*, *B. breve*, *B. infantis*, *L. paracasei*, *L. plantarum*, *S. thermophiles*)	Child suffering ASD	Twelve years old	The severe abdominal symptoms were alleviated. Enhancements were noted in core autistic symptoms. An increase was observed in the score of the social affect domain.	(Grossi et al., 2016)
DSF Vivomixx®	ASD children	18 to 72 months	Demonstrated positive impacts on core symptoms of autism. Marked enhancements were observed in gastrointestinal symptoms, multisensory processing, and adaptive functioning.	(Santocchi et al., 2020)
PD				
L. acidophilus, *B. infantis*	PD patients	Near about 76 years	Alleviate abdominal discomfort and bloating.	(Georgescu et al., 2016)
L. casei	PD patients	-	Improved bowel habits and stool consistency.	(Cassani et al., 2011)
Alzheimer's disease				
L. acidophilus, *L. fermentum*, *B. bifidum*, *L. casei,*	AD patients	60 to 95 years	Reduced level of MDA and serum hs-CRP. Improved MMSE score.	(Akbari et al., 2016)
Depression, anxiety and stress				
L. plantarum P-8	Stressed adults	-	Modulates the gut microbes. Reduced stress and anxiolytic effects. Enhance the synthesis of neurotransmitters and neuroactive metabolites.	(T. Ma et al., 2021)

Allergy

Allergy arises from an intensified hypersensitive reaction of the immune system to typically harmless substances (allergens) within the environment. Dysbiosis has been involved for the allergies development. Allergic diseases including asthma, allergic rhinitis, food allergy and atopic dermatitis could treat by the use of probiotics. *L. paracasei*, *L. salivarius* and *L. fermentum* as an anti-allergic has been used for children suffering with atopic dermatitis (I. -J. Wang and Wang, 2015).

Respiratory Diseases

During the last years, an increase in diseases like asthma and other respiratory diseases has been noticed in many industrialized countries. Studies suggested that asthmatic hyper-sensibility has been inhibited by *Enterococcus faecalis* FK-23. Probiotics appear to be more effective against particular respiratory tract diseases such as cystic fibrosis. (Alexandre et al., 2014).

Liver Diseases and Hepatic Encephalopathy

Cirrhosis appears to be linked with changes in the gut microbiota due to the occurrence of fecal *Bifidobacterium* species. In addition, modifications in intestinal microbiome have been noticed among chronic hepatitis B patients and hepatocellular carcinoma proliferation. Probiotics seem to be effective against cirrhosis. Hepatic encephalopathy is closely associated with gut microbiota. The toxic substances that are inactive in the liver produced due to the metabolic activity of the intestinal microbiota and cause hepatic encephalopathy. Lactic acid bacilli, specifically *Lactobacilli* and *Bifidobacteria,* appear to be more efficacious species for hepatic encephalopathy (Khungar and Poordad, 2012).

Oral Diseases

An inflammation restricted to the gingiva is called gingivitis. The inflammation process that has impacts on all tissues and the alveolar bone is periodontitis. The risk of both gingivitis and periodontitis can be decreased by *Lactobacillus salivarius* WB21 that regulates the oral microbiota. (Shimauchi et al., 2008).

Cancer

The major health issue is gastrointestinal cancer, which accounts for 20% of all cancers and 9% of all cancer death causes around the world. Probiotics show protection against development of cancer and it also decreased the postoperative inflammations incidence. In the cancer cells of both colon and gastric, *Lactobacillus paracasei* and *L. rhamnosus GG* strain show the effect against proliferation and pro-apoptotic effects. Furthermore, *Bacillus polyfermenticus* shows the antitumor properties (Orlando et al., 2012)

Osteoporosis

Probiotic intake could be a therapeutic approach in preventing and treating bone loss, they fortify bones and the skeletal structure. Furthermore, probiotics provide protection against deficiency of primary estrogen as well as secondary osteoporosis (Collins et al., 2017).

Diabetes

It seems that gut microbiome plays a significant role in diabetes development. As studies suggested, that risk of type 2 diabetes could be reduced and prevent by some species of *Lactobacillus* and *Bifidobacterium* (Bordalo Tonucci et al., 2017).

Obesity

Obesity is termed as functional and structural changes in the gastrointestinal ecosystem. In human beings, probiotics intake appears to lower values of metabolic parameters and results in the decreasing weight gain in obese adults (Z.-B. Wang et al., 2019).

Table 2: Therapeutic effects of different probiotics strains against different diseases

Probiotic Strain	Therapeutic Effects	References
Lactobacillus	Treat gastroenteritis	(Allen et al., 2010)
E. faecium, LGG, as well as *S. boulardii*	Prevent antibiotic-associated diarrhea	(Wilkins and Sequoia, 2017)
L. salivarius, L. paracasei, as well as *L. fermentum*	Act as an anti-allergic and has been used for children suffering with atopic dermatitis	(I. -J. Wang and Wang, 2015)
L. rhamnosus GG	Treat cystic fibrosis	(Alexandre et al., 2014)
Lactobacilli as well as *Bifidobacteria*	Treat hepatic encephalopathy	(Khungar and Poordad, 2012)
Lactobacillus salivarius WB21	Lower the risk of both gingivitis and periodontitis	(Shimauchi et al., 2008)
Lactobacillus paracasei and *L. rhamnosus GG*	Pro apoptotic and prevent cancer cells proliferation	(Orlando et al., 2012)
Bacillus polyfermenticus	Anti-tumor	(E. L. Ma et al., 2010)
Lactobacillus as well as *Bifidobacterium*	Reduce the risk of type 2 diabetes	(Bordalo Tonucci et al., 2017)
L. curvatus and *L. plantarum*	Helps in weight loss	(Kobyliak et al., 2016)
L. reuteri ATCC 6475	Defends against deficiency of primary estrogen and secondary osteoporosis	(Collins et al., 2017)

Consideration for Healthcare Professionals

The most vital region of concern with probiotic use is the threat of sepsis. The adherence of probiotics to the intestinal mucosa might enhance bacterial translocation and virulence. This discovery suggests that neonates with immune deficiencies may be particularly susceptible to probiotic sepsis. These theoretical concerns are underscored by recent case studies of probiotic-related sepsis in humans. Several reports have directly linked cases of Lactobacillus and other bacterial sepsis to the consumption of probiotic supplements. However, there is a need for comprehensive guidelines on the assessment of probiotics and their efficacy (Rautio et al., 1999).

Future Prospects and Research Avenues

Recent developments in microbiome science are advancing research on probiotics and prebiotics, revealing new types, mechanisms, and applications. Recent trends anticipate significant changes in the field, predicting a future of significant influence (Cunningham et al., 2021). Scientists are exploring novel strains of beneficial bacteria and yeasts with specific health-promoting properties. These next-generation probiotics may target conditions such as metabolic disorders, mental health issues, and immune system modulation more effectively (Hammes and Hertel, 2002). Modern approaches to investigating probiotics and prebiotics have expanded as a result of modernization of earlier methods. With the use of these high-throughput approaches, it is possible to rapidly establish maternal linkages with the fetus and determine the quantity and density of bacteria present in the newborn's stomach. It is guaranteed that the upcoming decade will bring heights to the targeted therapeutic modulation of the microbiota-gut-brain axis. Meticulously managed, extensive, long-term human clinical trials with stringent controls are urgently required to better understand both the mechanisms and therapeutic potential in neurological health and other diseases (Peterson, 2020).

Conclusion

Probiotics have shown promising neuroprotective effects by modulating gut microbiota, preventing inflammation and improving gut-brain bidirectional communication. These effects demonstrate significant therapeutic benefits for several neurological diseases. More research is recommended to fully understand their mechanism and efficacy.

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Chapter 19

Role of Prebiotics and Probiotics in the Management of Type 1 and Type 2 Diabetes Mellitus

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ABSTRACT

Type 1 Diabetes (T1D) is an autoimmune disease characterized by the destruction of pancreatic β-cells, leading to reduced insulin production. Genetic and environmental factors contribute to its development, with certain HLA class molecules heightening susceptibility. Environmental triggers prompt autoimmune responses, targeting β-cell components. Conversely, Type 2 Diabetes Mellitus (T2DM) arises from insulin resistance and elevated blood glucose levels. Gut microbiota, comprising various microbes, profoundly influence human health by metabolizing complex carbohydrates, producing short-chain fatty acids (SCFAs), and synthesizing essential vitamins. In T2DM, a decrease in butyrate-producing bacteria affects insulin sensitivity. Gut microbiota plays a role in both T1D and T2DM, influencing metabolic inflammation and autoimmunity. Prebiotics selectively stimulate beneficial bacteria, while probiotics offer health benefits, including improved gut integrity and reduced inflammation, potentially impacting diabetes prevention and management. Further research is needed to elucidate their mechanisms and safety.

KEYWORDS		
Diabetes type 1, Diabetes type 2, Prebiotics, Probiotics, Short Chain Fatty Acids (SCFAs), Gut Microbiota	Received: 12-June-2024 Revised: 18-July-2024 Accepted: 15-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder marked by elevated blood glucose levels and disrupted lipid and protein metabolism. It arises when the pancreas can't produce enough insulin or when cells resist its effects. The three primary types are: (1) Type 1; pancreatic insulin deficiency, (2) Type 2; insulin resistance and declining production, and (3) Gestational; during pregnancy, posing risks during and after birth. (Roglic, 2016). Persistent high glucose levels harm kidneys, nerves, heart, and blood vessels, elevating risks for conditions such as CVD, non-alcoholic fatty liver disease, erectile dysfunction, eye problems, and obesity (Kazi and Blonde, 2001). It may also contribute to an increase in the risk of communicable diseases like TB.

Humans are home to around 100 trillion symbiotic microbes that live in various sites of the body like the oral cavity, skin, gut, liver, and lungs. Alone the human gastrointestinal system is home to a diverse microbial population of over 100 trillion bacteria, making it one of the most populated areas of the human body. The human microbiota have a crucial role in food extraction, metabolism, and immune function. Furthermore, modifying microbiota in the human body may be essential for treating some illnesses (Wang et al., 2017).

The term "probiotics" refers to a product or preparation that contains a sufficient number of living, specified microorganisms that affect the microflora in a host compartment through implantation or colonization and positively influence the host's health. On the other hand, Prebiotics are non-digestible, fermentable meals that promote the development and activity of certain microorganisms in the gut (Quigley, 2019). In this chapter, we will specifically focus on the effects of prebiotics and probiotics on gut microbiota that help in the management of insulin dependent (type 1) and adult onset (type 2) diabetes.

Type 1 Diabetes

Type 1 Diabetes is a long-term autoimmune inflammatory condition that affects the pancreatic β-cells that produce insulin, which lowers insulin production (Notkins and Lernmark, 2001). T1DM links to autoimmune β-cell targeting and dysfunction, influenced by genetic and environmental factors, yet precise causes and mechanisms remain elusive (Ilonen et al., 2019).

Diabetes Type 2

Type 2 diabetes mellitus is a chronic metabolic condition marked by insulin resistance of the body cells and increased blood glucose levels. In T2DM, insulin levels are insufficient to satisfy the increased demand of the body due to insulin resistance (Chen et al., 2017).

Approximately 462 million population are diagnosed with T2DM which makes up almost 6.28% of the world's total population. The prevalence of T2DM increasing very rapidly as it ranked the 18th biggest cause of death in 1990 and in 2017 it ranked the 8th biggest cause of death (Khan et al., 2020).

Gut Microbiota

The microbiota is a term that encompasses all microorganisms that inhabit a certain place, including bacteria, archaea, viruses, fungi , and protozoans (Jandhyala et al., 2015). A healthy human GIT is host to almost 100 trillion microbes which exceeds the number of total human body cells. Bacteria are an important part of the gut microbe ecosystem (Zhang et al., 2015). The gut nurtures bacteria mainly from seven major groups: *Bacteroidetes, Firmicutes, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia,* and *Cyanobacteria,* with *Bacteroidetes* and *Firmicutes* predominating (Bäckhed et al., 2005). They help in the digestion of dietary fiber and polyphenols by utilizing a complex metabolic energy-scavenging mechanism based on syntrophic and co-metabolic activities. In exchange, commensal bacteria benefit from the host's protective and nutrient-rich environment (Zhang et al., 2015;Rashid et al., 2023). Other important functions that probiotic bacteria perform in the host body are shown in Fig. 1 (Cerdó et al., 2019):

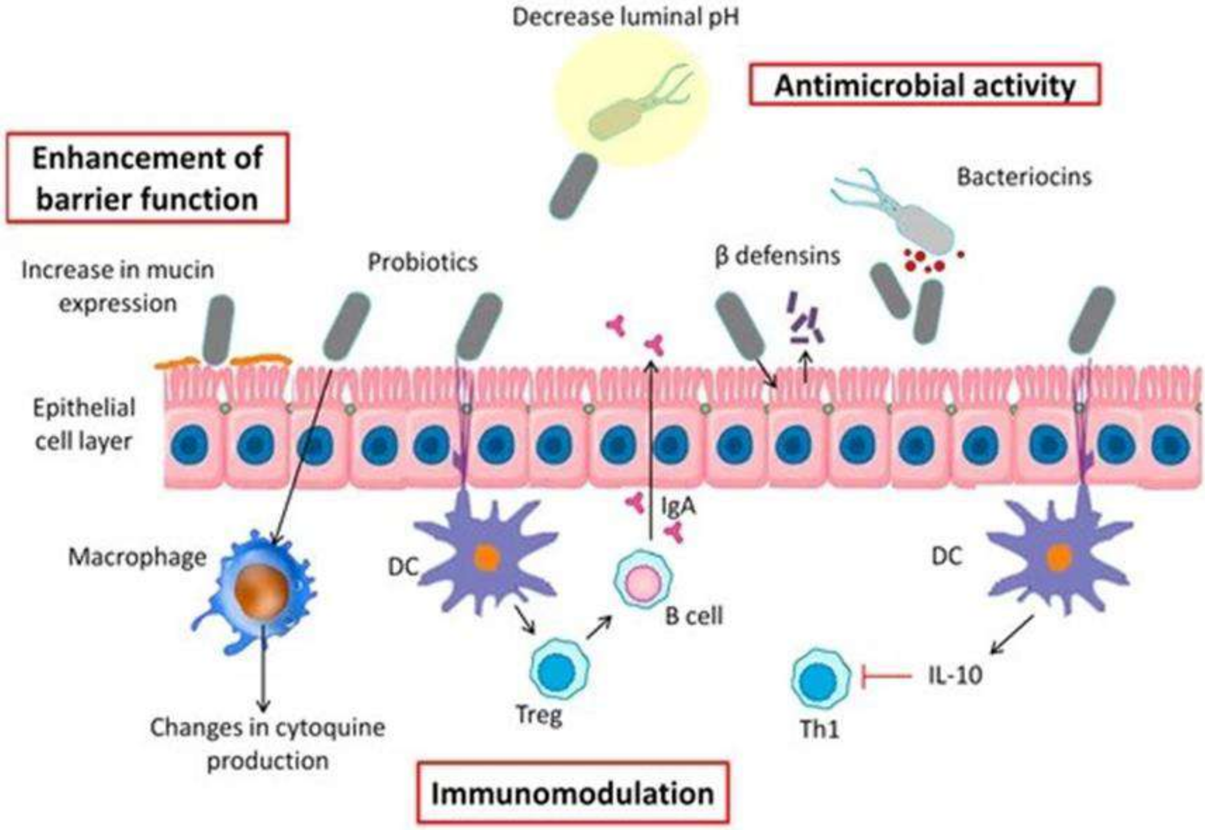

Fig. 1: Is a simplified illustration of the numerous tasks that probiotic bacteria may perform within the digestive tract. These functions include competing for attachment to mucosal and epithelial surfaces (exhibiting antimicrobial properties), inhibiting the growth of other microorganisms, promoting increased mucus production and barrier integrity (enhancing barrier effectiveness), and influencing immune system activity (modulating immune responses).

Gut Microbiota Role in Diabetes and Obesity

Obesity is one of the leading factors that enhance the likelihood of developing adult onset diabetes mellitus (T2DM) as part of the metabolic syndrome. It is a significant determinant for T2DM, causing 90–95% of all occurrences of the disease (Harsch and Konturek, 2018). Recently interest is increasing to find an association between obesity and gut microbiota. Early studies were focused on finding a difference in the composition of gut microbiota in obese and lean animal as well as human subjects and exploring relevance in these changes (Harsch and Konturek, 2018).

Gut Microbiota digest soluble dietary fibers and produce SCFAs like butyrate, acetate, and propionates (Tilg and Moschen, 2014). In addition to serving as a significant energy source, these SCFAs also play a part in controlling the amount of food and energy consumed (Hur and Lee, 2015). In comparison to healthy controls, T2 DM patients showed a decrease in butyrate-producing bacteria, including *F. prausnitzii* and *Roseburia* intestinalis, according to research by Qin et al. (Wang et al., 2012). In the Karlsson investigation, these results were validated in menopausal Type 2 diabetes mellitus female (Karlsson et al., 2013). In conclusion, T2 DM patients may lack gut bacteria capable of digesting plant polysaccharides into SCFAs.

Gut Microbiota Role in Type 1 Diabetes

Unlike obesity and T2DM, T1D is an autoimmune illness (Harsch and Konturek, 2018). Numerous theories have been put up regarding the role of gut microbiota in the etiology of insulin dependent diabetes (T1D). The mechanisms listed below describe the role that Microbiota play in type 1 diabetes;

- As one of the most significant consequences of microbial metabolisms, butyrate is involved in the stimulation of colonic T-regulator, the decreased activity of pro-inflammatory macrophages, and the improvement of gut barrier functionality by boosting the synthesis of mucin (Furusawa et al., 2013).
- Zonulin (a protein) is potentially a significant marker of digestive tract permeability and mucosal health. This protein alters the way macromolecules flow via intercellular connections.
- SCFA is created when the gut microbes consume and ferments fibers (Brestoff and Artis, 2013). By entering the bloodstream and influencing T-regulator differentiation, SCFAs suppress autoimmunity (Alkanani et al., 2015).

Prebiotics

The notion of prebiotics was initially presented by Glenn Gibson and Marcel Robertfroid in 1995 (Gibson and Roberfroid, 1995). Prebiotics, defined as non-digestible food ingredients that selectively stimulate beneficial microbes (bacteria) in the colon to enhance host health, have historically included only a few carbohydrates like lactulose, GOS, and β-fructans. The updated definition from the 6th ISAPP Meeting in 2008 expands this to "dietary prebiotics," which selectively ferment to alter gastrointestinal microbiota, benefiting host health. To qualify as prebiotic, a compound must resist stomach acidity, resist human enzyme hydrolysis, remain unabsorbed in the GIT, ferment by gut microbiota, and specifically enhance intestinal bacterial development or function (Gibson et al., 2010).

Mechanisms through which Prebiotic Effects Microbiota

Prebiotics can alter the makeup and activity of gut microbiota by giving these microbes energy sources (Flint et al., 2007). In phylogeny, distant bacterial species exchange their ability to routinely ingest a particular prebiotic (Scott et al., 2013).

However, according to some other research, a certain prebiotic can be degraded by a particular species. Bifidobacterium sp. fermentation of fructans (Maddalena et al., 2005) and starch (Alvaro et al., 2006) are two instances of this. Prebiotics can modify gut ecology by producing acidic fermentation products, reducing stomach pH. Even a minor pH shift from 6.5 to 5.5 can significantly alter gut microbiota composition (Duncan et al., 2009).

Prebiotic and Type 2 Diabetes

Nutrition treatment shows promise in managing and preventing type 2 diabetes. Diet is crucial for regulating blood glucose and metabolic abnormalities. Carbohydrate type, dietary fiber, food type, and specific components influence blood glucose impact. Studies indicate a negative correlation between BMI and dietary fiber intake. Therefore, a prebiotic diet rich in fiber may lower body weight and decrease type 2 diabetes risk. (Maki and Phillips, 2015).

A prebiotic diet may lower endotoxemia, a risk factor for diabetes (Cani et al., 2007). Regular fiber consumption can minimize glucose absorption, prevent weight gain, and boost essential nutrients and antioxidants, perhaps preventing diabetes (Slavin, 2013).

According to Hopping et al. (Hopping et al., 2010), those who take <15 g of fiber per day had a significantly low chance of developing T2D, indicating an inverse link. Another study found that those who consumed more insoluble fiber (>17 grams/day) or cereal fiber (>8 grams/day) had a lower chance of acquiring T2D (Meyer et al., 2000). Prebiotics by modulating the gut microbiota composition also have some other beneficial effects on body along with diabetes and weight management. Fig. 2 (S. O'Connor et al., 2017) highlights some of these beneficial effects of prebiotics.

Sources of Prebiotics

Prebiotics are found in many foods, including asparagus, beetroot, garlic, chicory, onion, Jerusalem artichoke, wheat, honey, banana, barley, tomato, rye, soybean, human and cow's milk, peas, beans, seaweeds, and microalgae (Varzakas et al., 2018). Due to their low values in foods, they are produced commercially. Prebiotics can be made from lactose, sucrose, or starch (Al-Sheraji et al., 2013).

Safety Levels for Prebiotics

High doses (40 to 50 grams/ day) and low doses (2.5 to 10 grams/ day) of prebiotics can result in osmotic diarrhea and flatulence respectively. It is to be noted that 2.5 to 10 grams of prebiotic is required to generate its beneficial effects on human health. The majority of prebiotic products on the market contain dosages ranging from 1.5 to 5 g per serving. In conclusion, prebiotics in their recommended range can cause mild to moderate side effects (Svensson and Håkansson, 2014).

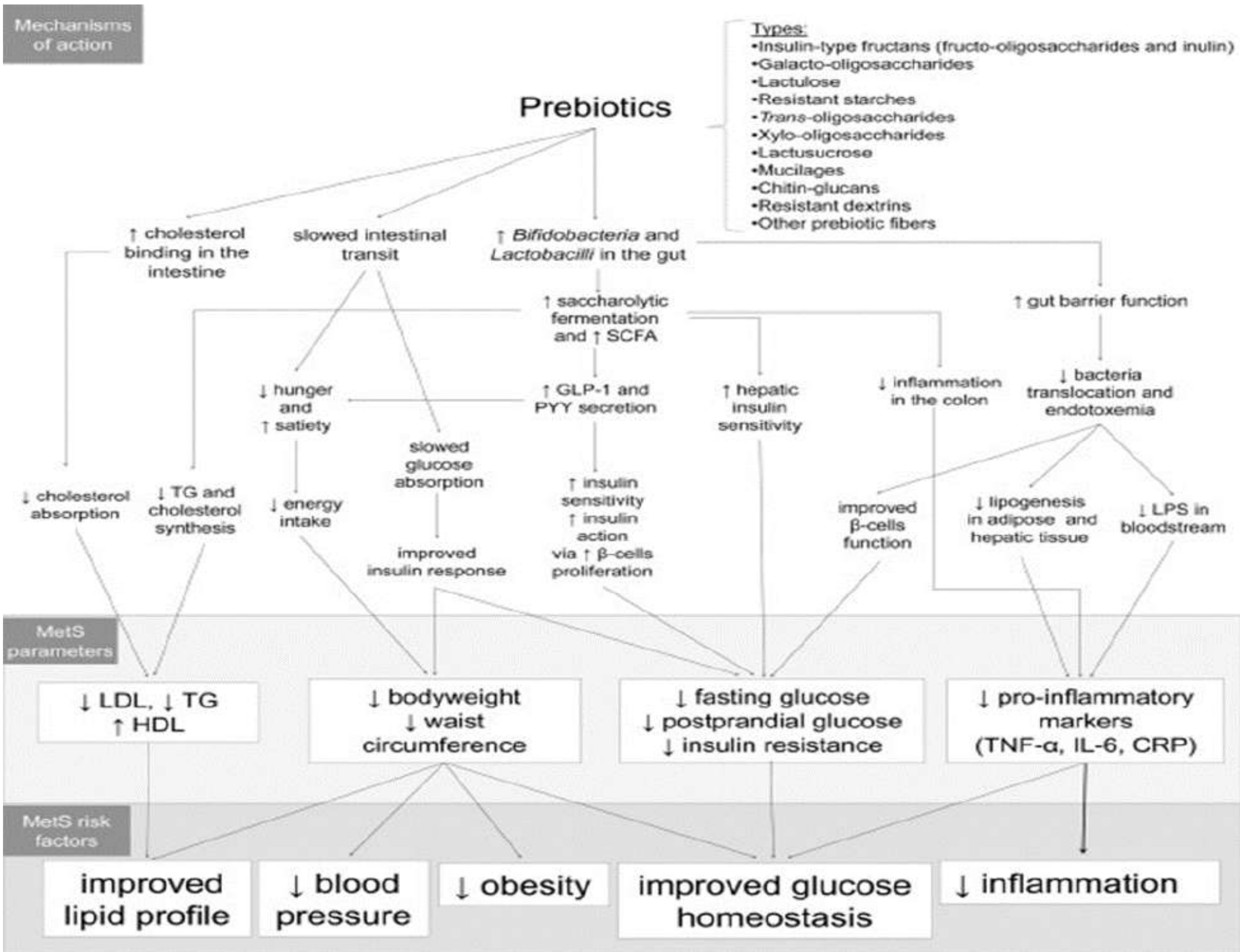

Fig. 2: Proposed mechanism of prebiotic activity in promoting health (S. O'Connor et al., 2017)

Probiotics

In 2001, the FAO/WHO Expert Consensus defined probiotics as living bacteria that provide health benefits when provided at suitable levels. Probiotics mostly consist of lactobacillus and bifidobacterium but also include yeast and bacilli (Meurman and Stamatova, 2007). Fermented foods, such as yogurt, and nutritional supplements sometimes include active living cultures.

Yeast, molds, and bacteria can all be probiotics. Probiotics are mostly bacteria, though. Among the list of bacteria lactic acid bacteria are the most common (Oyetayo and Oyetayo, 2005) in the production of probiotics.

Characteristics of a Good Probiotic

In 1989, Fuller (Oyetayo and Oyetayo, 2005) described the qualities of an excellent probiotic. 1) It must be a strain that can benefit the host animal by promoting better development or disease resistance. 2) It needs to be non-toxic and nonpathogenic. 3) It must exist as live cells, ideally in big quantities. 4) It must be able to endure and undergo metabolism in the environment of the gut, including being resistant to bile, low pH, and acidic substances. 5) It need to be stable in both field and preservation settings.

Food Sources and Uses

Probiotics may be found in foods including dairy products like (cheese, yogurt, and sour cream, ice cream) and non-dairy products include fruit smoothies, cereal, energy bars, baby formula, asparagus, and soybeans. Probiotics are beneficial for overall health, animal husbandry, soil fertility, and dental health.

Mechanism through which Probiotics Prevent Diabetes

Probiotics offer several benefits, and their methods of action in T2DM have been well examined (Panwar et al., 2013).

Numerous studies have explored the use of probiotics as a food supplement to prevent, treat, and manage metabolic complications, including diabetes (Bordalo Tonucci et al., 2017).Probiotics may improve T2DM by improving gut integrity, lowering LPS levels, increasing incretins, reducing ER stress, and enhancing peripheral insulin sensitivity (Balakumar et al., 2018).

Probiotics may help with diabetes by increasing glucose tolerance, modulating lipid metabolism, enhancing antioxidant status, gut flora, and SCFA configuration (Akbari et al., 2016). Probiotics can lower inflammation, immunological responses, and oxidative stress (Singh et al., 2017). The possible mechanism through which probiotics help in diabetes prevention is shown in figure 3 (Salgaço et al., 2019). Probiotics administered orally can affect microbes in the GIT, energy metabolism, and immune response. From this evidence, this can be concluded that probiotics can reduce T2DM incidence, delay and reverse its progression, and improve functionality at the start, development, and complications.

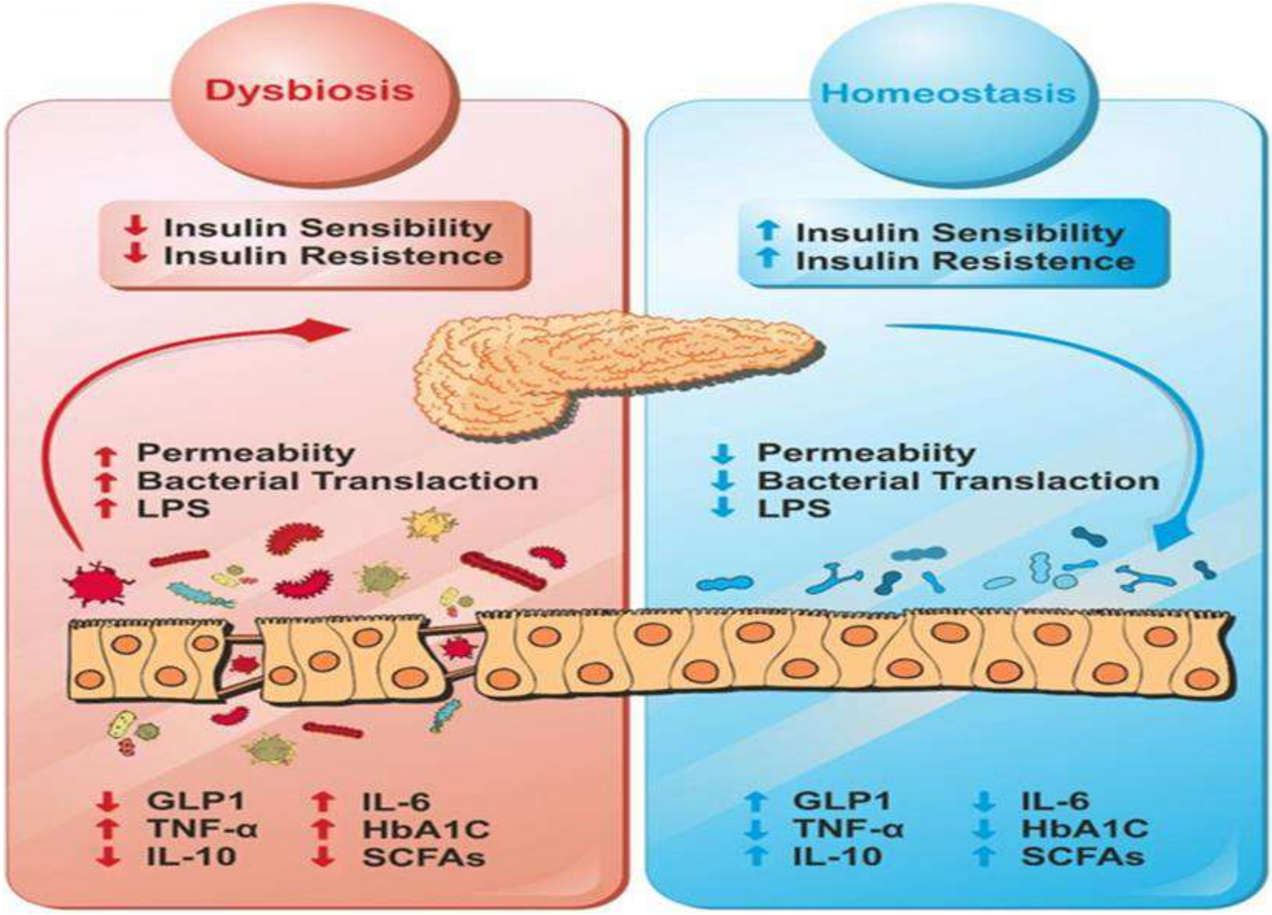

Fig. 3: The gut microbiota, in both balanced and disordered stages due to type 2 diabetes, influences the onset and prevention of the disease. Probiotics can improve the gut microbiota by increasing saccharolytic fermentation and short-chain fatty acid (SCFA) synthesis, thereby strengthening the intestinal barrier. Elevated SCFA levels promote the production of glucagonal peptide-1 (GLP-1), which is essential for regulating satiety, hunger, and insulin sensitivity, as well as reinforcing the intestinal barrier. This strengthened barrier reduces bacterial and lipopolysaccharide (LPS) translocation, lower inflammation causing markers such as interleukin-6 (IL-6) and tumor necrosis factor (TNF), increase anti-inflammatory markers such as interleukin-10 (IL-10), and increase glycosylated hemoglobin A1C (HbA1c).

Studies

Research suggests that consuming fermented dairy products, which include probiotics, is linked to a lower risk of T2DM (L. M. O'Connor et al., 2014). Other studies have found that probiotics help to prevent type 2 diabetes by enhancing glucose tolerance and insulin sensitivity (J. Chen et al., 2012). Lb. reuteri GMNL-263 delays T2DM occurrence by increasing the expression of PPAR-c and GLUT4, while decreasing the expression of lipogenic genes such Srebp-1c, FAS, and Elvol6 (Hsieh et al., 2013). According to (Gao et al., 2017), Lb. rhamnosus GG (LGG) reduces inflammatory cytokine expression, which is linked to diabetes development.

Probiotics Safety

Some probiotics may be able to prevent illnesses such as *C. difficile* and antibiotic-associated diarrhea, but there is lake of sufficient scientific data to support the widespread use of probiotics, particularly in healthy persons (Khalesi et al., 2019). Probiotics are promoted as having a range of health advantages, including supporting immunological, respiratory, digestive, cardiovascular, reproductive, and mental health.

The actual prevalence of accidental infections is unknown because of limited reporting during clinical trials and poor surveillance following product distribution. Serious adverse events, including fungemia and bacteremia, have been recorded (Cohen, 2018).

Conclusion

The intertwining relationship between gut microbiota and diabetes is multifaceted. In Type 1 Diabetes (T1D), autoimmune mechanisms target pancreatic cells, while in type 2 Diabetes (T2D), insulin resistance and metabolic disturbances prevail. Gut microbiota, essential for digestion and health, play a pivotal role in both conditions. The composition and functionality of gut bacteria influence diabetes development and progression. Prebiotics, non-digestible food compounds, and probiotics, living microorganisms, offer potential therapeutic avenues. Prebiotics, found in various foods, nourish beneficial gut bacteria, potentially reducing T2D risk and enhancing metabolic health. Probiotics, primarily lactobacilli and bifidobacteria, modulate gut flora, bolstering immunity and metabolic function. While promising, further research is needed to elucidate their full impact and ensure safety. Overall, harnessing the power of prebiotics and probiotics may offer novel strategies in the prevention and management of diabetes.

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Chapter 20

Probiotic and Prebiotic Modulation of Gut Microbiota in Individuals with Obesity

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ABSTRACT

Gut dysbiosis is implicated in the pathophysiology of obesity, which makes intestinal bacteria modulation an important therapeutic target. The intestinal microbiome features, such as its composition, relative levels, diversity index, and functional pathways of the microbiota are involved in obesity pathogenesis. Therefore, therapeutic options that target the modulation of these characteristics could have the potential to ease the prevalence of obesity/overweight. Gut dysbiosis is reported in obesity, modulating intestinal bacteria appears relevant in the treatment of the disease. From the human and animal trials investigated, various effects of prebiotics and probiotics have been reported. Reduction of body weight and body fat, suppression of serum lipids, improving glucose levels in plasma, suppression of pro-inflammatory cytokine expression and promotion of anti-inflammatory genes, preservation of gut barrier integrity, and enhancing the production of beneficial bacteria while reducing pathogenic microorganisms are some of the effects noted. However, there remains a gap in human trials of prebiotics and probiotics and more research can be done to determine their anti-obesity impact and how it relates to gut microbiota modulation to ensure the right bacterial composition balance and reduce dysbiosis. In this chapter, various prebiotics and probiotics have been assessed for their anti-obesity effect and the mechanisms of action of select supplements have been documented.

KEYWORDS Gut microbiota, Obesity, Probiotics/Prebiotics, Microbiome, SCFA	Received: 01-May-2024 Revised: 15-Jul-2024 Accepted: 04-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

The wide prevalence of obesity worldwide and its multifactorial pathophysiology complicate its treatment, with the disease also implicated as a risk factor for the development of other non-communicable conditions. Obesity is characteristically defined by an excess of body fat, but the age and rate of onset of excessive adiposity vary due to its complex and idiosyncratic etiology involving inherited, behavioral, and environmental factors (Archer and Lavie, 2022). It is mainly caused by imbalanced intake and expenditure of energy due to a sedentary lifestyle combined with overnutrition (Jia and Liu, 2021). Adipose tissues score excess nutrients in the form of triglycerides to be utilized by other tissues through lipolysis in conditions of nutrient deficiency. The efforts to curtail the increasing prevalence of obesity have not yielded much results, but research is advancing regarding developing a clearer understanding of the disease etiology and the impact it has on cardiometabolic diseases including type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), dyslipidemia, and hypertension (HNT) (Hayden, 2023). Obesity is not just associated with comorbid conditions but there is an increase in mortality linked to obesity and overweight and its comorbidities. There is growing interest globally in diagnosing obesity early as part of reducing its prevalence and the rates of morbidity and mortality caused by the disease. Global healthcare systems are considering all potential strategies that can combat obesity, ameliorate patient suffering, and reduce the social and treatment costs of the disease.

Gut microbial factors have gained prominence in disease pathology over the past years as researchers increasingly investigate the role of gut microbiota and its alteration in disease states. Human gut hosts a diverse microflora where Firmicutes and Bacteroidetes constitute up to 90% of the gut microbiome (Saravanan et al., 2023). Obesity impairs the composition of gut microbiota due to factors potentially linked to disease onset, such as excessively consuming high-energy foods, sugars, and fats, and low intake of fiber and physical inactivity (Tassoni et al., 2023). Due to the critical role of the human gut microbiota in obesity and its comorbidities via the mechanisms of adiposity and glucose metabolism, novel therapeutic approaches are trending towards modulation of the intestinal bacteria. Compare the diversity and stability of the gut microbiota in health versus the gut microbiome composition in obesity where the Bacteroidales genera and the ratio of Firmicutes to Bacteroidetes and the Enterobacteriaceae species are upregulated while Clostridia and Enterobacter spp. are downregulated. Targeting a modification of this imbalance is an important therapeutic goal in patients with obesity/overweight (Geng et al., 2022).

There remains a strong need for effective pharmacological treatment options for obesity as only a few drugs have shown sufficient efficacy and safety. Therapeutic alternatives have traditionally focused on optimizing lifestyle and behavior in obesity, but these have not been sufficient in most cases and considerations have been made to have surgical and pharmacological interventions that are necessary (Kloock et al., 2023). Indeed, bariatric surgery remains the most effective treatment option as it has shown efficacy in reducing body weight decreasing CVD-related deaths by 30%, and increased the overall life expectancy for patients by 3 years (Carlsson et al., 2022). However, bariatric surgery is still not accessible to patients in need, and it is associated with numerous adverse perioperative and postoperative outcomes, meaning newer therapeutic strategies are needed (Kosmalski et al., 2023). Due to the central role of the human gut microbiome in modulating obesity outcomes, considerations have been made for the use of prebiotics and probiotics as human and animal clinical trials have suggested potential beneficial effects on the physical, biochemical, and metabolic parameters related to obesity (Cerdó et al., 2019). Accordingly, this chapter will explore the use of various prebiotics and probiotics in treating obesity/overweight, with the investigation focusing on the mechanisms involved in this sort of treatment.

Composition of Gut Microbiota in Obesity

The Human Microbiome Project has aided the study of gastrointestinal (GI) microbiota composed of *Firmicutes*, *Bacteroides*, *Proteus*, *Actinomycetes*, *Verrucomicrobia*, and *Fusobacteria*, the prominent microorganisms in the gut of a healthy individual (Noor et al., 2023). The diversity and robust composition of intestinal microbiota are crucial for re-establishing equilibrium following disturbances and are indicative of a healthy gut. Any interference with the balance in the microbiome affects energy metabolism and the absorption of nutrients. Healthy gut microbiome is highly diverse and has a sign of dynamic equilibrium meaning it is capable of resisting perturbation and restoring its healthy state (Liu et al., 2021). Comparably, the gut composition of obese subjects based on the *Firmicutes/Bacteroidetes* ratio based on a mouse model indicated that *Firmicutes* were higher while *Bacteroidetes* were lower compared to lean subjects (Zsálig et al., 2023). However, the evidence is not conclusive, thereby warranting further research to confirm the association of intestinal microbiota with obesity.

Mechanisms of Gut Dysbiosis in Obesity

Gut microbiota dysbiosis causes disequilibrium in energy homeostasis leading to obesity. There are numerous mechanisms implicated in intestinal microbiota inducing obesity. Dahiya and colleagues (2017) describe the mechanism of gut dysbiosis in obesity using the mice model analysis of *ob/ob* mice versus lean *ob/+* and wild-type counterparts. They explain that genetically obese mice have significantly more *Firmicutes* and fewer *Bacteroidetes* compared with lean mice, and the *Firmicutes* are involved in drawing more calories from diet leading to obesity. In Pediatric obesity, *Firmicutes* were found to be more expressed while *Bacteroidetes* were less in the subjects' guts, and their development of obesity correlated with the high expression of short-chain fatty acids (SCFAs) noted in the stool of obese children, particularly acetate, propionate, and butyrate compared with normal-weight participants (Riva et al., 2017).

Bad gut microbiota is associated with high adiposity, insulin resistance, and dyslipidemia due to higher levels of *Firmicutes* versus low *Bacteroidetes* (Brenton et al., 2022). Gut dysbiosis is also linked with incretin impairment in overweight individuals as the less production of gut hormones like peptide YY (PYY), glucagon-like peptide-1 receptor (GLP-1), and neuronal nitric oxide synthase (nNOS) in the enteric nervous system contributes to the impaired gut-brain-periphery axis that controls insulin secretion and gastric emptying (Yamane and Inagaki, 2022). An impaired intestinal microbiome has been shown to exacerbate low-grade inflammation in the gut by releasing lipopolysaccharides (LPS) which can cross the intestinal epithelium and result in adiposity (Kang et al., 2023). Gut microbial diversity shifts worsen the level of triglycerides and cholesterol involved in adipogenesis, lipolysis, and fatty acid oxidation (Patra et al., 2023). All these mechanisms are important in the pathophysiology of obesity as well as its therapeutic approaches because rectifying gut microbiota dysbiosis and fostering a healthy intestinal microbiome is essential.

Probiotics Treatment in Obesity

Bifidobacterium

A human trial investigating the effect of *Bifidobacterium longum* BB536 and *Bifidobacterium breve* MCC1274 was done by Sato and colleagues (2024) where they assessed body composition outcomes in a randomized controlled investigation of candidates having age between 29 and 64 years. The 100 participants were assigned to 3 groups receiving *B. longum*

BB536, *B. breve* MCC1274, and control without *bifidobacteria* over a 16-week treatment trial. The basic result measurement was body composition where the abdominal fat was recorded, alongside the secondary outcomes of anthropometric data (weight, BMI, waist-hip circumference ratio, and waist circumference), and blood parameters (serum lipids, blood glucose modifiables, liver activity, and inflammation indicaters). According to the findings, probiotics reduced visceral fat area and total fat area significantly relative to control, while subcutaneous fat area did not change significantly. Body weight, BMI, fat percentage, and body fat mass all increased significantly in the control group between baseline and week 16, but not in the probiotic group.

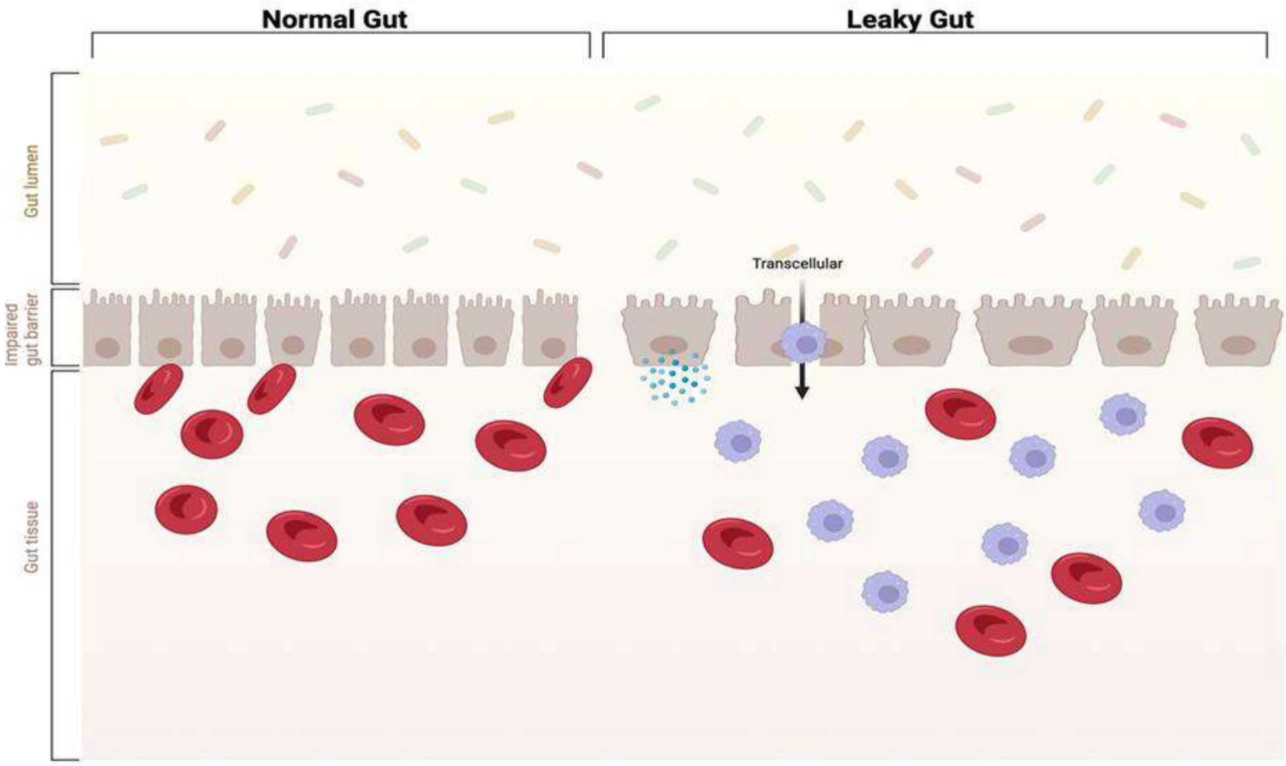

Fig. 1: Leaky Gut Promotes Weight Gain in Obesity due to Systemic Inflammation

Probiotics significantly suppressed BMI relative to placebo, and the serum triglyceride (TG) levels were further reduced markedly with probiotics administration compared to placebo. Overall, *B. longum* BB536 and *B. breve* MCC1274 intake significantly decreased visceral and total fat area in healthy normal, and overweight adults relative to placebo.

Sung et al. (2023) further administered *Bifidobacterium breve* B-3 (BB3) to healthy individuals and randomized them to placebo and treatment groups and the endpoints explored were changes in body mass, body fat percentage, changes in lean mass, total fat area, subcutaneous fat area, visceral fat area, and the visceral fat/subcutaneous fat area ratio. Based on the results, BB-3 administration significantly lowered body fat than baseline, and while the placebo group also experienced lower fat, the difference was not significant. The body fat was significantly lower in the treatment group vs placebo, and the body fat percentage decreased in both groups but the differences were not statistically significant. After BB-3 administration, BMI, waist circumference, and hip circumference were also significantly lower than before treatment. The decrease in visceral fat was thus associated with a reduction in body fat mass after BB-3 treatment.

Lactobacillus gasseri

Kawano et al. (2016) conducted an animal trial of the anti-obesity effects of the probiotic *Lactobacillus gasseri* SBT2055 (LG2055) where HFD-induced obese mice were treated for 21 weeks with the probiotic. The subjects were randomized into 3 groups: norma-fat diet (NFD), HFD, or HFD with LG2055 (HFD-LG) researchers explored the anti-inflammatory and gut barrier-saving consequences of the treatment. The outcomes of the experiment disclosed that LG2055 execution prevented rises in the body and adipocyte weights in HFD-LG in spite of similar energy consumption with the HFD and HFD-LG groups. Immune cell population analysis in the epididymal adipose tissue showed that the HFD-LG group exhibited significantly larger proportions of anti-inflammatory M2 macrophages and statistically lower M1:M2 ratio compared with the HFD group, confirming intake of the probiotic ameliorated HFD- incited temperament in macrophage composition to a pro-inflammatory rank. Further analysis was done for the T cell subpopulation being concerned with adipose tissue inflammation, where HFD was shown to reduce the CD4 T cell subpopulation compared to the NFD, with no difference in CD8-positive T cells. With LG2055 administration, there was a decrease in the proportion of CD8-positive cells meaning the probiotic- regulated segment of adipose tissue T cell subpopulation, thereby preventing inflammation. Subsequent analysis of intestinal permeability to FD-4 dextran revealed that the intestinal barrier was disturbed in the HFD group, as evidenced by greater plasma concentrations of FD-4 than in the NFD group. LG2055 administration exhibited a preventive effect against the increase in gut permeability caused by HFD as demonstrated by lower plasma FD-4 concentration than HFD and almost similar to that in the NFD group. LG2055 supplementation additionally protected against the entrance of bacterial lipopolysaccharides (LPS) into the portal vein in on the intestinal barrier.

Lactobacillus amylovorus

A randomized controlled trial on the effect of *L. amylovorus* CP1563 on lipid metabolism in overweight and mildly obese individuals was conducted by Nakamura et al. (2016) where 300 participants were assigned into two groups to take the probiotics vs placebo over 12 weeks of treatment. The assessment of BMI, body fat percentage, abdominal fat area, and whole body fat area was done and the researchers found time-dependent changes as the test group showed a significantly larger reduction than placebo. At the end of treatment, there were significant decreases in triglyceride and total and LDL-cholesterol levels in plasma, as well as significant differences in HDL-c between the placebo and probiotic groups, and CP1563 affected blood glucose, insulin, and homeostasis model assessment-insulin resistance (HOMA-IR) where significant differences were noted between treatment and placebo. Bilirubin and uric acid were significantly reduced by the probiotics. Overall, the study confirmed *L. amylovorus* had body fat-lowering effects and affected lipid, glucose, and uric acid metabolism in participants.

Sugawara et al. (2020) also explored *L. amylovorus* effects in healthy subjects to understand its ability to prevent obesity and affect the gut microbial community in pre-obese participants. In the experiment, 69 healthy subjects ingested beverages with or without the probiotic, and changes in abdominal, total, visceral, and subcutaneous fatty areas were measured, as well as the abundances of gut bacteria compared. It was shown that *L. amylovorus* significantly lowered TFA, VFA, and SFA at 12 weeks after the intervention compared to the control group, and the body weight and BMI were significantly lower in the treatment group than placebo. The fecal microbiota and SCFA analysis exhibited probiotic effects related to significantly greater alterations in *Roseburia* and *Lachnospiraceae* in the treatment group than placebo, and changes in *Collinsella were* significantly smaller in the treatment category compared to placebo. The findings were interpreted to mean butyrate-producing bacteria were enhanced in the subjects, with butyrate able to activate PYY and GLP-1 secretion through endocrine L-cells to inhibit lipid accumulation via suppression of insulin signaling.

Pediococcus pentosaceus

A study investigated the effects of *Pediococcus pentosaceus* LP28 effect in reducing body fat and weight where 62 subjects aged between 20 and 70 years were randomly assigned to 3 groups receiving living LP28, heat-killed LP28, and placebo powder once daily for 12 weeks (Higashikawa et al., 2016). In the clinical trial, the main study outcomes were BMI, body fat percentage, body fat mass, lean body mass, and waist circumference, as well as the main outcomes in serum variables. The findings showed that while BMI increased in the placebo group, there was a significant difference observed in BMI change between placebo and heat-killed LP28. In the heat-killed probiotics, there was a small reduction in BMI, as well as a decrease in the average body fat percentage observed. Body fat mass was reduced in the living LP28 group, and waist circumference decreased in the heat-killed LP28 group different from placebo. Additionally, the serum outcomes did not differ due to the administration of the probiotic showing no anti-obesity effect.

Prebiotics Treatment in Obesity

Whole-Grain Oat

Oats or oat-based cardiometabolic effects have been explored in research, such as in Connolly et al., (2016) RCT where they examined how whole grain oat Granola (WGO) impacts the human gut microflora and cardiometabolic danger elements in at-risk individuals. Over the course of two 6-week intervention periods separated by a 4-week washout period, 32 patients in the trial were randomized into two groups and given 45 g of either non-whole grain (NWG) or whole grain oatmeal (WGO) breakfast cereals daily. WGO contains β-glucan prebiotics which have been hypothesized to possess anti-cholesterol effects in metabolic conditions like obesity. Based on the analysis of fecal bacteria and SCFAs, it was shown that after WGO consumption for 6 weeks, bifidobacteria numbers and total bacterial community prominently raised in comparison to control, while NWG reduced bifidobacterial and total bacteria population over the same period. The blood lipid parameters also indicated WGO significantly reduce TC concentrations in contrast to NWG which significantly increased TC levels as well as plasma LDL-C. After measuring inflammatory indicators and insulin sensitivity/resistance, it was discovered that WGO cereal decreased fasting plasma glucose, which was lower at the conclusion of therapy than it was following NWG. Following treatment with either cereal, there was no change in the levels of inflammatory biomarkers, such as TNF-alpha, IL-6, CRP, and calprotein. GLP-1, PYY, BMI, weight, and fat mass were not altered significantly. Overall, the stimulation of gut bacteria by β-glucans was suggested as the mechanism of plasma cholesterol reduction when participants consume prebiotic oats.

Geliebter et al. (2015) assessed the satiety effects of a high-fiber cereal comparing oatmeal, isocaloric cornflakes, and water in 36 subjects who were assigned to three conditions on different days. The ratings of hunger and fullness were obtained with blood samples for measuring glucose, insulin, glucagon, leptin, and acetaminophen (gastric emptying tracer), and appetite assessed based on food intake. Subsequently, the results indicated for all subjects, oatmeal breakfast was associated with elevated fullness compared with water or cornflakes and the area under a curve (AUC) was small for hunger after oatmeal. AUC glucose was also higher for oatmeal than cornflakes, both greater than water, and the traditional glycemic area showed similar results as the glucose measure. There was no marked difference in insulin AUC values for oatmeal and cornflakes, both of which were greater than water. The other measures of leptin and acetaminophen did not differ by breakfast condition. Overall, satiety feelings were greater for oatmeal compared with corn flakes and water, which was attributed to potential slower gastric emptying due to beta-glucan and insoluble fiber in oatmeal. As confirmed in Mathews et al. (2023), cereal beta-glucan effect on body weight and BMI reduction is potentially associated with the mechanism of improving satiety perception, promotion of beneficial gut microbiota, slower gastric emptying, and the production of SCFAs involved in appetite and energy regulation.

Arabinoxylan Oligosaccharides

Arabinoxylan oligosaccharides (AXOS) supplementation in obesity has been shown to potentially have a beneficial effect as a new class of candidate prebiotics. Hosoda and colleagues (2017) conducted an animal trial of dietary steamed wheat bran (WB) for its effects in mice to understand the mechanism responsible for anti-obesity of arabinoxylan (AX) on postprandial energy metabolism and blood variables. Male mice were fed an isocaloric diet with or without WB and energy metabolism evaluated and plasma glucose, insulin, and glucose-dependent insulinotropic polypeptide (GIP) levels were measured. WB was shown to have significantly higher fat oxidation and lower carbohydrate oxidation compared with control. The postprandial blood total GIP was significantly lower in mice fed the WB diet compared with the control, while plasma glucose and insulin levels were not markedly different, although they were lower in the WB group. Arabinoxylan alone was also investigated for its effects and it was confirmed to affect the postprandial blood total GIP response which was significantly lower in the subjects, and the overall postprandial blood glucose response was also lower. The effect of AX on postprandial energy metabolism showed greater postprandial fat oxidation while carbohydrate oxidation was not significantly different. Taken together, the findings confirmed dietary steamed AX was responsible for the WB effect in increasing postprandial fat utilization and reduced postprandial blood GIP with the mechanism involved suggested to be related to the reduction of nutrient absorption and increasing the expression of proteins responsible for fatty acid oxidation and increasing lipolysis.

Oligosaccharides (OS)

Respondek et al. (2013) examined the modulatory influence of short-chain fructo-oligosaccharides (scFOS) on intestinal microbiota composition and host metabolic regulation using animal models of diet-originated obesity with humanized microbiota. In the experiment, 48 axenic C57BL/6J mice were introduced with fecal human microbiota samples and randomly allocated to 3 diets: control, HFD, or isocaloric HFD with 10% of scFOS (HF-scFOS). Following the statistical analysis of the data, the researchers found that the fecal microbiota of the subjects that received scFOS had a high proportion of Bifidobacteria and *Clostridium coccoides* but less *Clostridium leptum* group than control, and the *Bacteroide*s - *Prevotella* group/*C. coccoides* was markedly lower. As for feed intake, HF-scFOS mice ate less than HFD, and the HF-scFOS group weighed less than the ones on HFD alone, with the higher fat mass from HFD partly diminished by scFOS. The mice acquiring scFOS also presented massive empty and full caecum and the muscle mass was dense with HFD than the other two groups. Triglycerides were higher in HFD groups than in control, and adiponectin was significantly nether in HF-scFOS versus control leptin lower in HF-scFOS than the other groups, and insulin was higher for HFD without scFOS. Overall, the study confirmed that scFOS stimulated Bifidobacteria growth, increased *C. coccoides* while reducing *C. leptum*, decreased body weight and eliminated fat mass rise due to a lower feed intake, and lowered insulin concentration and blood leptin as scFOS promoted SCFA fermentation to induce leptin secretion from adipose tissue.

Nicolucci et al. (2017) further assessed the anti-obesity effects of prebiotics in a single-center, double-blind, placebo-controlled trial involving children aged 7-12 years who were given either oligofructose-augmented inulin or maltodextrin placebo once daily for 16 weeks. The researchers then analyzed blood samples for lipids, cytokines, LPS, and insulin, as well as bile acids profiled from fecal samples and microbiota composition analyzed. For body weight, it was shown that prebiotic intake lowered gain in weight in comparison to placebo, and the percentage of total body fat dropped noticeably, with OI. For the systemic inflammation measures, the prebiotics decreased serum CRP, which was improved in the control group, although the differences were not significant. IL-6 also significantly reduced from baseline with OI administration, and LPS decreased. The metabolic outcomes revealed prebiotic consumption significantly reduced serum TG, with no variations in fasting glucose, insulin, or HOMA-IR. Fecal bile acid analysis showed significant differences in cholic acid and chenodeoxycholic acid (CDCA) between OI and placebo. Gut microbiota changes further indicated escalated *Bifidobacterium* spp. in prebiotic and decreased *Clostridium* cluster XI in placebo.

Pectin

A study combining pectin (P) and oligofructose (OFS) was conducted to determine the effect on satiety and glycemic parameters based on reducing energy intake compared to control (Savastano et al., 2014). In the RCT, the researchers categorized the participants into OFS+P apex-dose, OFS+P bottom-dose, and maltodextrin control involving 96 subjects aged 18-60 years who were treated over 3 weeks. A shift in calorie intake from baseline served as the primary productive metric, and body weight, fasting and postprandial glucose levels, subjective appetite ratings, and insulin values represented as AUC were the secondary measures. Because of this, the researchers discovered no statistically significant variation in energy intake across the treatment groups, despite a minor decrease for both low-dose and high-dose OFS+P. Appetite ratings also indicated no statistically significant differences among the treatment groups. Minimal change was noted in fasting glucose from baseline for all groups, but the control group glucose AUC increased from baseline, while there was a larger reduction in FG in the apex-dose versus bottom-dose groups. Fasting insulin was greater in the control as compared to other groups at 3 weeks, while fasting insulin reduction was highest in high-dose compared with the low-dose group. Mean HOMA-IR was also minimized in the treatment groups with OFS+P compared with control, while body weight reduction was not statistically significant.

Treatment	Mechanism of Action	References
Probiotics		
Bifidobacterium	Fat reduction, BMI suppression, reduced serum TG, body weight reduction, improved glucose tolerance, FG reduction, lowered waist and hip circumference	(Sato et al., 2024; Sung et al., 2023)
Lactobacillus gasseri	Reduced body and adipose tissue weight, upregulated anti-inflammatory genes, T cell modulation, restored gut barrier integrity, LPS downregulation, microbiome modulation, cytokine upregulation,	(Kawano et al., 2016)
Lactobacillus amylovorus	Reduced TG and total and LDL-c levels, blood glucose, insulin, and HOMA-IR, decreased bilirubin and uric acid, lowered body fat, body weight, and BMI, SCFA production, microbiota modulation, activated satiety hormones	(Nakamura et al. 2016; Sugawara et al., 2020)
Pediococcus pentosaceus	BMI reduction, body fat percentage, body mass, waist circumference, *CD36* gene expression downregulation	(Higashikawa et al., 2016),
Prebiotics		
Whole-grain oat	Beneficial bacteria proliferation, reduced TC concentrations, reduced FPG, reduced insulin, systolic blood pressure, fat mass, LDL-c, promoted satiety, slower gastric emptying, SCFA production	(Mathews et al., 2023)
Arabinoxylan Oligosaccharides	Decreased energy density, reduced body weight gain, reduced fat mass, blunted caloric intake, the proliferation of beneficial bacteria and prevented pathogenic bacteria, satiety hormones, reduced hyperinsulinemia and HOMA-IR, increased ZO-1 expression, fatty acid oxidation, lipolysis	(Hosoda et al., 2017)
Oligosaccharides	Beneficial bacteria proliferation, reduced caloric intake, decreased body weight and fat mass, lower insulin concentration, SCFA fermentation, decreased inflammatory markers, serum LG reduction, bile acid synthesis	(Nicolucci et al., 2017)
Pectin	Lower energy intake, reduced FG, fasting insulin, HOMA-IR, reduced body weight gain and fat mass gain, satiety hormones, increased SCFA	(Adam et al., 2016)

Fig. 2: Probiotic/Prebiotic Treatment Action in Gut Microbiota Modulation

Similarly, Adam et al. (2016) administered high-fat control, high fat with high protein, or both diets with 10% pectin to adult diet-induced obese rats for 4 weeks to determine the mechanisms of anti-obesity of the prebiotic. The study's findings demonstrated that, in comparison to the control diet, HF with high casein (HFHC), or high pea protein alone (HFHP), the rats in the three pectin-containing diets saw slower body weight growth over the intervention period. Supplementary pectin and high protein reduced overall body weight gain and fat mass gain, with significant pectin x protein interaction noticed as pectin effects were greater with HFHC than with HFHP diet. In addition, there was no pectin x protein interaction and a substantial drop in final body weight, total body fat mass, and body fat percentage while lean percentage rose with supplemental pectin therapy but not with high protein. Daily food intake also decreased the most with HFHC+P in a time-dependent manner, showing pectin significantly reduced cumulative food intake but not by high protein, with significant pectin x protein interaction. PYY and total GLP-1 plasma concentrations rose in response to further pectin treatment, but not in response to high protein or the absence of pectin x protein interaction.

Similar to how plasma insulin fell with pectin and high protein administration without an interaction between the two treatments, plasma leptin reduced with pectin and high protein administration but not with high casein protein and no pectin x protein interaction. The glucose/insulin ratio was improved by pectin, but no PG was not significantly unalike between diet groups. Caecal SCFA analysis indicated increased SCFA concentrations in the three + P groups and the HFHP group but not dissimilar in HFHC in contrast to HF. Pectin increased the concentrations of acetate and propionate as well as by high pea protein, while high pea protein increased butyrate but decreased by pectin. Pectin increased succinate concentrations while decreasing BCFAs iso-butyrate, valerate, and iso-valerate while high pea protein increased their concentrations.

Conclusion and Future Research Directions

Obesity is highly prevalent worldwide and its multifactorial pathophysiology complicates treatment besides being a risk factor implicated in other noncommunicable diseases. However, advancing research on the link between gut microbiota and obesity has enabled a new therapeutic target in intestinal modulation with prebiotics and probiotics as part of alleviating disease and reducing the associated burden of mortality and morbidity. Bariatric surgery has been the most effective treatment option but its accessibility and adverse outcomes make it imperative to consider novel therapies for patients. In this chapter, it has been confirmed that modulating the gut microbiome with prebiotics and probiotics has the potential to effectively treat obesity. Based on experimental evidence in both human and animal trials, the mechanisms of action of select prebiotics and probiotics have been outlined in this research. The effects of body weight reduction and decreasing body fat by reducing energy intake, as well as lowering blood glucose and promoting the proliferation of beneficial bacterial strains have been documented. Prebiotics and probiotics also have anti-inflammatory effects in obesity and promote gut barrier integrity as part of its therapeutic impact in obese/overweight individuals.

Based on the research, future research directions could include more human trials to test the efficacy documented in preclinical studies using animal models. Prospective studies can also focus on specific mechanisms of action, like testing the impact on serum lipid concentrations alone, blood glucose, or body weight/fat as a way to clarify the therapeutic outcomes. The mechanism of achieving the right bacterial balance in the gut microbiome also remains to be studied conclusively and this can be the focus of future studies.

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Chapter 21

Yeast Based Therapeutics for Gastrointestinal Disorders

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ABSTRACT

Globally, gastrointestinal diseases (GIDs) exerts a major burden on healthcare systems, requiring novel treatment methods. *Saccharomyces cerevisiae*, along with other yeast strains, has encouraging probiotic effects through the restoration of gut microbial balance and the mitigation of inflammation, providing a new therapy option for GID. Furthermore, substances produced from yeast, such as mannan oligosaccharides and β-glucans, have shown to be effective in treating GIDs by binding pathogens and toxins and enhancing gut health. Moreover, recombinant proteins and monoclonal antibodies—which are yeast-based biologics—provide novel treatments for a range of gastrointestinal disorders (GIDs), including Helicobacter pylori infections and inflammatory bowel disease. Notwithstanding these developments, issues with efficacy, safety, and regulatory approval still exist, highlighting the necessity for more study to maximize the security and efficacy of yeast-based treatments for GIDs. Nonetheless, yeast-based therapeutics present a diverse and promising strategy for managing GIDs, highlighting the potential to improve patient outcomes and quality of life in individuals suffering from these debilitating conditions.

KEYWORDS		
Gastrointestinal diseases, Probiotics, *Saccharomyces cerevisiae*, Yeast, Yeast-Based Biologics	Received: 13-May-2024 Revised: 18-July-2024 Accepted: 19-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Gastrointestinal disorders (GIDs) refer to the disorders affecting the digestive system of individuals. Irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastrointestinal reflux disease (GERD), peptic ulcer disease, enteritis, diarrhea, constipation and leaky gut syndrome are some examples of gastrointestinal disorders (Han and Heitkemper 2019). The alternation in the composition of gut microbiota causes gastrointestinal disorders. Different individuals have different microbial communities in their digestive tract. The gut microbiota of humans is mostly populated by 99% of bacteria (Gorkiewicz and Moschen 2018). The traditional treatment mainly focuses on symptomatic treatment of GIDs without tackling the underlying causes. The recent advancements in medical research reveal the yeast-based therapeutics as a promising approach for gastrointestinal disorders. Yeast based therapeutics offer a novel approach for restoring the balance of gut microbiota. In this way the underlying causes of gastrointestinal disorders are being treated.

Yeast based therapeutics have probiotic properties, the probiotic property of various strains of yeast such *Saccharomyces cerevisiae* confers health benefits and restore the microbial equilibrium (Sen and Mansell 2020). In addition to probiotic properties, yeast-based therapeutics also have anti-inflammatory properties. Yeast derived compounds such as β -glucans modulate the immune response and reduce the inflammation of the gut. This anti-inflammatory property promotes healing and reduces the symptoms that are associated with gastrointestinal disorders. Moreover, yeast-based therapeutics also improve the barrier function of the gastrointestinal tract by improving the integrity of the gut barrier. In this chapter, we explore gastrointestinal disorders, yeast-based therapeutics such as yeast-based probiotics, yeast derived compounds and yeast-based biologics for the treatment of gastrointestinal disorders. This chapter also includes the advantages and limitations of yeast-based therapeutics for gastrointestinal disorders (GIDs).

What are Yeast Based Therapeutics?

Yeast based therapeutics can be defined as the production of biopharmaceuticals and vaccines by using different species of yeast. Yeast-based systems are particularly advantageous in drug development efforts for these complicated disorders, despite several drawbacks (Pereira et al., 2012). The common infection known as vulvovaginal candidiasis is primarily caused by *Candida albicans*, which disrupts the vaginal microbiota. *Saccharomyces cerevisiae*, a probiotic, has been shown to show promise in accelerating the clearance of Candida and suppressing virulence factors, especially when in live form (Pericolini et al., 2017). *Saccharomyces cerevisiae*, because of its ability to preserve cellular activities, is a useful model organism for researching human diseases such as cancer and dementia. Large-scale genetic and chemical experimental techniques have been made easier by its genetic simplicity, which has aided in the discovery of novel therapeutic targets and medications.

Types of yeast-based Therapeutics

Saccharomyces cerevisiae is especially useful for high-throughput settings for examining viral activity, drug screens, and vaccine development. Yeast is a popular model for *in vitro* and *in vivo* experiments because of its eukaryotic status, conserved cellular functions, simplicity of cultivation and upkeep, and genetic manipulation capabilities (Srivastava et al., 2024). Yeast's well-established fermentation processes and effective heterologous gene expression systems make it an ideal model for producing medically significant proteins. *Saccharomyces cerevisiae* and *Pichia pastoris* are two often used hosts for the generation of therapeutic proteins. Their non-toxic nature and genetic engineering skills make them suitable for prospective uses such as Yeast's applicability for protein expression and therapeutic development is further supported by its capacity to elicit immunological responses and its successful creation of commercial vaccines (Kumar et al., 2019). Compared to other biological systems like animal cells or plants, yeast is especially useful for the synthesis of VLPs because of its quick growth and stable expression levels because of their nonpathogenic nature, ease of handling, cost-effective medium culture, high expression capacity, appropriate protein folding, genetic responsiveness, and scalability. Compared to bacterial or mammalian systems, yeast species such as *S. cerevisiae* and *K. phaffii* are thought to be safe and effective hosts for the synthesis of VLPs because they do not produce endotoxins and have superior solubility and folding. Yeast is especially useful for the synthesis of VLPs because of its quick growth and stable expression levels (Srivastava et al., 2023).

Yeast-based Probiotics

Saccharomyces strains have the ability to attach themselves to enteropathogenic bacteria e.g. the binding of *Saccharomyces cerevisiae* Sc47, to salmonella particularly those expressing type I fimbriae, which causes the bacteria to fix onto yeast surfaces which may enhance their probiotic-like protective properties. Probiotic yeasts such as *Saccharomyces* exhibit less antagonistic action against pathogenic bacteria as compared to probiotic bacteria (Tiago et al., 2012). Probiotics are helpful living microorganisms as defined by the WHO and FAO. *Lactobacillus, Bifidobacterium*, and *Saccharomyces* yeast are among the bacterial strains that have shown potential health advantages. These can endure digestive challenges and improve gut health by controlling microbiota and adhering to gut cells (Staniszewski and Kordowska-Wiater 2021).

The ability of *Saccharomyces boulardii* CNCM I-745 to treat a range of conditions, such as Helicobacter pylori infections, bowel diseases, irritable bowel syndrome, candidiasis, dyslipidemia, and small intestine bacterial overgrowth in multiple sclerosis patients. The strain's fungal characteristics make it advantageous during antibiotic therapy; nevertheless, it also mentions the known incidences of fungemia and its general safety as a probiotic strain (Kaźmierczak-Siedlecka et al., 2020). Yeast with probiotic qualities such as *Saccharomyces boulardii* is advised for the treatment of both chronic and acute gastrointestinal disorders (Sen and Mansel 2020).

i) Probiotic yeast strains and their mechanisms of action: Probiotics are living bacteria, primarily *lactobacillus* and *bifidobacterium* found in fermented dairy products that offer health advantages when ingested in moderation. Safety considerations such as hemolytic activity are critical for the selection of probiotics. Certain strains of yeast, such as *Saccharomyces cerevisiae* and *Saccharomyces boulardii*, also function as probiotics, impacting gut flora (Ragavan et al., 2017)

ii) Prebiotic properties of certain yeast compounds: The breakdown of β-glucan from yeast, discovering that it is not hydrolyzed in saliva, stomach, or small intestine conditions but is broken down and metabolized by gut microbiota in the large intestine. β-glucan stimulates the development of advantageous gut bacteria such as Bifidobacterium and may function as a prebiotic to alter the makeup of the gut microbiota (Ahiwe et al., 2021).

iii) Yeast-derived bioactive molecules for gastrointestinal health: Yeast and its derivatives have long been utilized to supplement protein in cattle diets. However, they are also known to offer increased feed efficiency, a safer option to antibiotics, support for immune system and intestinal health (Patterson et al., 2023).

Yeast-Derived Compounds

Various yeast derived compounds are commercially produced and available in the market. Compounds like β-glucans and mannan oligosaccharides (MOS) derived from yeast are useful for treating gastrointestinal disorders (Shurson 2018). β -glucans have potential beneficial effects on gastrointestinal health and there is increasing utilization of these compounds. β -glucans treat the gastrointestinal disorders by increasing the binding of toxins, bacteria and viruses (Vetvicka et al., 2014). For evaluating the effects of using MOS in feed, Spring et al. (2015) reviewed results in reducing gastrointestinal disorders by binding of MOS with pathogens and limiting the colonization of pathogens (Spring et al., 2015). Other Yeast derived compounds such as Hydrolytic Enzymes (nucleases and proteases) are useful to treat gastrointestinal disorders by breaking the RNA, DNA and protein of viruses and bacteria. Nucleases break down the nucleic acids (DNA and RNA) into nucleotides and Proteases break down the proteins of pathogens into peptides and amino acid derivatives (Shurson 2018).

Carbon dots (CDs) with multiple uses, obtained from yeast metabolites, demonstrated antibacterial and antioxidant characteristics while posing minimal toxicity to human and animal cell lines. Additionally, these CDs were utilized to produce antimicrobial bio cellulose membranes, indicating that they may be exploited as packaging materials and additives with antibacterial and antioxidant properties (Ghorbani et al., 2022). Due to its probiotic, nutritional, and nutraceutical qualities, commercially manufactured yeast products and feed additives containing yeast are widely utilized in animal diets worldwide (Shurson et al., 2018). The benefits of yeast-derived β -glucans (Y-BG), which vary depending on dosage and health state, have been shown to have immunomodulatory properties that reduce infections and may have anticarcinogenic potential. The advantages of Y-BG against infections, stress-related cytokines, and in augmenting antitumor effects have been demonstrated in animal experiments (Samuelsen et al., 2014). Because of the advantages that yeast, and its derivatives provide for gut health, nutritional absorption, immunological adjuvant-like qualities, and humoral immunity, they are highly sought as feed additives for chicken. Because of these qualities, they present a viable substitute for artificial antibiotic growth promoters in the broiler feed sector, improving both animal welfare and production (Bilal et al., 2023). Nutritional supplements such as yeast and products derived from yeast improve health, performance, and immune function; these actions are mediated by immune stimulation through β-glucan and metabolic changes, which may improve the availability of energy for immune responses (Burdick Sanchez et al., 2021).

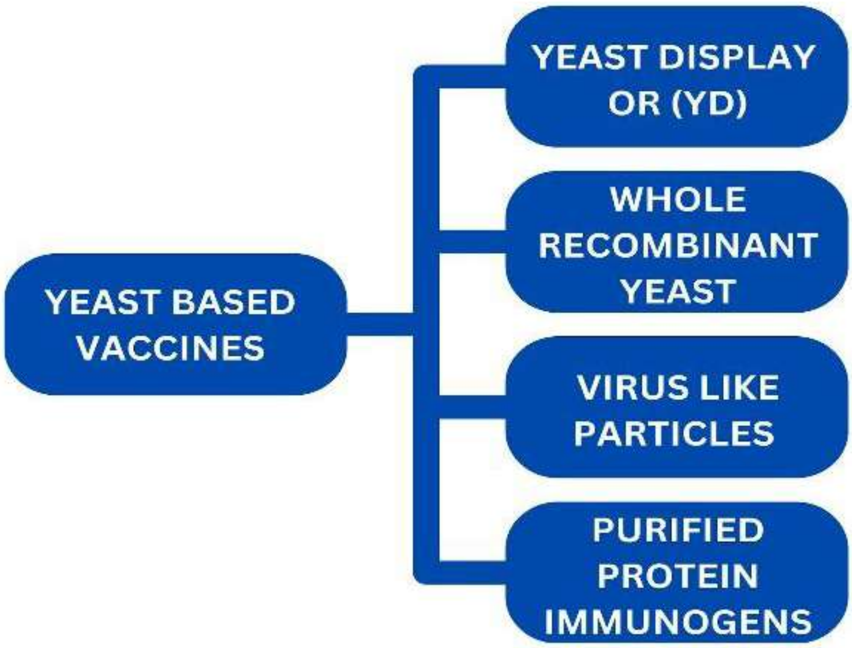

Fig. 1: Different ways in which yeast-based vaccines are used

Yeast-Based Biologics

Yeast-based biologics are pharmaceutical products such as recombinant proteins (vaccines), antibodies, enzymes and hormones derived through yeast-based expression systems (Love et al., 2018). The yeast-based expression system is extensively used in the production of recombinant proteins and various vaccines (shigella vaccine, *vibrio cholera* vaccine adenovirus vaccine etc.) have been produced commercially to treat gastrointestinal disorders by using yeast like *Saccharomyces cerevisiae, Pichia pastoris*, and *Hansenula polymorpha* (Roohvand et al., 2017). Fig.1 shows the different ways in which yeast-based vaccines are used.

Monoclonal antibodies (mAB) are biopharmaceuticals which are produced by using yeast-based expression system by expressing synthetic genes in suitable host and mAB widely used in various biologics-based analytical studies and monoclonal antibodies have various characteristics such as high specificity and binds exceptionally to target of interest (Das et al., 2024). *P. pastoris* is widely used for production of monoclonal antibodies. Several other species of yeast such as *Hansenula polymorpha*, *S. cerevisiae*, *Yarrowia lipolytica*, and *Schizosaccharomyces pombe* are used as hosts for the production of monoclonal antibodies (mAB).

Monoclonal antibodies like infliximab (Remicade), adalimumab and vedolizumab are useful in treating Inflammatory Bowel Disease. These mAB have affinities to bind with TNF-α and result in the production of regulatory macrophages which in turn produces the anti-inflammatory cytokines which reduces the inflammation of gastrointestinal tract (Ordás et al., 2012). Different mAB are used against different gastrointestinal disorders, for ulcerative colitis mAB like ustekinumab is used, for gastrointestinal reflux disease mAB like prolia is used and mAB like raxibacumab is useful in the treatment of *Halicobacter pylori* infection (Sofia and Rubin 2017).

The production of biologics is facilitated by the secretory capacity, genetic engineering potential, and ecologically benign processes of yeast species such as *S. cerevisiae* and *P. pastoris*. *P. pastoris* and filamentous fungi are two alternative expression methods that have been investigated for greater productivity and lower costs in the manufacture of biologics.

While yeast offers advantages like good expression levels and toxin-free synthesis, limitations such as limited secretion of big proteins (Garvey 2022). The poultry industry has recently shown interest in yeast-derived products because of their potential to improve productivity and prevent illnesses by altering the gut microbiota. A study that assessed the impact of several yeast-derived products on rooster cecal incubations of *Salmonella enterica* revealed promise for pathogen reduction without negatively affecting the cecal microbiota (Costello et al., 2023).

Advantages and Challenges

a. Benefits of yeast-based therapeutics

Owing to their diversity, safety, and manageability, yeasts are being studied intensively for natural and sustainable uses, such as probiotics, biofuels, protein synthesis, vaccinations, and environmental bioremediation (Tullio, 2022). Yeast models provide a number of benefits for research, including high-throughput screening and the creation of new treatment approaches e.g Alzheimer's disease (Epremyan et al., 2023). Yeast-based delivery methods can endure the harsh gastrointestinal environment and raise the rates of genetic material transfection for greater oral administration effectiveness; they present potential options for gene treatments and oral vaccinations (Alexander, 2021). Yeast is an excellent choice for small-market medications because it can produce recombinant protein therapies in an efficient and economical manner. Its fast process development capabilities and clean secreted product promote collaborations between industry and academics to enhance the production of yeast-based biopharmaceuticals (Love et al., 2018). Yeast based frameworks and nanoparticles such as nanogels and nano discs, have the potential to improve the clinical and pharmacological applications of curcumin by increasing its bioavailability and solubility (Rahmanian et al., 2023). Yeast-based transport of DNA and mRNA to immune cells, such as macrophages and dendritic cells, offers promise for triggering efficient cellular immune responses without need for additional proteins. This method, known as myconnection,' offers a superior and safe alternative to bacterial and viral systems for creating innovative live vaccines (Walch et al., 2012). Because of their adjuvant qualities and ability to transport nucleic acids for specific immune responses, yeasts have emerged as a potentially effective delivery system for RNA manipulation in vaccine development (Silva et al., 2023). Yeast G-protein coupled receptors used to generate biosensors, with a particular emphasis on cannabinoids because of their diverse range of actions such as being used to find novel Phyto cannabinoids, detect designer pharmaceuticals, and uncover chemicals (Miettinen et al., 2022). Vitamins, minerals like chromium and selenium, amino acids, and other nutrients are all provided by nutritional yeasts like *Saccharomyces boulardii* and *Saccharomyces cerevisiae*, which have significant positive effects on health. Vegans, athletes, and anyone with unique health issues can all benefit greatly from them. *S. boulardii* is used to cure illnesses including *Clostridium difficile* infections and antibiotic-associated diarrhea (Jach et al., 2018). In order to improve yeast's potential for creating therapeutic proteins with glycosylation similar to that of humans, a technique known as "yeast humanization" entails changing yeast genetics to resemble human cellular processes. Including modifications that increase yeast's capacity to produce functional recombinant therapeutic proteins at a reasonable cost and yield (Roohvand et al., 2020).

b. Challenges and limitations:

Yeast based therapeutics, despite having great potential for treating gastrointestinal disorders, also have some challenges and limitations like efficacy and specificity, safety concern, delivering of yeast-based therapeutics at intended site, and also in the production of recombinant proteins and monoclonal antibodies. The effectiveness of yeast-based therapeutics depends upon the specific strains of yeast and also depends upon the specific condition of GIDs (Kwak 2024). While considering the safety concern the yeast-based therapeutics can cause disease like fungemia (Roy et al., 2017). The minimum duration of yeast retention in the gut of the host limits the transportation of therapeutic molecules at targeted sites (Kim et al., 2023). The recombinant protein production is dependent on selection of host which is further dependent on the challenging environment (Brabander et al., 2023). The production monoclonal antibodies mAb have some limitations such as the existing process of purification of mAB limits the production capacity and leads to the increasing cost of production (Samaranayake et al., 2009).

Conclusion

In conclusion, by balancing the gut microbiota and reducing inflammation, yeast-based therapeutics provide a potential approach for treating gastrointestinal diseases (GIDs). *Saccharomyces cerevisiae* and other yeast-based therapies have the potential to alter immune responses and improve the function of the gut barrier due to their probiotic and anti-inflammatory qualities. Additionally, by binding with toxins and pathogens, yeast-derived substances improve gut health and lower the incidence of GIDs. Furthermore, recombinant proteins and monoclonal antibodies have promising approaches in the treatment of a number of GIDs, including *Helicobacter pylori* infections and inflammatory bowel disease. Even with these encouraging developments, there are still challenges, such as concerns about the effectiveness, safety, targeted distribution, and regulatory approval of treatments based on yeast. To overcome these obstacles and increase the promise of yeast-based therapies for GIDs, research is still being done. All things considered, yeast-based therapeutics are a useful and diverse strategy for treating gastrointestinal diseases, including advantages like safety, variety, and the possibility of developing novel treatments.

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Chapter 22

Probiotic Treatment in the Battle against White Nose Syndrome in Bats

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ABSTRACT

A fungus known as *Pseudogymnoascus destructans* (Pd), is the cause of a severe infectious disease known as White-nose syndrome (WNS), which affects bats during their hibernation period. Since it was first discovered in North America in 2006, White-nose syndrome has caused millions of bat deaths which has led to some species being threatened and has raised conservation concerns for scientists. This fungus infects the skin of bats, disturbs their hibernation patterns, and causes premature arousal, leading to increased mortality before spring. The fungus first emerged from the European states and was introduced accidentally by bats or fungal spore transportation. The effect of this disease on the bat population in North America has been significant, resulting in the death of several species, some of which are now on government records or considered for listing under the Endangered Species Act (ESA). The disease has spread to Europe and has an impact on 19 types of bats. One of the species, the greater mouse-eared bat, has shown high pathogen loads and lesion prevalence but it has also exhibited immunological tolerance which allows it to multi-host in nature. In this chapter, we will be reviewing the current understanding of White-nose syndrome (WNS), including its disease mechanism, Ecology, global distribution patterns, and conservation strategies. The fungus responsible for the disease is found in environment reservoirs, which contribute to multi-host nature. The disease affects the living skin layers of bats, negatively impacting ion balance, blood gas, evaporation water loss, and hibernation behavior. The seasonal pattern or disease is influenced by hibernation, with transmission peaking in late winter. We will also be exploring the limited research of homeopathic treatments for White-nose syndrome (WNS), including the use of natural treatment such as cold-pressed, terpeneless orange oil (CPT) that may inhibit the growth of *Pseudogymnoascus destructans* (Pd) in vitro. Probiotic treatments, such as *Pseudomonas flourescens*, have been shown to reduce disease impact and increase in survival of some bat species. The chapter advocated for further research on homeopathic treatments to address the critical conservation issue posed by white-nose disease and its impact on the population of bats.

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INTRODUCTION

The white-nose disease emerges in hibernating bats. It emerged in America in 2006 in a cave known as Hows's Cave (Tunner and Reeder, 2009). The cave has a lot of visitors every year because it is a popular tourist spot. The fungus called *Geomyces destructans*, later renamed *Pseudogymnoascus destructans* causes a whitish shaggy stuff on bat's wings or skin especially (Minnis and Linder, 2013). The disease infects the upper layers of the bat's skin, most often its wings and their membranes. It causes the bats to end their sleep very early during hibernation and they also die very early before the emergence of the spring season when the insects come for food (Lorch et al., 2011; Warneck et al., 2012). This disease is identified by the presence of whitish erosion on the skin, which results from an infection caused by fungus. This confirmation of disease is known by the detailed examination of the tissues obtained from the organ of the place where the disease occurs (Meteyer et al., 2009). Scientists think fungus came to North America from Eurasia, maybe hitching a ride on an infected bat or through fungal spores (Hoyt et al., 2021).

They found it in Washington states in 2016 and more recently in California, suggesting it's moving from the west. By 2024, Pd have been found in all the Rocky Mountain States, and only four western states have not reported it yet. This disease is causing major problems in the Eastern part of Europe. Due to this disease, some species are on the list of endangered species by the Endangered Species Act (ESA) (Kunz and Reichard, 2010, U.S. Fish and Wildlife Service 2022 a and b). *Myotis lucifugu, Myotis septentrionalis, Myotis sodalis, Myotis leibii, Myotis griescens, Eptesicus fuscus*, and *Permyotis subflavus* are the seven species of North America that have been found with severe symptoms of white-nose disease. Additionally, some other species have the fungus present on their skin which can be detected using PCR methods. Still, the specific symptoms of the disease have not been identified (Muller et al., 2013). 19 different species of bats hibernating in Europe are affected by this disease (Zukal et al., 2016, Hoyt et al., 2021). Mouse-eared bat (*Myotis myotis*) is the most affected bat species in the whole of Europe (Puechmaille et al., 2011). Mouse-eared bats have the highest amount of disease symptoms as compared to the other bats in the same hibernating place (Zukal et al., 2016). *Myotis myotis* bats and bats other than this species may survive without major harm from this fungus in Europe. This is because these bats have strong immunity developed in them (Lilley et al., 2019, Fritze et al., 2021a, Whiting-Fawcett et al., 2021). *Myotis lucifugus, Myotis septentrionalis*, and *Perimyotis subflavus* are three Canadian species that are said to be endangered in 2015 due to this disease. *Myotis sodalis* and *Myotis grisescens* are the two endangered species of bats reported by the government before this disease's emergence. This disease globally or regionally decreases the other species of bats (Frick et al., 2010; Langwig et al., 2012; Thogmartin et al., 2013). In this chapter, we look at what we currently know about white-nose disease. We focus on how the disease works, its impact on bats, where it is found globally, and how we can protect bats. Specifically, we are interested in how white-nose disease affects the region of America. We want to see if using homeopathic treatments could be a good and eco-friendly solution to help the bats. We start by explaining why it is important to talk about White-nose syndrome (WNS) in today's world. Then, we explore how the disease affects, including their body reactions. We also talk about where White-nose syndrome (WNS) is found, especially in Europe. Finally, we discuss ways people are trying to save and manage bat populations.

Disease Pathophysiology

Disease History

The white-nose disease was found in 2006 in the state of America first (Blehert et al., 2009). It is a fungal disease caused by *Pseudogymnoascus destructans* (Pd), and it's a huge threat to bats living in caves. This disease has become the most serious problem for bats that hibernate in many caries in Eastern North America, some bat species that were once widespread are now vanishing. (Ferik et al., 2015). This fungus is supposed to be found in contaminated mud and the other media present in the bat's resting spots (Puechmaille et al., 2011a; Linden et al., 2011; Loch et al., 2013a, b). This fungus will be grown from the sample collected at the end of the hot season when the bats are not in their resting place. The intensity of the bats affected by this disease varies a lot. Some bats don't show the symptoms of the disease, some bats show a less symptom, while others show severe symptoms that are the cause of their death (Cryan et al., 2010; Reeder et al., 2012). The three species of bats i.e., *Myotis lucifugus*, *Myotis septentrionalis* and *Perimyotis subflavus*, can get affected by the disease easily. In areas where the disease is found, the number of these bats during winter has decreased by more than 90% since white-nose disease was observed (Cheng et al., 2021). Figuring out if the sickness spears more when there are lots of bats together is crucial. It helps us to know if this disease will make bats disappear completely or if bat numbers will stay very low. When sickness spreads more when there are many bats, the chance of bats disappearing is lower because the sickness spreads slower when there are fewer bats (De Castro and Bolker, 2004).

***Pseudogymnoascus destructans* (Pd) Discovery**

Pseudogymnoascus destructans (Pd) was first time discovered in Europe by researchers when they looked at Mouse-eared bats in different regions of France (Puechmaille et al., 2010). The fuzzy whitish in color fungus appeared on the bat's nose. The researchers found that this disease covers up the 14 different countries of Europe. They got photographic proof of its presence in four more countries of Europe (Martinkova et al., 2010; Puechmilla et al., 2010, Kubatova et al., 2011; Simonovicova et al., 2011; Mestdagh et al., 2012; Wibbelt et al., 2010, 2013; Burger et al., 2013; Pavia-Cardoso et al., 2014; Shandwick et al., 2014). This is also a matter of concern for the researchers whether this fungus is mostly present in the states of Europe or if it is so because there are a lot of studies done in these areas (Puchemaille et al., 2010a). Research of Italy, Solvenia, and Sweden collectively found that the presence of the white nose disease and the strain of its fungus varies in different parts of European states (Voyron et al., 2010; Nilsson et al., 2010; Puechmaille et al., 2011a; Mulec et al., 2013). Researchers across the world can study this fungal infection only on bats but an exceptional case was done in Estonia. In Estonia, the fungus was collected from the walls of the caves where bats used to rest. It is the emergence of the first isolated spore of this fungal strain in European states (Puechmaille et al., 2011a). About 66% of the species of bats known as Mouse-eared bats are commonly affected by the white nose disease as compared to other species of bats (Martinkova et al., 2010; Puechmaille et al., 2011a). *Myotis dasyceme, Myotis mystacinus, Myotis blythii, Myotis daubentonii, Myotis brandtii, Myotis emarginatus, Myotis nattereri, Myotis bechsteinii, and Myotis escalerai/species* are the nine species of myotis bats found in Europe are also affected by this fungus (Cheng et al., 2021).

Disease Dynamic Decoding

The germ-causing sickness, a cold-loving fungus called *Pseudogymnoascus destructans* (Pd), goes deep into a bat's living skin layers during hibernation. It affects the bat's noses, ears, limbs, and membranes (Pikula et al., 2017). This illness can make it difficult for the bat's body to balance ions and blood gases (Verant et al., 2014). It also makes the bat lose water through evaporation (Willis et al., 2011) and changes how the bat behaves during hibernation (Bohn et al., 2016). White-nose disease has a key feature that it affects many types of bats, making it a multi-host disease. This means that the fungus causing White-nose disease can infect various bat species. When a pathogen, like *Pseudogymnoascus destructans* (Pd), lingers in the environment, it is more likely to lead to species extinction because even a few remaining individuals can get infected (Redder et al., 2012).

White-nose disease-affected bats can utilize all their energy in waking up from deep sleep during their resting days (Redder et al., 2012). After their rouse, when they wake up, the body of bats tries to fight the fungal disease which affects their body more (Meteyer et al., 2012).

White-nose syndrome (WNS) is a sickness that happens during certain times of the year. Langwig et al. (2015) have researched how sickness can cause damage to hibernated bats. At the start of the winter, the disease spread fast and bats started dying due to this disease. During this time, the disease has reached its peak and at that, most of the bats have caught the fungal disease (Puechmaille et al., 2011a).

Disease Enigma

Before White-nose disease showed up in regions of Europe, all sick bats that hibernate in the northern United States were doing well in terms of their population getting bigger (Frick et al., 2010; Langwig et al., 2012). But when White-nose disease appeared *Myotis septentrionalis, Myotis lucifugus, Myotis sodalis,* and *Perimyotis subflavus* faced a real threat and their species declined a lot while *Myotis leibii* and *Eptesicus fuscus* were not affected much (Langwig et al., 2012). Corynorhinus genus of bats species was not affected by the white-nose disease, even though they live in caves affected by White-nose disease in states like West Virginia and Virginia. Figuring out why some bats are more affected than others is still an important topic of research, but we don't have a clear answer yet. A study by Langwig et al. (2012) found that differences in where bats rest (like temperature and humidity) were linked to how much White-nose disease affected them. It is also important to figure out if the germ-causing White-nose disease sticks around in the environment, as it helps us to understand how the disease spreads and how much risk it is for bats to die out from it (De Castro and Bolker, 2004).

Rectification of Disease

Not much research has been done on using homeopathic treatments for White-nose syndrome (WNS) in bats. Yet, some studies found hopeful outcomes with different natural treatments. One such treatment is cold-pressed, terpeneless orange oil (CPT), which has shown effectiveness against various forms of *Pseudogymnoascus destructans* (Pd) in lab tests, potentially lessening the impact of this disease (Nicholas et al., 2016). Meteyer et al. (2011) discovered that taking good care of little brown bats helped them get better. In separate studies, Cheng et al. (2017) and Hoyt et al. (2019) use a bacteria called *Pseudomonas flourescens* as a treatment for White-nose disease which can give positive results for this disease. Another study by Boire et al. (2016) showed that a type of orange oil can stop the fungus from growing. These studies suggest that natural treatments, including homeopathic options, might be useful in managing White-nose disease in bats. Cheng et al. (2017) discovered that giving little brown bats bas prebiotic treatment with Pseudomonas fluorescence helped to make the disease less severe, and the bats were more likely to survive. Another study by Hoyt et al. (2019) also supported this treatment, showing a five-times increase in survival for bats that fly freely during winter. Rocke et al. (2019) looked into using vaccines that are carried by viruses, and these vaccines caused bats to have a better immune response against the fungus.

Probiotics Help in Disease Recovery

Cheng et al. (2017) and Hoyt et al. (2019) discovered that by using a helpful bacterium called *Pseudomonas flourescens* can make White-nose disease less severe in bats. *Pseudomonas fluorescens* is a simple group of bacteria often used in farming to control fungi, and it is used to treat a disease called chytridiomycosis in amphibians (Bangera and Thomashow 1999; Gram et al., 1999; Myers et al., 2012). A researcher tested in past studies that the various strains of *Pseudomonas flourescens,* which were collected from different types of bats and found that these strains had different levels of effectiveness against the fungus in lab testing. One particular strain taken from hibernating *Eptesicus Fuscus* bat in Virginia was able to decrease the number of skin lesions caused by the fungus (Cheng et al., 2017).

Another study by Joseph et al. (2019) tried two ways to help bats with White-nose syndrome (WNS). They used the same bacterium, *Pseudomonas flourescens*, and also a chemical called chitosan. They did two experiments at the same time, one with bats in cages and the other with free-flying bats, all at a mine in Wisconsin, USA. They wanted to see if these treatments could reduce the impact of White-nose disease on bats. In the experiment where bats were flying freely, those treated with *Pseudomonas flourescens* had a much higher chance of surviving the winter five times more compared to the group that did not get treated. Their survival rate went up from 8.4% to 46.2%. This happened because the treated bats took 30 days longer to come out of their hibernation spot. The bats treated with chitosan while flying freely had a survival rate of 18%, which was about the same as the bats in the control group (those not treated), but it was not significantly different.

In a test where bats were kept in cages, those treated with chitosan had a much better chance of surviving until they were released on March 8th (53%) compared to the control group and bats treated with *Pseudomonas flourescens* (both 27%). The white-nose disease doesn't show fewer disease attacks because of the disturbance produced inside the cage. Researchers were shocked when they treated the bats with chitosan treatment and the bats showed higher ultraviolet inflorescence (Heather et al., 2019). But, according to Hoyt et al. (2019), things like disturbing bats in their cages can affect the results. McGuire et al. (2017) also talked about how White-nose disease increases the working capacity of bats, and they lose more water. Puechmaille et al. (2011) pointed out that White-nose disease is a big problem for bats in Europe, and we need more research and efforts to help them. Even though using probiotics to treat White-nose disease looks helpful, we still need to do more things to fully fix how the disease hurts bat populations.

Cold-Pressed Terpenless Orange Oil (CPT) Treatment

There might be another solution for wns which is pressed, terpenless orange oil (CPT). This special oil has been discovered to stop the growth of *Pseudogymnoascus destructans* (Pd) completely when tested in a controlled environment (Padhi et al., 2018). In a study by Nicholas et al. (2016), they looked at how cold-pressed orange oil works against different types of this fungus in a lab setting. They used a Kirby-Bauer diffusion test for all their experiments. They made spore mixtures, adjusted them to a specific level, and spread them on plates. The temperature provided to the plates for 6 months is 15°C or 4°C, and the items have fungal growth on them and are being observed. (Sean et al., 2016). After allowing the controls to grow, they measured the areas where the growth was stopped (in millimeters) on the test plates. The results were compared with the antifungal-treated drugs.

All fungal isolates are treated with 100% CPT with an incubation period of one month to stop them, without their temperature. This whole inhibition remains as such for up to six months after a single exposure at this concentration. Amphotericin B, an antifungal drug also shows effectiveness on this fungus. It's worth noting that despite being tested at the highest concentration (100%), CPT did not have a major impact on a range of other environmental organisms, such as various microorganisms (Joshua et al., 2016). Since CPT is not very harmful, it might be possible to use all-natural mixtures as pre-treatment in the environment to get rid of *Pseudogymnoascus destructans* (Pd) in bat homes. However, more studies are necessary to check if CPT has any bad effect on how bats act and stay healthy, and if it affects other parts of the connected environment (Rick et al., 2016). This natural substance could be a way to treat the environment before the fungus appears. But we need more research to make sure it won't harm bats or the whole system.

Conclusion

White-nose disease poses a life-taking threat to hibernating bat populations in North America, leading to significant declines, species endangerment, and ecological imbalances. The nature of *Pseudogymnoascus destructans* (Pd) fungus is infectious and has fueled a devastating epizootic, with millions of bats succumbing to the disease since its emergence in 2006. The extensive spread of White-nose syndrome (WNS), including recent detections in Western states, underscores the urgency of understanding and addressing this ecological crisis. The pathophysiology of White-nose syndrome (WNS), which is characterized by skin lesions, disrupted hibernation patterns, and immunological responses, highlights the complexity of the disease's impact on bats. The multi-host nature of *Pseudogymnoascus destructans* (Pd) and its persistence in environmental reserves contribute to the challenges in developing effective conservation strategies. Some species, such as *Myotis myotis* in Europe, display immunological tolerance, offering insights into potential mechanisms of survival. The chapter highlights the critical need for conservation efforts, emphasizing the importance of understanding disease mechanisms, global distribution patterns, and the varying impacts on different bat species. The alarming declines in bat populations have led to several species being listed or considered for listing under conservation regulations. Exploring potential treatments, the chapter introduces promising findings regarding homeopathic solutions, particularly the inhibitory effects of cold-pressed-terpeneless orange oil (CPT) on *Pseudogymnoascus destructans* (Pd) growth. Probiotic treatments, like Pseudomonas fluorescence, also promise to mitigate disease severity. However, the research in this area is still limited, highlighting the necessity for further investigation into homeopathic treatments and their important role in the conservation techniques and management strategies of White-nose disease. As bat populations face the looming threat of extinction, collaborative efforts between scientists, conservationists, and policymakers are crucial to developing effective strategies that address the complexities of White-nose disease and secure the future of these vital species in our ecosystems.

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Chapter 23

Therapeutic Potential of Prebiotics in Fish Husbandry

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ABSTRACT

As the aquaculture business expands to satisfy rising seafood demand, it becomes increasingly important to preserve farmed fish's maximum health and performance. In recent years, prebiotics have emerged as a viable method for improving the gut health, growth performance, and disease resistance in aquatic creatures. The therapeutic potential of prebiotics in fish husbandry explains their role in boosting fish health and well-being. The prebiotics are non-digestible chemicals that preferentially nourish beneficial gut bacteria, promoting a healthy gut microbiota in farmed fish. The prebiotics increase growth performance by increasing food absorption and strengthening the immune system. This chapter investigates the processes by which prebiotics influence gut microbiota composition and immunological responses in fish. It also covers practical topics including prebiotic kinds, dose, and delivery techniques in fish diets. Finally, this chapter emphasizes the importance of prebiotics as sustainable alternatives to antibiotics in aquaculture, providing prospective pathways for improving the health and production of farmed fish while preserving environmental sustainability.

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INTRODUCTION

Aquaculture, a critical component of the global food supply, presents enormous issues in ensuring the health and productivity of farmed fish. Among these issues, illness outbreaks pose significant economic and environmental risks, necessitating the use of antibiotics to control infections (Boyd et al., 2020). However, the excessive use of antibiotics in aquaculture has prompted concerns about antibiotic resistance, food safety, and environmental sustainability. The antibiotic use in aquaculture as a preventive measure has been linked to the evolution and spread of several resistant human pathogens, including *Aeromonas sp., Escherichia tarda, Escherichia coli, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio cholerae*, and others (Allameh et al., 2016; Brogden et al., 2014). In response, there is a rising interest in creating alternative techniques to promote the sustainable aquaculture operations while protecting the health and welfare of farmed fish. One such technique is to use prebiotics, which are nondigestible food substances that selectively encourage the growth and activity of beneficial gut microbes. The prebiotics have emerged as attractive antibiotic alternatives in fish farming due to their capacity to regulate the gut microbiota, increase nutritional absorption, and improve disease resistance (Lakshmi et al., 2013). It is critical in fish husbandry to keep farmed fish in peak health and output. The disease outbreaks present considerable issues, including economic losses and environmental concerns. The gut microbiota is a critical component of fish health, influencing nutrition absorption, immunological function, and disease resistance. The dysbiosis, or an imbalance in intestinal microbial communities, can harm the fish health by increasing susceptibility to infections. The use of prebiotics as therapeutic agents in aquaculture represents a substantial change toward more sustainable and environment friendly operations.

Prebiotics

The words "pro" and "bios" signify "before life" in Greek.

The prebiotics can be digested by helpful intestinal microbes; they also resist gastric acidity and promote the growth of beneficial microorganisms that support human health (Guerriero et al., 2017).

"A prebiotic is a substance that has been selectively fermented to allow for specific changes in the activity and/or composition of the gastrointestinal microflora, which benefits the host's health and well-being."

The prebiotics are indigestible food ingredients that specifically promote the expansion and function of good bacteria in the digestive system (Wang 2009; Mussatto and Mancilha 2007; Ouwehand et al. 2005).

The prebiotics are largely undigested by helpful bacteria in the colon, where they are fermented by them (Al-Sheraji et al., 2013; Ogueke et al., 2010). They defy digestion in the upper gastrointestinal system. The short-chain fatty acids (SCFAs) and other metabolites are produced during this fermentation process, and they have a number of health advantages, such as

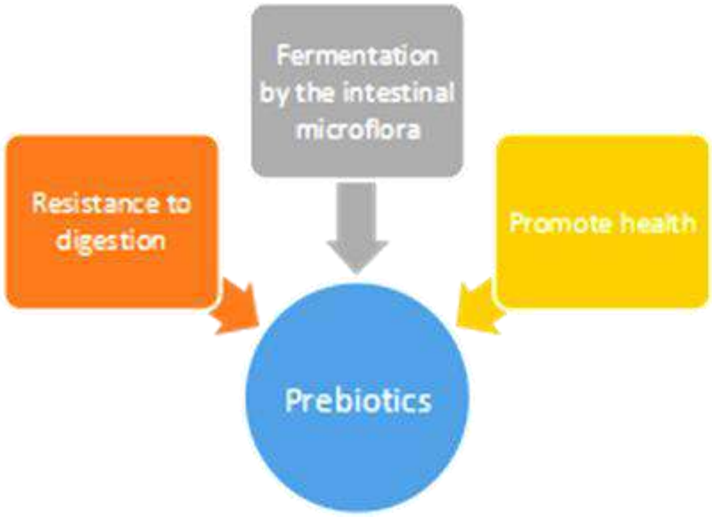

Fig. 1: Some uses prebiotics.

1. Supporting the gut health,
2. Boosting the immune function, and
3. Improving the nutrient absorption.

The prebiotics selectively stimulate the growth of lactobacilli and bifidobacteria, two types of helpful bacteria, while suppressing the growth of pathogens, which helps maintain a healthy gut microbiota (Hutkins et al., 2016).

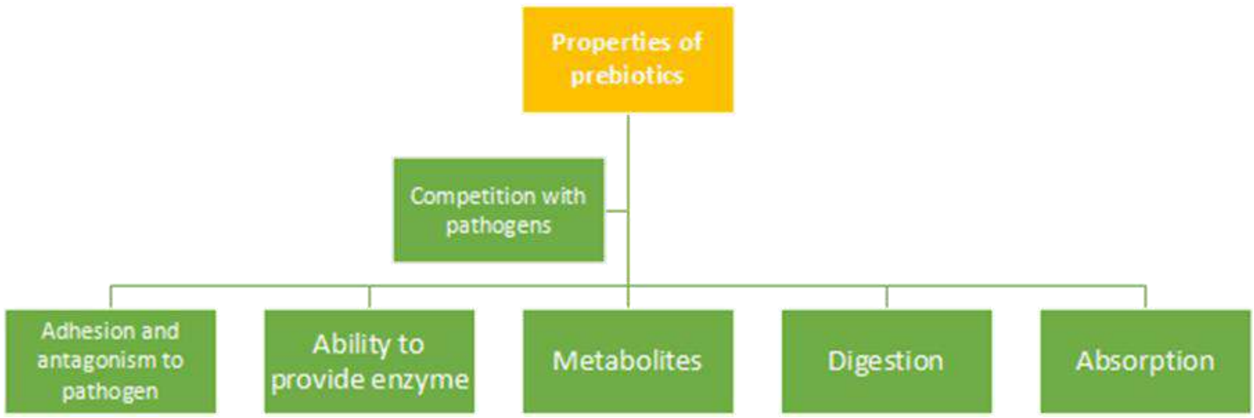

Fig. 2: Properties of prebiotics

Sources

Because they support a healthy gut microbiota, the prebiotics in aquafeeds are essential for improving the health and performance of aquatic species. The prebiotics can come from a variety of sources in aquafeeds (Wang 2009; Mussatto and Mancilha 2007; Ouwehand et al. 2005), including:

- Fructooligosaccharides (FOS)
- Galactooligosaccharides (GOS)
- Insulin
- Mannan oligosaccharides (MOS)
- Beta-glucans
- Phenolics and phytochemicals
- Polyunsaturated fatty acid (PUFA)
- Xylooligosaccharide (XOS)
- Conjugated linoleic acid (CLA)

Mechanisms of Action of Prebiotics in Fish

Prebiotics improve the health of fish through a number of methods, the main ones being immune system stimulation and gut microbiota modification.

Short-chain Fatty Acid (SCFA) Production

The SCFAs including acetate, propionate, and butyrate are produced when prebiotics are fermented by good gut bacteria. By giving intestinal epithelial cells an energy source, encouraging the formation of mucin, and preserving the integrity of the gut barrier, these SCFAs are crucial for gut health (Macfarlane, 2011).

Table 1: Some sources of prebiotics in aqua-feeds (Ganguly et al., 2013)

<table>
<tr><th colspan="6">Some sources of prebiotics in aquafeeds</th></tr>
<tr><td colspan="2">1. Fructooligosaccharides
✧ FOS, or short-chain carbohydrates, made up of molecules of fructose connected to one another.
✧ They are frequently obtained from foods like sugar beet, chicory root, and specific fruits and vegetables.
✧ FOS has been demonstrated to specifically promote the growth of lactobacilli and bifidobacteria, two types of good gut bacteria, in fish.</td><td colspan="2">2. Galactooligosaccharides
✧ GOS are a different class of oligosaccharide consisting of galactose molecules connected to one another.
✧ They can come from foods including milk, some cereals, and legumes.
✧ Due to its prebiotic qualities, GOS can help fish's gut health and general performance by encouraging the growth of good gut bacteria.</td><td colspan="2">3. Insulin
✧ A polysaccharide made of chains connecting fructose molecules, insulin is known as such.
✧ It is frequently found in plants including garlic, Jerusalem artichokes, and chicory root.
✧ As a prebiotic in aquafeeds, inulin has been shown to promote the development of advantageous gut flora and enhance fish nutrition absorption.</td></tr>
<tr><td colspan="3">4. Mannan oligosaccharides
✧ Oligosaccharides called MOS are extracted from the cell walls of some algae and yeast.
✧ They have been demonstrated to have prebiotic benefits on fish by strengthening the immune system and encouraging the growth of good gut flora.
✧ Additionally, MOS can lessen the chance of infections by preventing harmful bacteria from adhering to the gut epithelium.</td><td colspan="3">5. Beta-glucans
✧ Polysaccharides called beta-glucans are present in the cell walls of several fungi, yeast, and cereals.
✧ It has been demonstrated that they exert stimulatory effects in fish, boosting resistance to illness and immunological responses.
✧ Because they enhance gut health and encourage the growth of good gut bacteria, beta-glucans can also function as prebiotics.</td></tr>
<tr><td colspan="3">6. Arabinoxylans
✧ The polysaccharides known as arabinoxylans are present in the cell walls of cereal grains, including rye, wheat, and barley.
✧ The arabinoxylans function as prebiotics in aquafeeds by giving good gut bacteria fermentable substrates to grow on, which promotes the synthesis of SCFAs and changes the gut microbiota.</td><td colspan="3">7. Lactulose
✧ Galactose and fructose combine to form lactose, a synthetic disaccharide.
✧ Because lactulose can promote the growth of good gut bacteria, it is utilized as a prebiotic in aquafeeds even though it isn't found naturally in diet.
✧ In aquatic species, lactulose improves gut health, immune system performance, and nutrient absorption through encouraging the growth of beneficial bacteria.</td></tr>
</table>

Immune System Augmentation

Prebiotics have the ability to directly affect the fish immune systems by inducing the synthesis of immunoglobulins, cytokines, and antimicrobial peptides. These elements are essential for both the innate and adaptive immune responses, which aid fish in protecting themselves from harmful infections by inducing the synthesis of cytokines, antimicrobial peptides, and other immune mediators, prebiotics improve fish innate immune responses (Akhter et al., 2015). These elements are essential to the early defense against pathogens because they aid in the eradication of invasive microbes and the avoidance of illnesses. The prebiotics help fish identify and react to pathogenic risks by increasing the activity of innate immune cells including neutrophils, macrophages, and natural killer cells. This improves fish immunity to disease overall (Hoseinifar et al., 2015).

Modulation of Innate Immunity

The natural killer cells, neutrophils, and macrophages are examples of innate immune cells that prebiotics have the ability to activate. These cells are essential for identifying and getting rid of infections, which improves the fish immunity in general.The prebiotics can influence fish's adaptive immunity while also enhancing innate immune responses. By phagocytosis and inducing inflammation, among other primary responses, the innate immune system offers a first line of defense against microorganisms (Broz and Monack, 2013).

Regulation of Inflammatory Responses

Prebiotics control the generation of cytokines that are both pro- and anti-inflammatory through immunomodulatory actions. Prebiotics aid in the prevention of excessive inflammation, which can be harmful to fish health, by encouraging a healthy immune response. The T and B lymphocytes are activated as part of adaptive immune responses, and they are essential for identifying and getting rid of particular infections. The prebiotics have been demonstrated to influence T and B cell activity and proliferation as well as the synthesis of cytokines, antibodies, and other immunological components (Akhter et al., 2015).

Enhancement of Mucosal Immunity

An essential part of fish mucosal immunology is the gut-associated lymphoid tissue (GALT).The prebiotics can boost GALT function and development, which will strengthen the mucosal immunity and improve defenses against intestinal infections. (Yu et al., 2020)

Antioxidant Effects

The prebiotics can reduce oxidative stress in fish tissues by scavenging free radicals and exhibiting antioxidant capabilities. The prebiotics support fish immunity generally and maintain health by reducing the oxidative damage (Devi et al., 2019).

Indirect Effects on Immunity

The immune function is indirectly impacted by prebiotics' modification of the composition of the gut microbiota (Liu et al., 2022). The immune homeostasis is facilitated by a healthy gut microbiota, which also strengthens host defenses against infections and reduces inflammation linked to dysbiosis (Yahfoufi et al., 2018).

Improvement of Gut Health and Integrity

By encouraging the growth of good bacteria that compete with harmful germs for resources and adhesion sites in the gut, prebiotics help to maintain gut health. By strengthening the intestinal epithelial barrier, prebiotics can lessen the amount of pathogens and inflammatory stimuli that enter the bloodstream. This lowers the risk of inflammatory illnesses in fish and helps prevent systemic infections (Ashaolu, 2020).

The fish gut microbiota composition is modulated in large part by the prebiotics. The Prebiotics selectively encourage the growth and activity of specific microbial populations while preventing the spread of harmful species by acting as substrates for the helpful bacteria.

The improved gut health, nutritional absorption, and general host well-being are linked to this modulation, which results in a more diversified and balanced gut microbiota (Vieira et al., 2013). The selective activation of beneficial bacteria in fish guts is one of prebiotics' main effects. The beneficial microorganisms with a reputation for improving health, such as lactobacilli, bifidobacteria, and other lactic acid bacteria, find fermentable substrates in prebiotics. The prebiotics support a healthier gut microbiota and better host health by increasing the quantity and activity of these beneficial bacteria (Vargas-Albores et al., 2021). Additionally, prebiotics have the ability to drive viruses out of fish guts through competitive exclusion. Prebiotics work by encouraging the development of good bacteria, which makes the environment less conducive to the colonization and spread of harmful microbes. By preventing pathogens from adhering to the gut epithelium, this competitive exclusion mechanism lowers the likelihood of infections and increases fish disease resistance generally (Denev et al., 2009).

Effects on Mucosal Immunity

The prebiotics can also affect the fish mucosal immunity, which is essential for warding off pathogen invasion of mucosal surfaces like the skin, gills, and stomach. The mucosal immune cells assist preserve the integrity of mucosal barriers and stop pathogen colonization. Examples of these cells are mucin-secreting goblet cells, intraepithelial lymphocytes, and secretory IgA-producing cells (Lazado and Caipang 2014).

Prebiotic Index

The prebiotic index, which is a measurement of the relative development of desirable and undesirable bacteria in relation to changes in the microbiota's overall population, can be used to choose prebiotics. The ability of a substrate to support the growth of particular bacteria in comparison to the growth of autochthonous gut microbiota in glucose is reflected in the prebiotic score. With a prebiotic score of -0.09 for fructooligosaccharide (FOS), the pathogen is likely to develop more than the targeted bacterium, *Lactobacillus plantarum*, and less than glucose in FOS. These numerical measurements aid in directing the choice of suitable prebiotics that promote the development of advantageous gut flora while simultaneously inhibiting the spread of harmful strains (Roberfroid, 2007).

Impact of Prebiotics on Growth Performance

Promotion of Growth and Feed Efficiency

It has been demonstrated that prebiotics increase fish development and feed efficiency. The prebiotics maximize the utilization of dietary resources by regulating the gut microbiota and improving nutrient absorption, which raises growth rates and improves feed conversion ratios. Furthermore, prebiotics promote the gut's synthesis of short-chain fatty acids (SCFAs), which provide the host with energy and enhance growth performance (Helal et al., 2015).

Influence on Nutrient Digestibility and Absorption

The prebiotics help fish absorb and digest nutrients better by fostering the growth of good gut bacteria and enhancing the gut health. Beneficial bacteria can help break down complex carbs, proteins, and other food components into more easily absorbed forms by producing enzymes through the fermentation of prebiotics. The fish growth performance and feed efficiency are improved as a result of this better nutrient consumption (Ringø et al., 2010).

Effects on Metabolic Parameters

Additionally, prebiotics can affect fish metabolic characteristics like insulin sensitivity, energy balance, and the metabolism of fats and carbohydrates. The prebiotics assist control metabolic processes in the host by altering the gut microbiota and encouraging the synthesis of SCFAs, which improves metabolic health and performance. According to Nagashimada and Honda (2021), prebiotics may have anti-inflammatory properties that lower the risk of metabolic diseases including fatty liver disease and obesity in fish.

Optimization of Production Efficiency

In general, prebiotics' effects on growth performance help aquaculture systems maximize output efficiency. The prebiotics enhance the growth rates, feed conversion ratios, and nutrient use of farmed fish, hence optimizing production and reducing input costs and environmental implications. Furthermore, prebiotics provide long-term substitutes for antibiotics in aquaculture to support the development and well-being, lowering the need for antimicrobial agents and assisting in the adoption of more eco-friendly production methods (Rohani et al., 2022).

Stress Alleviation and Welfare Benefits

Role of Prebiotics in Stress Response

The prebiotics are essential for controlling how fish react to stress. The fish undergo physiological and behavioral changes that can have an adverse effect on their health and wellbeing when they are subjected to stressful situations, such as handling, illness problems, or changes in their environment. By adjusting the gut-brain axis and controlling the release of stress-related chemicals like cortisol, prebiotics help lessen the harmful consequences of stress. The prebiotics help fish maintain homeostasis and general well-being by supporting a balanced gut microbiota and improving the gut health. This helps fish withstand stresses. (Burokas et al., 2017).

Mitigation of Stress-Related Disorders

The prebiotics have been demonstrated to help fish suffering from stress-related conditions like stunted growth, weakened immune systems, and increased illness susceptibility (Naiel et al., 2022). The prebiotics support the synthesis of short-chain fatty acids (SCFAs) and the activity of beneficial gut bacteria, both of which are critical for immune system function and gut health and the ability to withstand stress. The prebiotics may also have anti-inflammatory properties that lessen the inflammatory response brought on by stress and shield fish from developing illnesses linked to stress (Serradell et al., 2020).

Improvement of Fish Welfare

In general, the enhancement of fish welfare in aquaculture systems is facilitated by the reduction of stress and the amelioration of pathologies associated with stress. The prebiotics support the physical and mental health of farmed fish by fostering a healthy gut microbiota and boosting resistance to stresses (Wang et al., 2023). Reductions in the death rates, improved growth rates, and greater resilience to disease are examples of improved welfare outcomes that result in more ethical and sustainable aquaculture methods. The prebiotics also provide long-term advantages for fish welfare and overall production efficiency by lowering the requirement for stressful management techniques like antibiotic treatments or overcrowding (Merrifield and Ringo, 2014).

Applications of Prebiotics in Fish Husbandry

Larviculture and Early Life Stages

The prebiotics are vital for boosting nutrition absorption, gut health, and survival rates in fish reared in the larviculture and early life stages. To give fish larvae and juveniles the nutrients they need to develop a healthy gut microbiota, prebiotics can be added to starter meals (Kotzamanis et al., 2007). The prebiotics assist fish become more resilient during the crucial early phases of development by boosting the growth of beneficial bacteria and strengthening immunological function. This results in increased survival rates and improved growth performance (Borges et al., 2021).

Grow-Out and Grower Fish

The prebiotics continue to help fish during the grow-out stage of fish production by boosting the disease resistance, increasing the feed efficiency, and encouraging the growth. According to Sealey et al. (2009), prebiotics can be added to grow-out feeds to improve nutrient utilization, lower the frequency of digestive diseases, and enhance the general health and performance of grower fish. The prebiotics improve growth rates, survival rates, and production efficiency in grow-out systems by regulating the gut microbiota and boosting the immunological function (Adhikari and Kim, 2017).

Broodstock Management

The prebiotics can also be used to improve the quality of eggs, progeny survival rates, and reproductive performance in broodstock management. The prebiotics can be added to broodstock fish diets to boost overall reproductive health, improve nutrient absorption, and improve gastrointestinal health (Abu-Elala et al., 2021). The prebiotics help increase spawning success rates and enhance the quality of progeny in broodstock management programs by supplying vital nutrients for the development of healthy eggs and larvae (Sumon et al., 2022).

Future Perspectives and Research Directions

Optimization of Prebiotic Formulations and Dosages

The prebiotic formulations and dosages for various fish species, life phases, and production systems should be the main focus of future studies. This entails figuring out the ideal inclusion levels to optimized health advantages and performance outcomes in fish, as well as researching the synergistic effects of prebiotics with other dietary supplements and feed additives.

Exploration of Novel Prebiotic Sources

Investigating novel prebiotic supplies from a variety of sources, including aquatic plants, algae, and by-products from the food and feed sectors, is necessary. Finding prebiotics with distinct chemical compositions and useful qualities that can improve the health and provide sustainability benefits for aquaculture should be the goal of research.

Integration of Omics Technologies

The metagenomics, metatranscriptomics, metabolomics, and proteomics are a few examples of omics technologies that provide strong instruments for examining the intricate relationships that exist between prebiotics, gut microbiota, and fish host health. In order to better understand the prebiotics' mechanisms of actions, find indicators of immune system and gut health, and optimize prebiotic therapies for better fish welfare and performance, future research should incorporate omics techniques.

Addressing Knowledge Gaps and Emerging Challenges

In the area of the prebiotics in fish husbandry, there are still a lot of unanswered questions and developing issues that must be resolved. This entails knowing how prebiotic supplementation affects fish health over the long run, looking at how prebiotics affect non-target organisms and ecosystem dynamics, and evaluating how environmentally sustainable prebiotic production and use in aquaculture are.

Conclusion

Prebiotics, in summary, have promising uses in fish husbandry due to their ability to improve growth performance, strengthen the immune system, support gastrointestinal health, and lessen the need for antibiotics. Important research results show that prebiotics are essential for regulating the gut microbiota, improving nutrition uptake, and lessening the harmful effects of stress on fish. The prebiotics also lessen nutrient contamination in aquaculture systems, enhance water quality, and lessen reliance on antibiotics, all of which contribute to the environmental sustainability. The prebiotics have a lot of consequences for the aquaculture sector since they provide environmentally benign and long-lasting ways to enhance fish welfare, health, and productivity. The farmers can improve growth performance, lessen the environmental effect of traditional aquaculture operations, and increase the resistance of their fish populations to disease by adding prebiotics to aquafeeds. The prebiotics also aid in the economy by increasing the marketability of fish products raised on farms, decreasing input costs, and increasing production efficiency. The prebiotics in fish husbandry have promising futures if prebiotic formulations and dosages are optimized, new prebiotic sources are investigated, omics technologies are integrated, and knowledge gaps and new obstacles are addressed. More study is required to fully comprehend prebiotics' mechanisms of action, evaluate their long-term impacts, and tailor their applications for various fish species and production systems in order to optimize their potential for enhancing the fish health, welfare, and sustainability. Furthermore, in order to advance sustainable practices in the global seafood supply chain and encourage the use of prebiotics in aquaculture, cooperation between researchers, industry stakeholders, and policymakers is crucial.

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Chapter 24

Synergistic Effect of Probiotics and Prebiotics on Shrimp Culture

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ABSTRACT

Shrimp aquaculture has grown quickly to become a major global industry that employs tens of thousands of skilled and knowledgeable people in addition to offering financial gain and high-quality food. The prevalence of diseases poses a significant risk to shrimp aquaculture, potentially leading to substantial declines in production and financial setbacks. Antibiotics are among the many conventional techniques that have been used over the years to control infections, yet unsuccessful. Dietary and water mixed supplements have served as substitutes, with probiotics and prebiotics emerging as particularly beneficial options for treating bacterial, viral, and parasitic diseases, and enhancing the shrimp production. Probiotics are live microbial feed supplements, whereas prebiotics are indigestible food components that benefit the host by promoting the development and activity of beneficial gut flora.

KEYWORDS
Aquaculture, Shrimp culture, Antibiotics, Prebiotics, Probiotics

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INTRODUCTION

Aquaculture refers to the culture of aquatic organisms under controlled or semi-natural conditions. Shrimp aquaculture stands out as a primary focus within the industry, mainly because of the high nutritional value associated with shrimp (Table 1). Shrimp culture is prevalent in developing nations around the world, providing vital support to rural communities for their livelihoods and contributing to poverty reduction efforts (FAO, 2016). It accounts for more than half of the world's shrimp production, as wild-caught alone cannot meet the worldwide demand for exports. Consequently, shrimp farming is recognized as one of the most lucrative sectors within the aquaculture industry. In many countries, black tiger prawn (*Penaeus monodon*) has been the primary cultured species. However, the emergence of viral pathogens has prompted several farmers to shift to the imported *Litopenaeus vannamei* (Pacific whiteleg prawn). Since 2003, this species has undergone genetic selection and domestication, making it the preferred choice for many aquaculturists (Jamal et al., 2019). One way to boost production efficiency is to intensify the process, but this approach may also heighten the vulnerability of the cultured organism by the declining water quality and escalating stress levels within the aquaculture system. However, the rapid expansion of aquaculture brings forth challenges like widespread epizootics, inadequacies in feed utilization, suboptimal growth progress (Flegel, 2012), and disease outbreaks alongside the increased mortality, that contribute significantly to the overall poor growth experienced in aquaculture. In the aquaculture industry, poor growth and the development of drug-resistant pathogens stand out as crucial concerns. The industry strives to enhance the growth or survival rates, optimize feed efficiency, and bolster the resistance of aquatic organisms (Joseph, 2017).

Control of Shrimp Disease

Currently, the primary concern revolves around effectively controlling and preventing disease outbreaks in shrimp populations. Traditionally, the management of bacterial infections in shrimp has mainly relied on the use of chemical additives, antimicrobial disinfectants, or antibiotics (Karunasagar and Ababouch, 2012). Many farmers widely use antibiotics in significant quantities preventively, even in the absence of apparent infections (Jamal et al., 2019). Consequently, this practice has led to a rise in several antibiotic-resistant strains of highly virulent pathogenic vibriosis through genetic mutations. This increase is associated with the ability of marine vibriosis to use plasmids to spread antibiotic resistance genes throughout the dense bacterial population in ponds (Akhter et al., 2015). The *Vibrio parahaemolyticus*, a marine pathogen becomes severe through the utilization of a plasmid that expresses a lethal toxin (Jamal et al., 2019). The development of biofilm on surfaces allows Vibrio species to proliferate even in the presence of antibiotics which poses a serious problem for controlling the shrimp diseases. This protective mechanism shields bacteria from antibiotics, complicating their eradication. The utilization of chemical agents has faced challenges due to the consequential severe environmental effects (Newaj-Fyzul et al., 2014). Its accumulation in the shrimp can render them unattractive for export. To address the challenge of managing shrimp diseases effectively, there is a growing need for Alternative management and control strategies. This has prompted a concerted effort to explore environment-friendly alternative treatment options in aquaculture. These substitutes aim to address the risk of antibiotic resistance while guaranteeing a safe, secure, and nutritious food supply for the world's growing population. An effective alternative involves the utilization of antibacterial probiotics and prebiotics, which offer an environmentally friendly approach to disease management in aquaculture.

Table 1: Nutritional information of shrimp

NUTRITION FACTS		
Per 100g Serving		
Total Calories 118 .8 (15.3From Fat)		% Daily Value *
Total Fat	1.6g	3%
Saturated Fat	0.5g	3%
Cholesterol	210.6mg	70%
Sodium (Na)	947.2mg	29%
Potassium (K)	170.6 mg	50%
Total Carbohydrates	1.5g	1%
Proteins	22.4 g	
Vitamin A		6%
Calcium (Ca)		9.1%
Iron (Fe)		1.8%

* % Daily Values are based on a 2000 Calorie Diet.

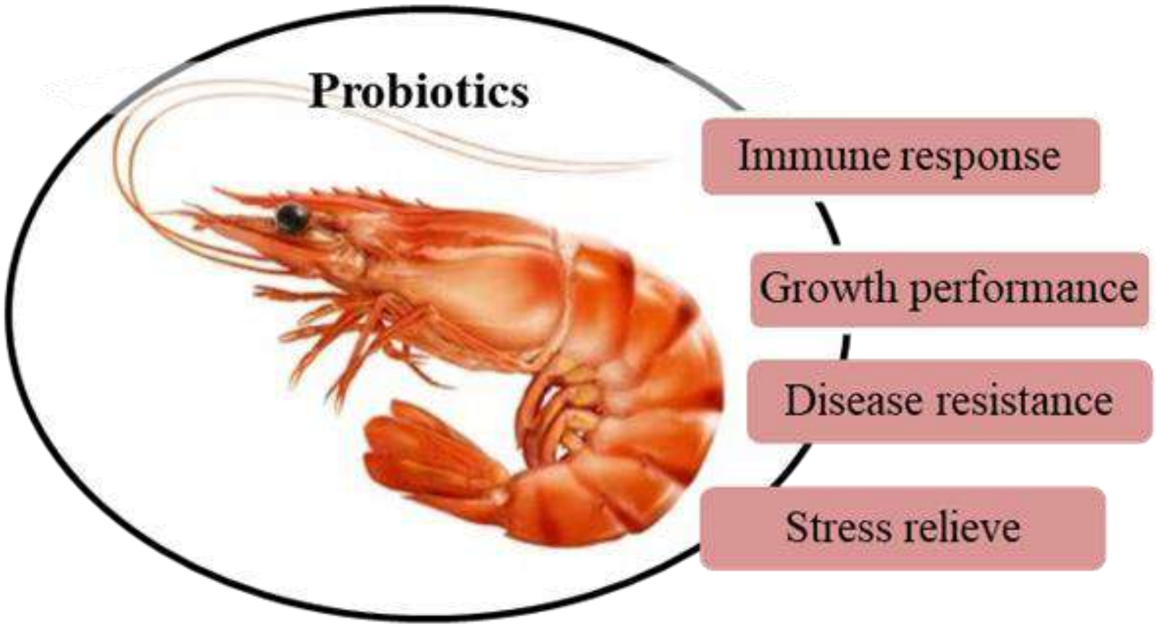

Fig. 1: Effects of probiotics in shrimp culture

Source and Potential Needs

Probiotics

Probiotic was referred to "as a live microbial audit which has a beneficial effect on the host by modifying the host-associated, ambient microbial community through improvement of its feed or enhancing its nutritional value and also by enhancing the host response toward disease, or by improving the quality of its ambient environment" (Verschuere et al., 2000). Probiotics improve the shrimp health by struggling against pathogen colonization through competitive exclusion, releasing metabolites that inhibit pathogen growth, and thereby bolstering shrimp resistance to diseases (Fig. 1). The utilization of probiotic bacteria has gained popularity for its benefits in enhancing nutrition, promoting gastrointestinal health, and preventing diseases. It has been effectively used in many developing countries to control the disease in shrimps and other aquatic animals (Newaj-Fyzul et al., 2014).

Prebiotics

A prebiotic is any compound, substrate, long-chain sugar, nutrient, or fiber that acts as food for beneficial microorganisms within the digestive system of a host (Mountzouris, 2022). Additionally, a prebiotic is characterized as a substance that withstands the acidic environment in the stomach, is fermentable by gut microbiota, and stimulates the growth of beneficial gut microbiota, thus enhancing the overall health of the host (Davani-Davari et al., 2019). In general, prebiotics are complex, long-chain carbohydrates that supply energy to beneficial microorganisms, or probiotics, thereby improving the health of an organism. Prebiotics are primarily sourced from plant-based products and edible mushrooms, with fewer derived from animal dairy products (Fig. 2). They occur naturally in a variety of foods such as vegetables, fruits, beans, seaweed, microalgae, and animal milk (Elumalai et al., 2021). It can serve multiple functions in the gut, including, facilitating the elimination of harmful microorganisms from gut epithelial cells by acting as a receptor, modulating the host immune system, and controlling inflammation (Mohammadi et al., 2021).

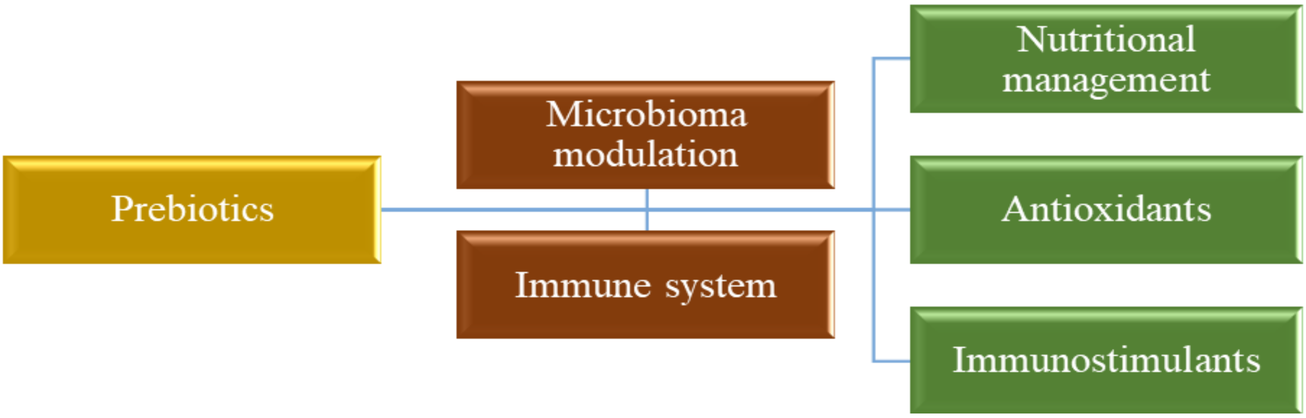

Fig. 2: Role of prebiotics in shrimp culture

Mechanism of Action

Shrimp are susceptible to several bacterial and viral epizootics. In recent years, several infections and diseases have been predominant in shrimp farming. Periodic damage can lead to widespread mortality and significant economic losses. It is estimated that shrimp disease outbreaks result in global losses of around 3 billion US dollars (Lundin, 1995). It was inevitable that new approaches and strategies would emerge. Probiotics and prebiotics were therefore created and successfully applied in shrimp farming. Probiotics' mode of action has been suggested by a few theories recently, but it is unclear how exactly they work in aquaculture. Some hypotheses suggest that the key mechanisms by which probiotics exert their effects include the stimulation of immunity, enhancement of growth and tolerance, antagonising infections, and changing the microbiota in the gut.

Prebiotics' mechanisms of action have been seen in a variety of shrimp species, including improved resilience to disease (Sun et al., 2019), immune system stimulation and modulation (Miandare et al., 2017), intestinal microbiota enhancement, change in GI microbiota, improved host species growth and survival (Li et al., 2019), antioxidant activity, and valuable changes in enzyme activity (Fig. 3) (Hu et al., 2019).

Modes of Application of Pro and Prebiotics

Probiotics can be classified into several types depending on their application (Fig. 4);

a) Probiotics that are collected from the gut and can be orally administered in combination with feed to improve the beneficial microflora of the intestine.

b) Those that are administered as water additives and can grow in the water medium by absorbing all the ingestible food thus, resulting in starving of pathogenic bacteria due to malnutrition.

c) Which can be administered through micro-encapsulation, and have a direct and positive impact on water quality, physical parameters, and shrimp health.

d) In the form of living cells.

e) Those that can be administered via probiotic-rich Artemia or microalgae and can promote growth and survival during the nursing phase.

However, prebiotics are a popular set of alternative disease management strategies that promote non-specific immune responses. There are several varieties of prebiotics, depending on their application (Fig. 4). For example,

a) Supplementing an aquatic diet with prebiotics can increase glucose uptake, and bioavailability of trace elements.

b) Prebiotics when used as a water additive for a specific period stimulate immune responses and enhance resistance against diseases.

c) Bio-encapsulation of Artemia species with prebiotics provided appropriate size for consumption, high nutritional value, and promoted larval growth.

d) Raw addition of prebiotic-rich *Artemia nauplii* increased shrimp larval and post-larval survival.

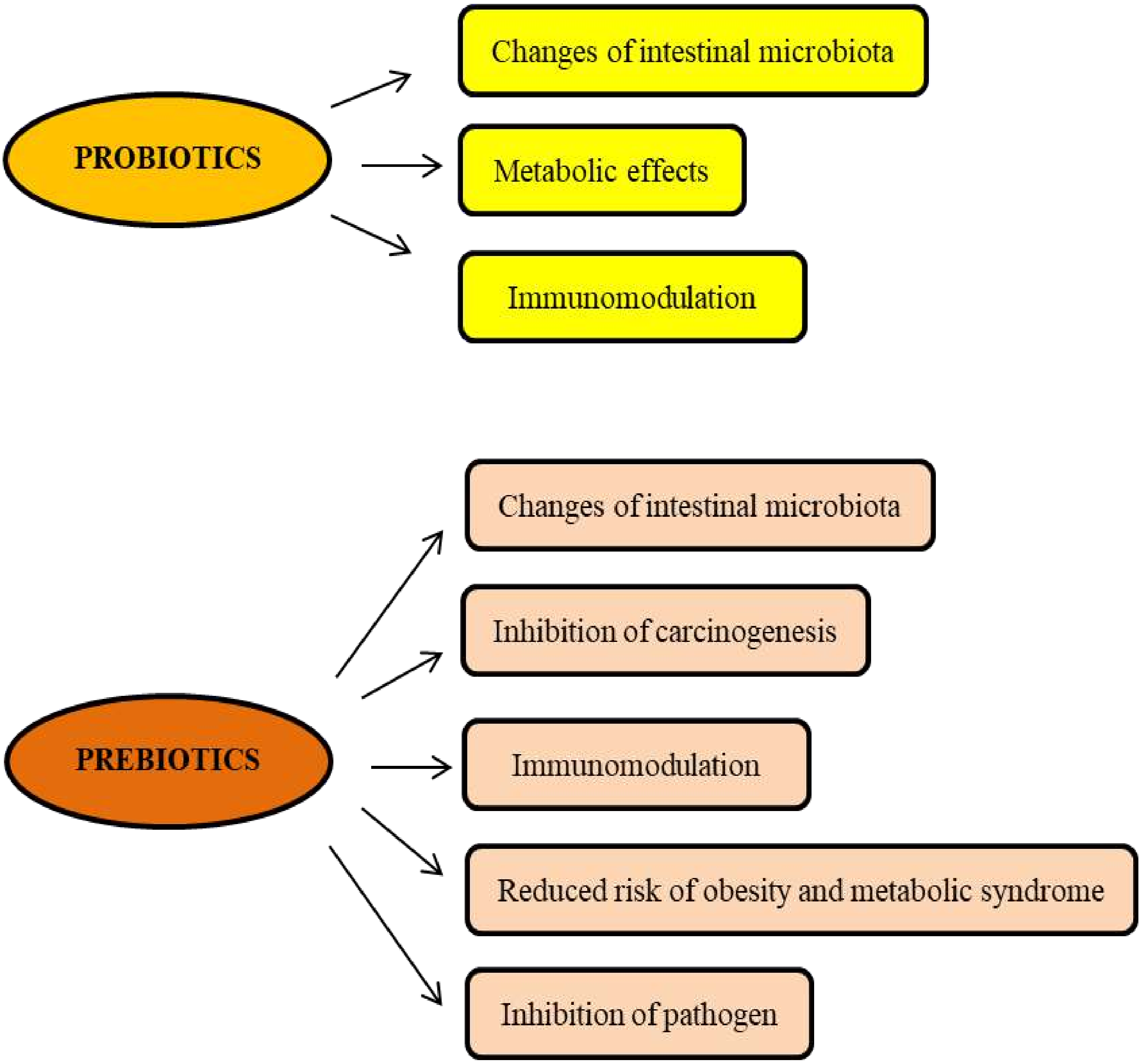

Fig. 3: Mode of action of pro- and prebiotics

Immune Modulation and Disease Resistance

The major mechanism of probiotics as an immunostimulant is to stimulate phagocytosis, an inflammatory response before antibody production and antibacterial activity in the host (Mohapatra et al., 2013). In particular, probiotics can effectively modulate the growth of anti-inflammatory cytokines such as IL-10 and Transforming Growth Factor b (TGF-b) and pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-12, and tumor necrosis factor-a (TNF-a) in several life forms. Several Bacillus probiotic species are known for enhancing the host's innate and adaptive immunity by immunostimulatory effects and stimulation of beneficial gut microflora. *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus coagulans*, *Bacillus pumilus*, *Bacillus amyloliquefaciens*, *Bacillus velezensis* and *Bacillus megaterium* are the most used Bacillus species in shrimp cultures (Suva et al., 2017). The probiotic treatment has been proven to be broadly effective in enhancing disease resistance by improving serological immunity and competitive exclusion in the shrimp's gut (Table 2).

The ability of species to resist certain bacterial and viral loads depends on their antagonism capacity triggered by genetic makeup as well as certain immune parameters. Shrimps normally possess non-specific immune genes which exert defensive responses against pathogenic strains such as recognition, phagocytosis, melanization, cytotoxicity, and cell to cell communication in hemocytes (Cheng et al., 2005).

Table 3 shows the efficiency of prebiotics as immunostimulators in aquaculture (Abarike et al., 2019). A diet supplemented with Bio-Mos® and β-1,3-D-glucan, promoted growth and survival of *Penaeus latisulcatus* and increased the efficiency of immune parameters. The use of xylooligosaccharides in shrimp, promotes growth factors the feed conversion ratio (FCR), protection against disease and potential immune responses (Table 3) (Wang et al., 2010)

Table 2: Applications and mechanism of some probiotics

Sr. No.	Probiotics	Applications	Mechanism	Citation
1	*Shewanella algae*	stress tolerance	(i) Bio-active compound (ii) catalase and hydrolytic enzymes	(Ariole and Ekeke, 2016)
2	*Bacillus subtilis* E20	Regulation of immune molecules	Increased digestion of glutamine (a fuel for immune cells)	(Tseng et al., 2009)
3	*Bacillus subtilis*	Control Vibrio parahemolyticus	Increased regulation of prophenol oxidase (immune-related genes)	(Interaminense et al., 2019)
4	*Lactococcus lactis*	antimicrobial activities against Vibrio parahaemolyticus	Increased regulation of two *Litopenaeus vannamei* prophenol oxidase (LvproPO 1, and LvproPO 2) Transcripts.	(Chomwong et al., 2018)
5	*Lactobacillus plantarum*	Heighten the survival rate of Pacific white shrimp	Vibrio spp. counts in the midgut	(Constanza Bolívar Ramírez et al., 2017)
6	*Enterobacter hominis*	Promoting rapid growth	Digestive enzymes secretions absorption ratio	(Zuo et al., 2019)
7	*Bacillus* sp. NP5 RfR	Intestinal Microbiota Diversity	By promoting the proliferation of OUT (operational taxonomic unit)	(Hasyimi et al., 2020)
8	*Bacillus subtilis* AQAHBS001	Improved resistance against Vibrio infection	Increase in microvillus and intestinal wall thickness	(Kewcharoen and Srisapoome, 2019)
9	*B. subtilis* L10 and G1	Improved digestion and immune response	manipulating the shrimp microbiota by inducing the immune gene expression	(Zokaeifar et al., 2012)
10	*Lactococcus lactis* D1813	Assessment of Immunomodulatory Role	Increased regulation of lysozyme gene expression as well as anti-lipopolysaccharide factor,prophenoloxidase, superoxide dismutase and increased level of toll-like receptor.	(Maeda et al., 2014)
11	*Pediococcus acidilactici* GY2	Reduced mortality during *Aeromonas Hydrophila*	Improved digestion and immune response	(Miao et al., 2019)
12	*Streptococcus phocae* PI80	Inhibition against vibriosis	Bacteriocin activity	(Swain et al., 2009)

Table 3: Prebiotics along with their descriptions

Sr. No.	Prebiotics	Description	References
1	β-glucan	Byproduct of wine brewing industry (produced through hydrolysis of barley gums using *Bacillus amyloliquefaciens*)	Canal-Llauber̀es (2010)
2	Inulin	Plant elecampane, *Inula helenium* derived carbohydrate (found in onion, garlic, barley)	Meyer and Blaauwhoed (2009)
3	Arabinoxylan oligosaccharide (AXOS)	Commercially produced from wheat bran using *Bacillus subtilis*	Swennen et al., (2006)
4	Mannanoligosaccharide (MOS)	Commercially derived from yeast (*Saccharomyces cerevisiae*) cell wall, is an indigestible short chain polymer derived from hydrolysis of glucomannan and galactomannan	Merrifield et al. (2010)
5	Galactooligosaccharide (GOS)	Non-digestible prebiotic derived from milk	Muzaffar et al., (2021)
6	Oligosaccharides	Found naturally in plant legume seeds. Due to the absence of α-1,6 galactosidic activity in small intestine, it can't be digested by monogastic animals	Cruz-Requena et al. (2019)
7	Fructooligosaccharide (FOS)	Synthesized through hydrolytic activity of β – fructofuranosidases. Found naturally in onion, garlic, banana, asparagus and many others. There is another type of prebiotic under FOS group known as the short chain FOS (scFOC) that derived from barley and wheat	Zhou et al., (2007)
8	Galactoglucomannans (GGM)	GGM is a hemicellulose substance from plant cell wall. GGM is suitable to promote growth of probiotic, Bifidobacterium species. It can be found naturally from wood-based plant	Polari et al., (2012)
9	Somaltooligosaccharide (IMO)	Prebiotic derived from cornstarch	Li et al., (2009)
10	Xylooligosaccharide (XOS)	Prebiotic derived from defatted rice bran	Sun et al., (2019)

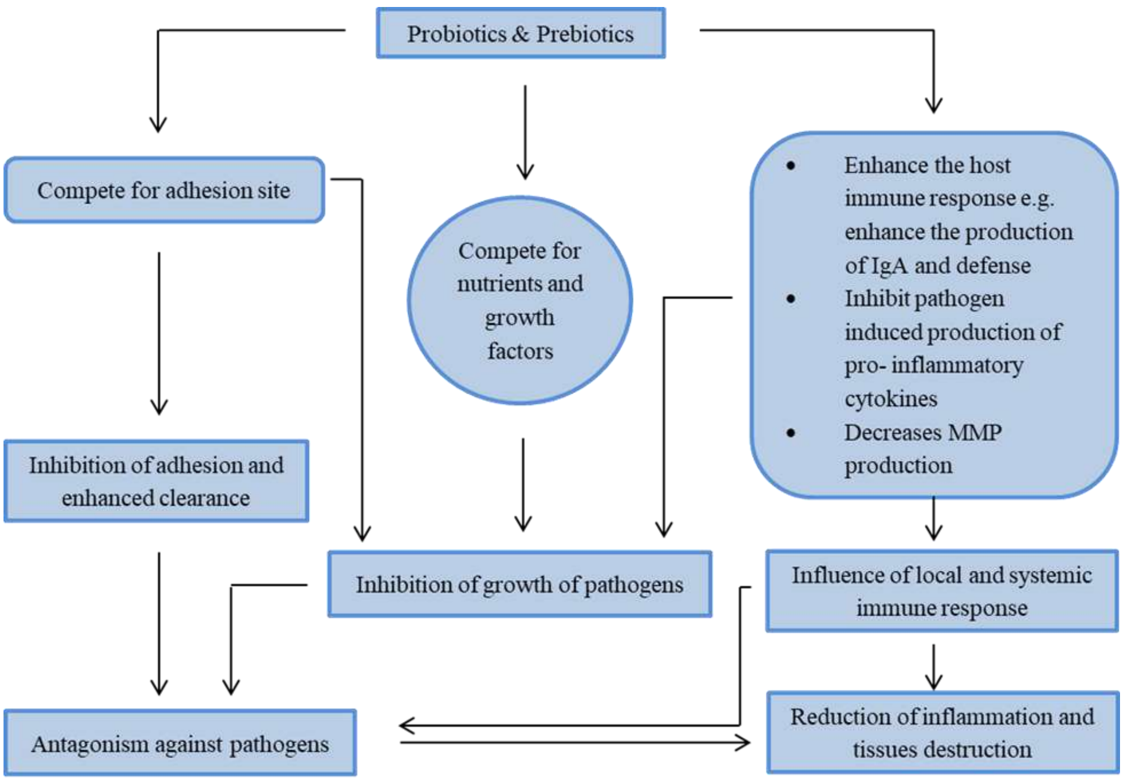

Fig. 4: Applications of Probiotics and prebiotics in shrimp culture

Competitiveness against Pathogens

Probiotics are well known for their antagonism against pathogens in the host species as well as in the culture system. Several mechanisms are defined by which probiotic bacteria can induce bacterial antagonism such as the production of siderophore substances, but more importantly, antimicrobial agents (antibiotics, antimicrobial peptides). The application of probiotics can further induce a competitive exclusion process, preventing pathogens from developing vital resistance genes. This ecological process can be intentionally altered to modify the microbial composition of pond water and soil (Amin et al., 2015). Probiotics are cheap, non-pathogenic and mostly non-toxic sources of antibiotics, having the ability to synthesize a variety of metabolites with antibacterial function, thus are worthwhile for commercial production (Abarike et al., 2018).

Production of Inhibitory Compounds

The production of inhibitory biological compounds such as antibiotics, antibacterial substances, siderophores, bacteriolytic enzymes, proteases and protease inhibitors, lactic acid and other organic compounds like bacteriocins, hydrogen peroxide and butyric acid are widely studied and well-documented functioning mechanism of probiotics (Kesarcodi-Watson et al., 2008). Probiotic microbes possess the potential to inhibit or even eliminate some pathogenic bacteria. However, in vitro inhibition may not be observed during in vivo experiments, due to incessantly changing physicochemical environmental factors (Pandiyan et al., 2013). The production of bacteriocins, inhibition of virulence gene expression, and lytic enzymes such as β-1,3-glucanase that inhibit and lyse the cell wall of the pathogens, protease, chitinase, and cellulose have been widely observed (Table 2). Other antibacterial compounds such as organic acid and hydrogen peroxide may also have an inhibitory effect due to the residual activity after catalase and acid treatment (Miandare et al., 2016).

Competition for Nutrients and Adhesion Sites

The potential probiotic bacterium is usually able to colonize and adhere to the intestinal mucosa as it prevents the adhesion of pathogens through the inhibition of etiological agents. It also eradicates pathogens from the infected GI tract and prevents disease occurrence by interfering with the disease cycle. Several gram-positive, gram-negative, and lactate-producing probiotics have superposed other bacteria in adhesion capacity (Lara-flores, 2011). Probiotic Bacillus can replace Vibrio through competitive exclusion for nutrients and adhesion sites, thereby becoming a prepotent component of intestinal microflora. Some potential strains are able to attach to the mucus by dislocating pathogenic bacteria to compete for essential nutrients and space. The adhering mechanism of probiotics can be summarized in the following steps; (a) it starts with attraction (b) binds to surface-secreting gel and (c) ends with attachment to animal tissue cells (Balcazar et al., 2007). However, constant exposure to inadequate feeding ratios may lead to a reduction in probiotic efficiency as well as intermittent production of organic matter and nutrients.

Alteration in Gut Microflora

The complex polymicrobial ecology of the alimentary canal of shrimp is an interface between the external environment and the body and has an important influence on health and disease. The intestine performs multiple functions such as supporting digestion and absorption of feed, maintaining osmotic balance, regulating endocrine and immunity. The alimentary microbiota of adult penaeid shrimp (*Penaeus chinensis*) may serve as an additional source of food, vitamins, and necessary amino acids and enhance microbial activity in the gastrointestinal tract (Wang et al., 2000). This microbiota can be favored by many factors, for example, increased population, density and types of microbes and the complex interactions between them (Martinez Cruz et al., 2012). It has become a common practice to alter shrimp's gut microbiota by adding probiotics in feed or culture systems to improve digestion, growth, and survival. Some microorganisms participate in the digestion processes by producing extracellular enzymes, such as proteases and lipases, and can provide necessary growth factors. Similarly, probiotics can consume carbohydrates from the intestine for self-growth and produce various digestive enzymes such as amylase, protease, lipase, increasing growth rate, and pre-digestibility of secondary compounds (EL-Haroun et al., 2006).

Prebiotics regulate local cytokine and antibodies to increase intestinal SCFAs (short chain fatty acids) production and improve the binding capability of SCFAs to G-protein coupled receptors on leukocytes and carbohydrate receptors on the intestinal epithelial (Seifert and Watzl, 2007). Supplementation of Previda (a commercially produced prebiotic) modulated intestinal bacterial community and stimulated immunity of shrimp (Anuta et al., 2016). Prebiotic administration further stimulated nutrient absorption and improved homeostasis stability. However, improper doses may result in the proliferation of intestinal microbiota.

Stimulation of Growth and Survival

Both dietary supplementation and oral administration of probiotics enhanced the growth of the target species by providing the necessary nutrients and improved feed utilization and digestibility by increasing digestive enzymes (Table 2) (Reda and Selim, 2015). Bacillus generally facilitates nutrient assimilation by using a variety of nutrients for their own growth and simultaneously releasing necessary digestive enzymes for the host, resulting in higher growth and survival (Lara-flores, 2011). However, the application of microencapsulated and freeze-dried Bacillus has shown no significant effect on shrimp larvae but increased the growth and survival of Post Larvae (PL) remarkably. This uncertainty may be associated with shrimp's exposure time to probiotics. A wide variety of prebiotics are administered as feed supplements to attain better growth activity in treated shrimp (Das et al., 2017). Live feed has also been reported to promote the growth of shrimp larvae, specifically Artemia enriched feed due to its high nutritional value and digestibility, for instance, bioencapsulation of Artemia species with MOS, improved survival of *Litopenaeus vannamei* while enhanced growth and FCR when supplemented with diet (Table 3). Prebiotic application may also enhance the survival rate by stimulating several immune parameters such as phagocytic, bactericidal and phenoloxidase activities (Li et al., 2019).

Enzymatic Activities

Application of probiotics in aquaculture can help attain higher enzymatic activities and enhance feed utilization and digestive capacity by modulating extracellular and antioxidant enzymes (Table 2). Glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) are commonly found antioxidant enzymes in aquatic species. SOD helps decompose harmful oxygen molecules (O_2^-) into H_2O_2 while CAT catalyzes the dismutation of H_2O_2 into H_2O and O_2. Antioxidants enzymes are also known to counteract the damage caused by reactive oxygen species (ROS) thus protecting the host against oxidative stress (Wang et al., 2017). The secretion of digestive enzymes is another characteristic of Bacillus species. Usually, Bacillus species can modulate almost all major digestive enzymes such as protease, amylase, trypsin, and lipase. In particular, *B. coagulans* have been reported to enhance the digestive enzyme activity of freshwater prawns (Gupta et al., 2016). Similarly, Bacillus PC465 improved feed absorption of *Litopenaeus vannamei* by increasing digestive enzyme activity. Administration of prebiotics has also been helpful to make enhancements in enzymatic activities (Table 3). Dietary supplementation of beta-glucan as prebiotic may result in significant enhancement of protease and amylase activities, increased nutrients and feed assimilation in the host, alteration in Bacillus and Geobacillus microbial communities and a reduction in lipase levels through hypolipidemic activity (Zhou et al., 2016). The combination of β-glucan (prebiotic) and Bacillus (probiotic) can enhance digestive enzymes as Bacillus triggers extracellular enzymes in the shrimp's colon, such as protease and amylase (Abdollahi-Arpanahi et al., 2018). The commercially available prebiotic immunogen may also increase digestive enzymes in Pacific white shrimp *Litopenaeus vannamei*. However, excessive dosage of prebiotics may result in reduced enzymatic activities such as glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) (Hu et al., 2019).

Conclusion

Shrimp aquaculture has been dominated by various pathogenic and viral diseases over the past few decades. The use of antibiotics, to counter these diseases, backfired as it equally threatened the health of the host and the consuming bodies. Besides resisting the pathogens inappropriate antibiotic application led to bacterial resistance in shrimp and human consumers. Considering the gradual spike in the global demand and preference for healthy and hygienic shrimp, there is a need for natural alternatives such as pro-, and prebiotics as dietary supplements to improve the competitive exclusion of pathogens from the system and to enhance the immune parameters of shrimp without affecting its health. However, to achieve optimal protection, a clear understanding of these immunostimulants is necessary. As per the experiments conducted in recent decades, pro-, and prebiotics could be considered a better alternative, as compared to antibiotics and similar products, for achieving protection while maintaining environmental stability thus, increasing the shrimp yields. The addition of these supplements in shrimp feed can significantly decrease the occurrence of disease and increase enzymatic activities, feed consumption, growth and survival of cultured shrimp. In conclusion, a methodical and comprehensive understanding of the utilities of pro-, and prebiotics in the field of shrimp farming can be achieved through profound knowledge of the genetic makeup and transcriptomic and proteomic profiling of these products.

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Chapter 25

Nutritional Importance of Probiotics in Fisheries/Aquaculture

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ABSTRACT

The Aquaculture sector is the fastest production unit for food in the world and it plays a major role to fulfill the rising demand of the food. It is not the only source of food but also a good source of earning and hence human development. Meanwhile, the aquaculture sector is facing challenges against different disease outbreaks that not only hampers its growth but also reduces its production and economic values. To overcome these challenges there is a new emerging technique by probiotics that is now in trending as a feed supplement with different benefits. Probiotics are a vast range of organisms that include yeast, bacteria, fungus, microalgae and their products. The term "probiotic" refers to a relatively recent classification of bacteria that are linked to advantageous outcomes for their host. Probiotics are considered best feed supplements that enhance the growth, modulates the immune response, cure different infectious diseases, increase the digestibility, reduces stress and improves the water quality. Probiotics used in the aquaculture and different foods have historically been considered safe and no dangers in organism have been identified, which is still the best evidence of their security.

KEYWORDS
Probiotics, Nutritional values, Fish health, Feed efficiency, Feed supplementation, Disease resistance, Antibiotic alternatives

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INTRODUCTION

Aquaculture being the fastest developing production unit of food globally plays a vital role to meet the rising demands of food. It is not only restricted to overcome food shortage, but also a valuable source for more earnings and human development as well. However, the major challenge in the growth of aquaculture is the spreading of diseases in aquaculture species that not only affect their growth but also hampers the economic values as well as socio-economic development of several countries (Bondad-Reantaso et al., 2009). The term "probiotic" refers to the beneficial bacteria that enhance a host's health when provided in sufficient amounts (Harsh and Sangita, 2022).

In first carried out application, *Bacillus* spp. spores was used as a feed additive to speed up growth. *Bacillus* spp. was also tested for their capacity to raise *Penaeus monodon* farming productivity and enhance water quality by lowering ammonia and nitrite concentrations (Edun and Akinrotimi, 2011).

The term "probiotic" originated from a phenomenon observed in co-cultured organisms, when certain microorganisms generated compounds that promoted their own growth, thereby augmenting the development of the host. Probiotics are microorganisms that can improve an animal's general health and prevent disease by assisting the body in fighting off pathogens in the gastrointestinal system. For instance, probiotics are beneficial due to their low production costs and numerous uses in a range of host species (Rashid et al., 2023).

One important way to increase the demand for plant and animal proteins is through aquaculture, which is also a promising industry for promoting human food security and adequate nutrition. As a component of agriculture, aquaculture primarily aims for making food production sustainable and has a strong knowledge of the food chain. From 14% in 1986 to 47% in 2006, the average use of aquaculture items has improved, and in the upcoming years, a further 50% increase is predicted (Asghar et al., 2022).

Humans mostly obtain their protein from fish, which are also used as bio-indicators within a biome. Fish are low in fat and abundant in protein, vitamins A, B, D, and E, minerals calcium, phosphorus, iron, zinc, and iodine. Fish are gradually producing at a high level on an industrial and economical scale. Asian countries including China, India, Indonesia and Bangladesh have fish farming systems that are used to cultivate high-value and highly suitable fish (Roos et al., 2007).

However, in some of these countries, the main obstacles to aquaculture are outbreaks of various diseases, poor fish growth and survival rates caused by harmful pathogens (bacteria, fungi, viruses, protozoa, and different parasites) and an unsanitary aquatic environment that can contribute to a decline in supply chain (Wang et al., 2008).

When given in adequate quantities, probiotics, which are live bacteria, offer health benefits to the host. Probiotics are added to feed as a means of increasing the number of good bacteria, improving growth efficiency, and lowering the quantity of enteric pathogens. Probiotics are often derived from the bacterial genera *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Bifidobacterium*, and *Bacillus* (Mehmood et al., 2023).

Another negatively impacting factor that is antibiotic resistance as a result of overuse of antibiotics, according to the conducted research of Friedman et al. (2016). Now, probiotics are being used in aquaculture as a new method to reduce the spread of harmful microorganisms. Probiotics have been used in aquaculture to reduce the use of harmful anti-microbial agents, including certain antibiotics, as well as to improve the appetite and bio growth performance of farmed species in a sustainable and environment friendly way (Wang et al., 2005).

Challenges to the Aquaculture

Nomoto (2005) stated that global outbreaks of bacterial, fungal, and viral diseases have resulted in enormous financial losses and it has been documented that unfavorable environmental conditions, imbalanced diet, toxin production and hereditary variables all contribute significantly to stock mortality. The use of different medications, particularly antibiotics, has been the main strategy for preventing and controlling animal diseases in recent decades. However, this approach poses serious threats to health of the community because it encourages the choice, spread as well as perseverance of bacterial resilient strains.

It is anticipated that aquaculture would supply the constantly increasing need instead of catch fisheries. In their aquatic culture ponds, fish growers raise the stockings to unachievable levels due to the excessive demand. They become more vulnerable to opportunistic infections and disease outbreaks as a result of the stress caused by overcrowding. Antimicrobial resistance develops and spreads in aquatic systems as a result of excessive administration of antibiotics prompted by the increasing incidence of number of diseases and therapeutic interventions (Nayak et al., 2023).

Need for the Probiotics

Nair et al. (2017) explained the idea to overcome all the above-mentioned challenges with a new emerging approach that is being used named as probiotics. The definition of probiotics is, a live microbial supplement that exerts beneficial effect through various mechanisms by modifying the microbial community associated with the host, improving the feed utilization, increasing its dietetic value, boosting the host's response to diseases or enhancing the overall quality of the host's ambient environment.

Probiotics are a vast range of organisms that include yeast, bacteria, fungus, microalgae and their products. Probiotics are becoming more and more popular as a preventive and therapeutic measure because of the growing risk of antimicrobial resistance brought on by the widespread applications of antibiotics (Hasan and Banerjee, 2020).

Probiotics are being used more frequently and have been shown to be helpful in reducing the incidence of disease through a number of mechanisms, including better nutrient utilization through the breakdown of complex substances in the environment and feed, the mineralization process, participation in the biogeochemical cycles, preservation of water quality, and enhancement of immunological parameters (Melo-Bolivar et al., 2020).

Probiotic benefits its host as well as the environment through different ways as elaborated in this definition. Probiotics always prefer the importance of health for their hosts along with the characteristics i.e., their proper utilization to feed, better nutrition and a host’s healthy environment. According to the updated definition, the idea of possible uses of probiotics and their advantages are illustrated more precisely even from the diverse context (Anadon et al., 2019).

Properties of the Probiotics

Special qualities that are much needed for product to maintain health, are found in probiotics. During the whole digestion process, probiotics always facilitate to easily pass through the body to reach intestinal tract where they can directly alter the immune functions in a positive way. They also help to increase the balance of gut micro-biomes, immune reactions and control inflammation (Aureli et al., 2011).

Additionally, probiotics have anti-pathogenic qualities that help to maintain gut health, stop the growth of dangerous bacteria, and possibly even prevent infections. Furthermore, their stability during storage and processing is essential to preserving their effectiveness in fortified meals or supplements. Probiotics are defined by their many characteristics as living things that work in harmony with their host to promote better health (Fig. 1) (Raheem et al., 2021).

Applications of Probiotics in Aquaculture

Research on probiotic use in aquatic organisms is being stimulated by the need for sustainable aquaculture practices. Although, new aspects are being discovered like the impact on stress tolerance and reproducing ability but further research is required. Initially, the main focus was on using them as growth promoters and to enhance animal health (Patricia et al., 2012).

The *Lactobacillus*'s antioxidant qualities support the body's defense against pathogen invasion. Lactic acid bacteria have been reported to have probiotic properties when they are present in milk and epithelium (Ullah et al., 2023). Various applications of species specific probiotic in aquaculture has been summarized in Table 1.

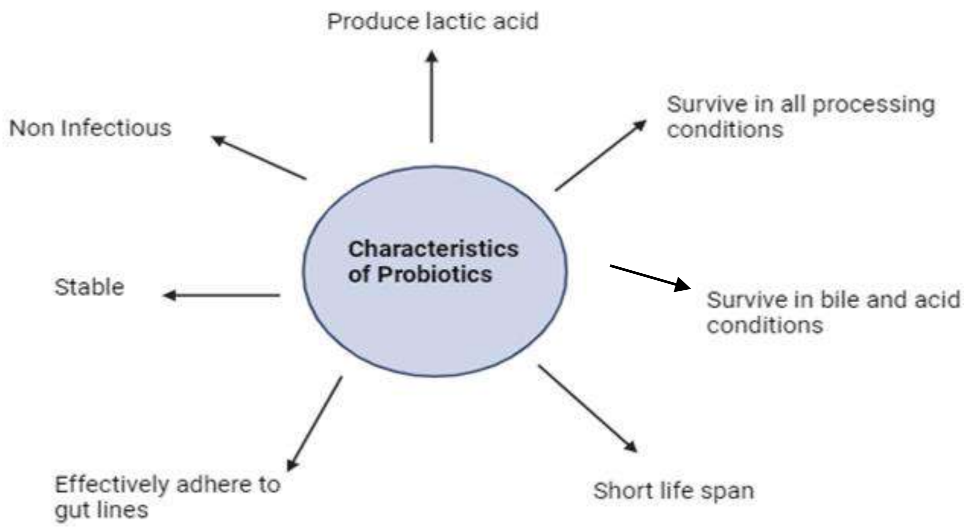

Fig. 1: Characteristics of an ideal probiotic

Table 1: Different Applications of Specific Probiotics in Aquaculture

Application	Specific Probiotics	Target specie	Reference
Growth promoting agent	*Bacillus sp.*	*Cyprinus carpio*	Lin et al., 2012
	Lactobacillus helveticus	Catfish	
	Lactobacillus lactis	*Macrobrachium rosenbergii*	
	Streptococcus thermophilus		
Prevention of Infections	*Saccharomyces cerevisiae*	*Oncorhynchus mykiss*	(Sun et al., 2012)
	Pseudomonas fluorescens	*Anguilla anguilla*	
	Lactococcus lactis	*Epinephelus coioides*	
Development in digestion of nutrients	*Bacillus sp.*	*Macrobrachium rosenbergii*	(Tapia-Paniagua et al., 2012)
	Lactobacillus acidophilus	*Clarias gariepinus*	
	Shewanella putrefaciens	*Solea senegalensis*	
water quality improvement	*Bacillus sp.*	*Penaeus monodon*	(Taoka et al., 2006)
	Lactobacillus acidophilus	*Clarias gariepinus*	
Tolerance of stress	*Bacillus sp.*	*Paralichthys olivaceus*	
	Lactobacillus acidophilus		
Effect on aquatic species reproduction	*Bacillus subtilis*	*Oecilia reticulate*	(Abasali and Mohamad 2010)
	Lactobacillus acidophilus	*Xiphophorus helleri*	
	Bifidobacterium thermophilum		

Growth-promoting Agent

Irianto and Austin (2002) reported the applications of probiotics in aquaculture sector to boost the body growth of different cultured fish and shrimps, but it remained unclear that either these items actually stimulate hunger or enhance the digestibility by natural ways. These reporters believe that there might be a combination of these elements; additionally, it would be crucial to ascertain whether probiotics are palatable to aquaculture species.

There have been reports by El-Haroun et al. (2006) that probiotics being used to boost edible fish growth. The probiotic *Streptococcus* strain added to the diet of Nile tilapia (*Oreochromis niloticus*) resulted in a considerable rise in the fish's crude protein and crude fat content. In just nine weeks of culture, the fish weight improved from 0.154 g to 6.164 g. Owing to the species' commercial significance, probiotic food supplements increased the body weight of fish by 115.3% as a result, when a commercial formulation with 2% concentration of probiotics was employed.

Probiotics have also been effectively tested and exemplified by Macey and Coyne (2005) in the culture of shellfish. Two yeasts and one bacterium strain were extracted from the digestive system of abalone (*Haliotis midae*). Three potential probiotics were combined to create a diet. To make a final dry diet, each probiotic was used in equal concentrations. In eight-month cultures, the growth rates of small (20mm) and big (67mm) abalone were enhanced by 8% and 34%, respectively (a diagrammatic scheme shown in Fig. 2). Additionally, probiotic-supplemented abalones exhibited a 62% survival rate against the pathogenic bacterium *Vibrio anguillarum*, in contrast to a 25% survival rate for untreated animals.

Prevention from Infections

For many years, aquaculture sector employed antibiotics to avoid crop diseases. But this led to a number of concerns, including the development of bacterial resistance mechanisms, the accumulation of antibiotic remains in animal's body tissues, and an imbalance in the gut microbiota of aquatic species, all of which had a negative impact on the health of the animals. Probiotic usage is therefore a practical substitute for controlling disease and inhibiting infections in aquaculture species (Patricia et al., 2012).

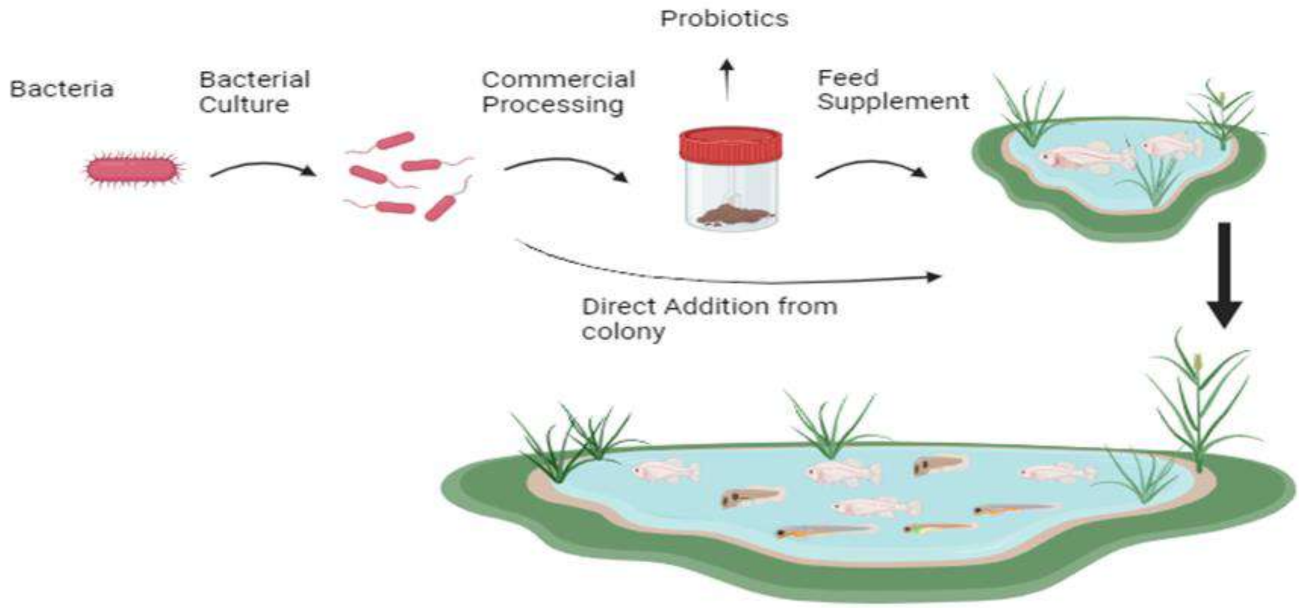

Fig. 2: Diagrammatic series of probiotic application in pond culture system as a growth promoter

By releasing chemicals that have a bactericidal or bacteriostatic impact on pathogenic bacteria present in the host intestine, probiotic microorganisms can act as a barrier to prevent the growth of opportunistic infections. The direct mode of action against prevalence of infections is the formation of bacteriocins, antibiotics, siderophores, hydrogen peroxide and different types of digestive enzymes along with changes in intestinal pH caused by the production of organic acids are often responsible for the antibacterial action (Nair et al., 2017). Additionally, it has been discovered by Ghosh et al. (2008) that probiotic concentrations of 106 to 108 cells/g enhance the growth of friendly micro biota inside the intestinal tracts of decorative fishes belonging to the *Xiphophorus* and *Poecilia* genera, thereby reducing the number of hetero trophic microbes.

In the connection with public health, probiotics have been investigated primarily as a way to lower enteric infections and meat contaminations. These are living, non-pathogenic microorganisms that may be advantageous for the microbiota in the intestines, immunomodulators, and early colonization of beneficial bacteria when added (Gul and Alsayeqh, 2022).

Development in Digestion of Nutrients

According to a previous study, probiotic strains give growth factors including vitamins, fatty acids, and amino acids as well as produce extracellular enzymes like lipases, proteases, and amylases, which may benefit aquatic animals' digestive systems (Balcazar et al., 2006).

As a result, administering probiotics to the meal improves the efficiency of nutritional absorption. Research on juvenile common *Dentex dentex* showed that *Bacillus cereus* strain in a small quantity as food additives to the diet boosted fish growth. Similar outcomes were seen in the case of rainbow trout when *B. subtilis*, and *B. licheniformis* were given for ten weeks in addition to the fish diet (Merrifield et al., 2010).

Probiotic yeast (*Saccharomyces cerevisiae*) has been added to the diet of European sea bass larvae (*Dicentrarchus labrax*) in an experimental trial in which the fish were evaluated for growth parameters, and the appearance of change in key anti-oxidative enzymes. The presence of the yeast was found to be responsible for differences in enzyme activity and gene expression patterns between the probiotic-supplemented and non-supplemented treatments (Tovar-Ramirez et al., 2010).

Water Quality Improvement

Water quality was monitored by Martinez et al. (2012) in an experiment when probiotic strains, particularly those belonging to the gram-positive species were added. This type of bacterium converts organic stuff into CO_2 more effectively than gram-negative bacteria. It has been proposed that fish farmers can reduce the organic carbon in growing season by keeping high probiotic levels in their production ponds. Furthermore, this can maintain a balance in phytoplankton production (Balcazar et al., 2006).

In order to enhance water quality for fish culture and prevent *Aeromonas hydrophila* growth, many bacillus strains were isolated from *Cyprinus carpio*. In addition, concentrations of nitrates, phosphates and ammonia, were decreased more than 70% for three of the nine isolates that had a strong capacity to suppress the pathogen. On the other hand, when a commercially available probiotic examined in catfish (*Ictalurus punctatus*), a substantial increase in net fish output and survival was observed (Lalloo et al., 2007)

Tolerance for Stress

Cultured species are stressed by aquaculture methods because they require intensive yields in shorter amounts of time (Vianello et al., 2003). Since the hormone cortisol is directly related to the animal's reaction to stress, it was measured in body tissues of fish as a stress marker in order to assess the growth improvement. The fish treated with cortisol had cortisol levels that were considerably lower than those of the control group (Carnevali et al., 2006). The examination for stress were conducted till the half of the colony perished, at which point the mean lethal time (LT50) was determined both without and with the addition of a commercial probiotic including *Lactobacillus acidophilus*, *Saccharomyces cerevisiae* and *Bacillus subtilis*. In the stress test, the probiotic treated group demonstrated higher tolerance than the control group (Taoka et al., 2006).

Effect on Aquatic Species Reproduction

At the reproductive stage, the culturable species show high dietary requirements. According to the reports of Izquierdo et al. (2001), adequate concentrations of all dietary components are necessary for reproductive capacity. Additionally, the interaction between these elements affects reproduction in a number of ways, including fertility, fertilization, larval birth, and development. Currently, "brood stock diets," which are simply larger-sized meals, are commercially available for the majority of cultured fish species. In actual, a lot of fish hatcheries feed their brood stock only fish waste or/and in addition to commercially available diets to enhance the nutrition of the fish (Ghosh et al., 2007).

Mechanism of Action

Probiotic defends against various infections by competing with them for vital nutrients and attachment sites, generating an unfavorable environment for them to thrive in, or adjusting the immune system (Balcazar et al., 2006).

Synthesis of Inhibitory Substance

Numerous bacterial strains are capable of producing one or more antimicrobial compounds in vitro, including bacteriocins like pediocin and streptococcins (Fig. 3), as well as low molecular-weight molecules like ethanol, carbon dioxide and lactic acid (Sadeghi et al., 2022). In nature, antagonistic relationship between microbes is common, such as *Vibrio* spp. isolated from Japanese Flounder were shown to exhibit antibacterial properties that prevents the growth of *Pasteurella piscicida*. Furthermore, the gut of dragonets contained *Bacillus* sp., which was extracted and produced inhibitory compounds that were efficacious against *Vibrio vulnificus* (Sugita et al., 2002).

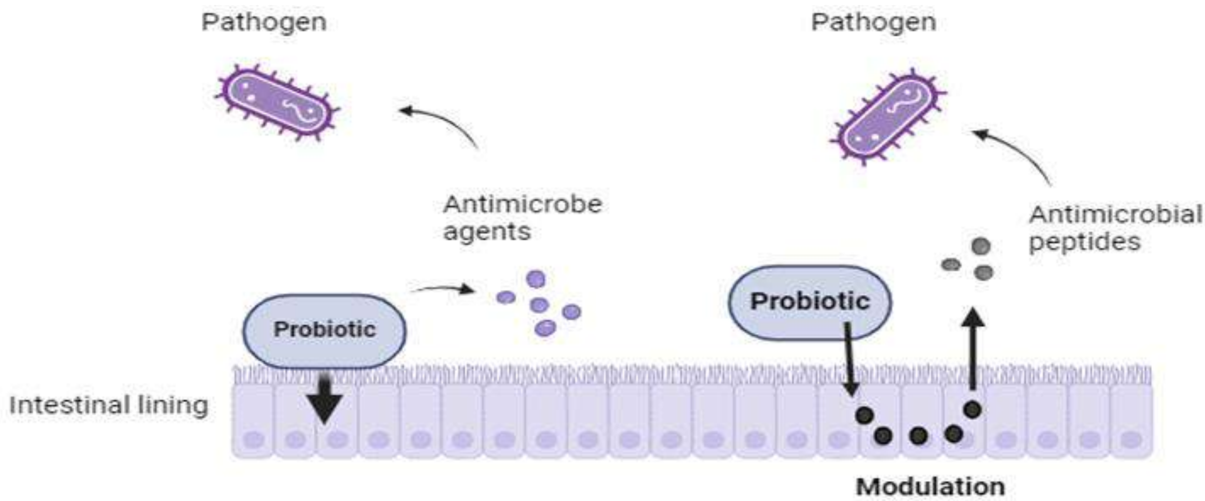

Fig. 3: The intestinal protection through antimicrobial agents produce by probiotics and by modulation of immune response to combat with pathogens (Balcazar et al., 2006).

Low molecular weight compounds like carbon dioxide, ethyl alcohol, caprylic acid and caproic acid; bacteriocins such as pediocin, sakocin and streptococcins are formed by numerous bacterial colonies. These antimicrobial substances have negative impact on pathogens. For example: Intestinal bacteria, obtained from Juvenile and larva of Japanese flounder (*Paralichthys olivaceous*) are studied and depict antibacterial capacity. Similarly, *Pasteurella piscicida* growth is retarded by 53.3%. Moreover, *Vibrio vulnificus* multiplication is hindered by *Bacillus,* so the strain which were obtained from the intestine of dragonets proved that it has the ability to reduce the number of pathogenic bacteria by inhibition in the body (Sugita and Ito, 2006).

Contest for Vital Nutrients

Microorganisms compete fiercely for few nutrients, and their interactions are influenced by factors such as growth rate, intrinsic metabolic velocity, nutrient absorption velocity, and inhibitor secretion. Research on probiotics may benefit from this competition. For instance, iron is necessary for the growth of all microbes and is well acknowledged to play a significant role in bacterial interactions and virulence. A variety of bacteria, including certain *Pseudomonas* species, secrete siderophores in order to absorb iron. The purpose of one experiment was to determine whether *Pseudomonas fluorescens*, is a good probiotic for rainbow trout by examining its effect on development of *Vibrio anguillarum* in both iron-rich and iron-limited environments (Luis et al., 2006).

Contest for Binding Sites

Competition for attachment sites on gut linings is one potential defense against pathogen colonization. Probiotic strains colonies mucosal surfaces without inducing illness, and they may even form a symbiotic association through immunomodulation, pathogen displacement, or digestive support. Pathogens attach with adhesion site located on the intestinal mucosa or epithelial surfaces. If their attachment is prohibited, growth of aquaculture will be increased. Probiotics show symbiotic association with adhesion site and speed up not only the process of digestion but also restricted pathogens adherence (Chabrillon et al., 2005).

Regulation of the Immunological Reactions

Probiotic strain supplementation modulates the immune response, which is another way to stop pathogen infection. Different host immunological responses, both specific and non-specific, can be triggered by components of bacterial walls (Fig. 4). While lipopolysaccharide is expressed exclusively by Gram-negative bacteria, peptidoglycan is one of those essential components that is found in bacteria. After two weeks of feeding, a strain of *Lactobacillus rhamnosus*, a species commonly seen in probiotic preparations, have the ability for respiratory burst activity stimulation in rainbow trout (Nikoskelainen et al., 2003).

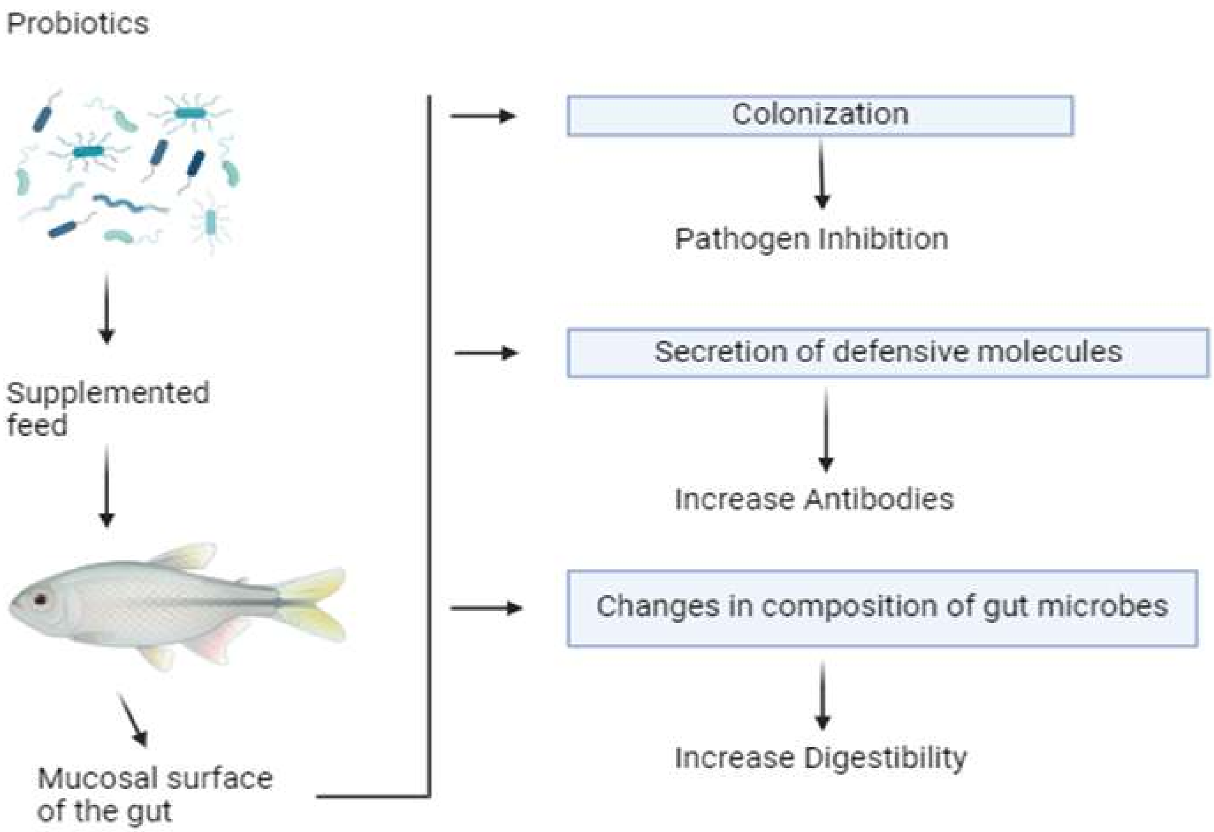

Fig. 4: Properties of Probiotics as immunity modulator in the gut of fish (Banerjee and Ray, 2017)

Effects as Antivirals

Although the mechanism of interaction was not characterized, it has recently been observed that probiotic bacteria (*Vibrio hepatarius*, *Vibrio* sp., *Bacillus* sp., and *Vibrio alginolyticus*) given in the diet of juvenile prawns decreased the white spot virus syndrome. According to other research, probiotics and native bacteria can create soluble molecules that alter the intestinal surface's glycosylation, which in turn alters the structure of the viral receptor and hinders the virus's ability to recognize and enter the body (Panigrahi et al., 2005). The synthesis of biological materials, includes the extraction from algae and bacterial extracellular agents, is another process that inactivates viruses. According to reports, *Vibrio* spp. obtained from black tiger shrimp hatcheries shown antiviral efficacy against both *Oncorhynchus masou* virus (OMV) and infectious hematopoietic necrosis virus (IHNV), with percentages of plaque reduction ranging from 62% to 99% (Freitas et al., 2003).

Safety Considerations of Probiotic

Probiotics used in the food business have historically been considered safe; in fact, no organism's dangers have been identified, which is still the best evidence of their security. Concerning the safety of aquaculture products, farms in Asia that regularly utilize probiotics based on *Bacillus subtilis* have reported cases of bacterial white spot syndrome (BWSS) in *Penaeus monodon* cultures. The patches resemble those caused by the deadly White Spot Viral Syndrome (WSS), which spreads quickly and kills large numbers of prawn and shrimps. Lesions from BWSS, a non-systemic infection in *P. monodon*, typically go away after molting; in this state, cultures remain active and develop normally with little to no mortality. It is concerning, because the majority of farmers are unable to differentiate between BWSS and WSS. Farmers are needed to send samples to a laboratory for a second opinion in case of suspicion. It has also been questioned if leftover probiotics could infect the final user because certain aquaculture foods are eaten raw or only partially cooked. Probiotic use in mice has been shown to be safe through studies utilizing mice as experimental animal that were provided probiotic bacteria to approach the LD50 value for *Shewanella algae*. The conclusion based on this data by using *S. algae* is safe for those who consume prawns, including employees of farms and processing facilities.

Futuhre Perspectives

Probiotic use as a preventive measure has been shown to enhance the health and productivity of aquaculture species. Probiotics have a lot of potential because of the amazing advancements produced in the cultivation of fish, mollusks, crustaceans, and live food. The use of probiotics is considered as a type of guarantee that it will reduce the disease outbreaks in aquaculture but, there are still many questions about its use such as; what is the efficiency of probiotics in a stressful situation? Are probiotics able to create the pathogenicity? The actual mechanism in which probiotics regulates its action is still unclear. The answers to these concerns lie in developing, preparing, and storing probiotics suitable for large-scale usage in aquaculture as well as in establishing guidelines for quality control (Edun and Akinrotimi, 2011).

The aquaculture industry, which mostly raises fish, mollusks, and crabs, is the one that is providing food at the fastest rate in a number of nations, including China, Norway, India, Malaysia, Brazil, USA, Japan and Sri Lanka. In aquaculture, bacterial illness is a prevalent issue that results in a significant loss of life for both farmed and wild fish. Farmers typically employ antibiotics that are not beneficial to the environment, to lower the danger of pathogenic germs and to produce results quickly. Antibiotics also place new drug-resistant bacteria under selective pressure, which increases the risk of transmission from food chains to humans (Hoseinifar et al., 2018). Although several probiotic candidates and mixes are now being identified, the aquaculture industries are still constrained to use these developments. Moreover, farmers are unaware of the value of probiotics and application to add them into ponds. Therefore, the only method to train fish producers to replace antibiotics with probiotics is through regular government campaigns. Probiotics have been shown to improve fish fertility, and their function in reproduction is under research at the current situations (Banerjee and Ray, 2017).

Concluding Remarks

Probiotics have been shown in a prior study to have encouraging effects on fish and shellfish disease resistance. As a result, it is possible that this environmentally beneficial dietary supplement will become more well-known as an aquaculture antibiotic substitute. It is important to remember, that prior studies' findings indicated that probiotics have species-specific benefits. Due to the immune systems of aquatic species and their susceptibility to disease outbreaks, it is crucial to develop efficient and environment friendly methods of disease bio-control. The findings of above-mentioned studies motivate for additional research on the use of probiotics in aquaculture for parasite bio-control. Furthermore, compared to other immunity stimulants, there is a lack of study on probiotics potential as a bio-control measure against viral and parasite diseases in aquatic organisms, despite the positive results gained. Finally, there is still much to learn about the processes by which probiotics affect the immune system. Particularly with regard to the molecular mechanisms underlying the interactions that occur between the host and probiotic, further research is needed.

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Chapter 26

Probiotics and Prebiotics, Boosting Fish Gut Health, Immunity and Disease Resistance

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ABSTRACT

Probiotics are defined as live microorganisms that help in the prevention and management of particular pathological conditions. Probiotics also help to maintain a healthy balance of gut microbiota, supporting digestive and immune system functions. Prebiotics are indigestible fiber that serves as food for probiotics and other healthy microorganisms that already exist in the gut. The local or native anaerobic fauna of the alimentary tract shows resistance against pathogens. Probiotics and prebiotics have been considered for enhancing health and resilience in fish within the aquaculture system. In more important ways, these supplements modulate the structure of the microbial community for the creation of a beneficial bacteria-conducive environment and at the same time, can repress the spreading of the pathogenic strain. Such microbial modulation allows the fish better nutrient uptake, digestion and utilization for better growth performance and feed efficiency. Besides, probiotics and prebiotics are also known to have an immunomodulatory effect and have been upregulated in both innate and adaptive immune responses in fish for the decrease in incidences of infectious diseases. The use of probiotics and prebiotics in aquafeeds is an area that holds a substantial framework for enhancing the sustainability and productivity of aquaculture enterprises through improvements in gut health, immunity and disease resistance. Further, the sensible use of these supplements meets the command to reduce dependence on antibiotics and chemical interventions to maintain environmental compatibility in aquaculture practices.

KEYWORDS		
Probiotics, Prebiotics, Fish health, Immunity, Disease resistance	Received: 12-May-2024 Revised: 19-July-2024 Accepted: 22-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Prebiotics and probiotics are two main components that promote gut health and the overall well-being of a variety of organisms including animals and humans. Probiotics are defined as live organisms that, when added in adequate quantities, give health benefits to the host by regulating the balance of intestinal microbiota and enhancing immune function. These healthy bacteria can be obtained from supplements or naturally occurring in fermented foods (Venema and Do Carmo 2015). Prebiotics, on the other hand, are undigestible fiber that serves as food for probiotics and other healthy microorganisms that already exist in the gut. Prebiotics aid in developing a positive gut setting by increasing the development and activity of helpful quantities of bacteria. Probiotics and prebiotics have been gaining popularity in recent years, with an increase in research on these supplements. These may give positive outcomes to an organism's health because they work together to develop healthy gut microorganisms (Holzapfel, 2006).

Brief Overview of the Importance of Fish Gut Health

Gut health, immunity and disease resistance are very important to the health and survival of fish. The gut microbiome is of extreme importance in the maintenance of these aspects of fish health. The gut microbiome is a complex community of microorganisms symbiotically living in the gut of fish and interacting with the host. Some of their functions are digestion, nutrient absorption and regulation of the immune system. A healthy gut microbiome can help prevent pathogenic bacteria colonization and improve fish disease resistance. On the other hand, an altered gut microbiome could cause dysbiosis (an imbalance in bacterial composition) which may lead to an increase in diseases among fishes. (Gómez and Balcázar 2008).

Explanation of the Role of Probiotics and Prebiotics in Fish Gut

Probiotics and prebiotics contribute to gut health, immunity and disease resistance. Probiotics regulate the gut bacteria, help the immune system to boost itself and assist in treating several disorders related to digestion. Prebiotics help bind and remove extraneous pathogenic bacteria from the gut and preserve the health of the fish through improved gut integrity, digestion, nutrient absorption and immune function. Prebiotics promote healthy, balanced, diverse populations of gut bacteria, or healthy microbiomes (Merrifield and Ringo 2014).

Non-digestible carbohydrate fractions like inulin, oligosaccharides (galactose, fructose or mannose), β-glucans, organic acids, fructo-oligosaccharides (FOS) and mixtures of these components are the main sources of prebiotics (Van Doan et al., 2020). Further development in sustainable aquaculture develops because these prebiotics promote the overall health and growth of fish, reducing the application of antibiotics and vaccinations. Many fish species such as rainbow trout, brook trout, sturgeon, common carp, koi, African catfish, European sea bass and sea bream have been regarded to derive the advantages out of the application of prebiotics on gut morphology, pathogen-binding capability, immunostimulant property and nutrient digestibility (Hasan et al., 2023). Both probiotics and prebiotics work in conjunction to regulate the gastrointestinal microbiota (Kalita et al., 2023).

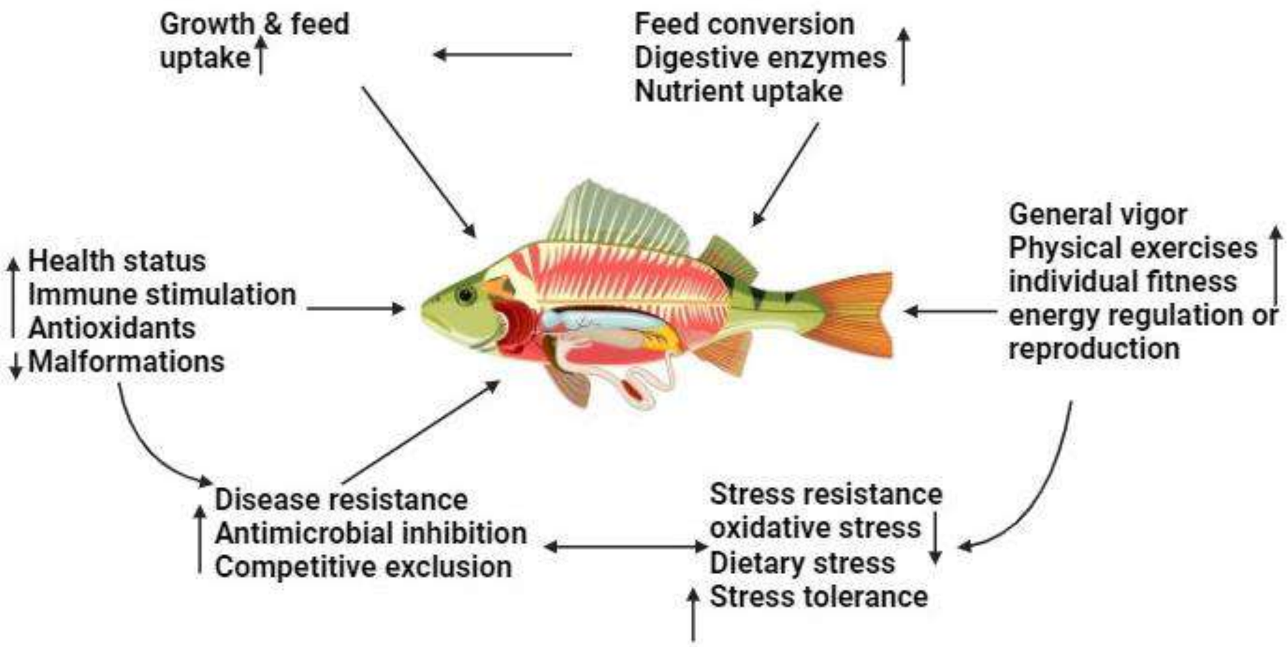

Fig 1: Diagrammatic view of the role of Probiotics and Prebiotics in fish health (small upward arrows show an increase in growth, FCR, General vigor, stress tolerance, health status, disease resistance and small downward arrows show a decrease in dietary and oxidative stress and malformations).

Mechanism of Action of Probiotics in Fish Health

Probiotics support fish gut health through many ways. Among them is the alteration of the gut microbiome. Probiotics can colonize the gut, thereby reducing the number of harmful bacteria through nutrient and binding site competition. They may also produce inhibitory substances that completely inhibit the growth of harmful pathogens (Plaza-Diaz et al., 2019). The other way is through enhancement of the gut immune system. Probiotics can also enhance fish resistance to infection by elevating immune cell production and cytokines. It boosts mucus production and tight junction proteins, which are substances that block dangerous bacteria from getting into the blood and toxins from entering the gut. These probiotics shall also increase the uptake and utilization of nutrients in the fish, thus resulting in improved growth performance of the fish (Loh, 2017).

Beneficial Examples of Probiotics in Fish Health

A large number of probiotics useful for fish have been studied and developed for aquaculture industries. For instance, probiotics benefit the utilization of feed and resist diseases, as well as improve growth in fish cultured.

1. Lactobacillus spp: *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus casei* (Wuertz et al., 2021).
2. Bifidobacterium spp: Both *Bifidobacterium longum* subsp. *Longum*
3. Enterococcus species: *Enterococcus faecium* and *Enterococcus faecalis* (Sayes et al., 2018).
4. Streptococcus species: *Streptococcus thermophiles*.
5. Bacillus species are the *Bacillus subtilis* (Martínez et al., 2012).

These probiotics can be given to fish via feed, water or direct application to their skin or gills.

Beneficial Examples of Prebiotics in Fish Health

Some of the prebiotics studied in fish are fructooligosaccharides (FOS), β-glucan (GLU), chitosan (CTS), mannan-oligosaccharides (MOS), and xylooligosaccharide (XOS) (Wee et al., 2022). All these reports showed that the prebiotics enhanced disease resistance and antioxidant potential in hybrid grouper, grass carp and Nile tilapia (Li et al., 2021). However, the effectiveness of prebiotic supplementation depends on the prebiotic structure, dosage, supplementation period, fish species and age/stage/weight. (Amillano-Cisneros et al., 2023).

Effects of Probiotics on Fish Gut Microbiota Composition

Some probiotics introduced into new fish species result in the alteration of gut microbiota and lead to changes in both growth and immunity. For example, the use of probiotic supplementation (Bacillus velezensis and Lactobacillus sakei) to rainbow trout increased beneficial microorganisms while reducing harmful bacteria, hence the regulations in growth and immunity (De Marco et al., 2023). In addition, probiotics were able to modulate the metabolome (total number of metabolites present in an organism) and therefore affect a wide range of metabolic processes in fish. All these findings emphasize the importance of probiotics for the fish gut microbiota and fish health, thus making them quite important tool in aquaculture for improving the growth and disease resistance of the host (Rohani et al., 2022). Probiotics help in the absorption and effective digestion of nutrients in fish (Wuertz et al., 2021).

Probiotics can increase the length of the intestine villi, increase the growth of beneficial microbes and decrease the load of pathogenic bacteria in the intestine with improved nutrient absorption and digestion in the fish (Assan et al., 2022). The activities of digestive enzymes in all species of fish are further boosted by probiotics, thus improving digestion and nutrient absorption (Ghori et al., 2022). Moreover, probiotics have also been shown to enhance growth and survival, as well as the intestinal morphology of fish, thereby increasing nutrient digestibility and nutrient metabolism of the organism (Gaffar et al., 2023).

Probiotics and Prebiotics in Enhancing Fish Immunity

Probiotics and prebiotics maintain healthy gut microbiota, promote beneficial bacteria, such as Lactobacillus and Bifidobacterium and suppress the growth of pathogens. Alteration of the gut microbiota supports digestion and an increase in nutritional absorption, thus resulting in better health and an increased immunity in fish. This enhances the gut barrier by stimulating mucin production and tight junction protein that serves to exclude pathogens and toxins from reaching the systemic circulation. A healthy gut barrier is very important in the maintenance of fish free from infection and diseases (Akhter et al., 2015).

Table 1: The Effects of Probiotics (gram-positive and gram-negative bacteria) against pathogens in fish.

Sr.#	Probiotics	Fish Species	Pathogens	Beneficial Effects	References
	Gram Negative Bacteria				
1.	"Pseudomonas spp.	"Rainbow trout	"F. psychrophilum	"Low mortality rate.	"(Korkea-aho et al., 2011).
2.	*P. aeruginosa*	Rohu	*Aeromonas hydrophila*	High survival rate.	(Giri et al.,2012).
		Zebrafish	*Vibrio parahaemolyticus*	Improves defence mechanism	(Vinoj et al., 2015).
3.	*P. chlororaphis*	Perch	*A. sobria*	Control "*A. sobria*" infection	(Chi et al.,2014).
4.	*P. fluorescens*	Rainbow trout	*V. anguillarum*	Reduced mortality rate.	(Capkin and Altinok, 2006).
5.	Aeromonas hydrophila	Rainbow trout	A. salmonicida	Low rate of infections.	(Kim et al.,2010).
		Goldfish	*A. salmonicida*	High level of infections control.	(Wu et al.,2015).
6	*A. sobria*	Rainbow trout	*Lactococcus garvieae* and *Streptococcus iniae*	Increased disease resistance.	(Giri et al.,2012).
		Rainbow trout	*A. Bestiarum*	Protection against several pathogens.	(Vinoj et al.,2015).
7.	*A. veronii*	Common carp	*A. hydrophila*	High rate of disease resistance. .	(Chi et al.,2014).
8.	*Shewanella putrefaciens*	Gilthead seabream	*Vibrio anguillarum*	Reduced mortality rate.	(Chabrillón etal., 2006).
		Senegalese sole	*Photobacterium damselae* sub sp. Piscicida	Improved growth and disease resistance	(Diaz-Rosales et al., 2009).
9.	*S. xiamenensis*	Grass carp	A. hydrophila	Increase immunity.	Wu et al., 2015
10.	*Enterobacter cloacae*	Rainbow trout	*Yersinia ruckeri*	High survival rate.	(Capkin and Altinok 2006).

11.	*Enterococcus faecalis*	Rainbow trout	*A.salmonicida*	Low mortality rate.	(Rodríguez-Estrada etal., 2013).
12.	*Enterobacter amnigenus*	Rainbow trout	*Flavobacterium psychrophilum*	Improvement in infections control.	(Burbank et al., 2011).
13.	*Roseobacter sp*.	Turbot	*V. anguillarum*	Infection control.	(Planas et al.,2006).
14.	*Vibrio alginolyticus*	Atlantic salmon	*A. salmonicida*	Reduction in cumulative mortality.	(Hjelm et al.,2004).
15.	*Flavobacterium sasangense*	Common carp	*A. hydrophila*	Immunity increase.	(Chi et al.,2014).
16.	*Zooshikella* sp.	Olive flounder	Streptococcus inane	Increased immunity.	(Kim et al.,2010).
17.	*Phaeobacter gallaeciensis*	Cod larvae	*V. anguillarum*	Reduction in death rate.	(D'Alvise et al., 2012).
Gram-Positive Bacteria					
18.	*Carnobacterium divergens*	Atlantic cod	*V. anguillarum*	Reduction in Vibriosis.	(Al-Dohail et al., 2011).
19.	*Carnobacteria. inhibens*	Atlantic salmon, rainbow trout	*A. salmonicida, Vibio ordalii, Yersinia ruckeri*	Reduced mortalities.	(De la Banda et al., 2012).
20.	*Lactobacillus rhamnosus*	Rainbow trout	*A. salmonicida*	Decreased mortality.	(Nikoskelainen et al., 2001).
21.	*L. sakei*	Rock bream	*Edwardsiella tarda*	Reduction in cumulative mortality.	(Harikrishnan et al., 2011).
22.	*L. acidophilus*	Nile tilapia	*Pseudomonas fluorescens, Streptococcus iniae*	Strong immunity.	(Aly et al.,2008a).
23.	*L. lactis*	Olive flounder	*Streptococcus iniae*	Activated "innate immune system" and protection against pathogens.	(Kim et al., 2013).
24.	*L. plantarum*	Rainbow trout	*Lactococcus* (*Lc.*) *garvieae*	Reduction in death rate. "	(Vendrell et al., 2008).
25.	*L. pentosus*	Japanese eel	*Edwardsiella tarda*	Improve immunity.	(Lee et al., 2013).
26.	*L. brevis*	Tilapia	*A. hydrophila*	Reduction in death rate.	(Liu et al., 2013).
27.	*Leuconostoc mesenteroides*	Rainbow trout	furunculosis	High Disease resistance.	(Balcázar et al., 2007).
		Brown trout	*Aeromonas salmonicida*	Increased immunity and disease resistance.	(Balcázar et al., 2009).
28.	P*ediococcus acidilactici*	vertebral column compression syndrome (VCCS)	*Pediococcus acidilactici*	Increase survival.	(Aubin et al., 2005).
29.	*P. pentosaceus*	Grouper	*V. anguillarum*	Reduction in cumulative mortality.	(Huang et al., 2014).
30.	*Enterococcus faecium*	European eel	*Edwardsiella tarda*	Reduced edwardsiellosis.	(Aubin et al., 2005).
31.	E. *casseliflavus*	Rainbow trout	*Streptococcus iniae*	Improve growth rate.	(Safari et al., 2013).
32.	*E. gallinarum*	Sea bass	*Vibrio anguillarum*	Protection against pathogens.	(Sorroza et al., 2013).
33.	*Bacillus pumilus*	Tilapia	*A. hydrophila*	Increased immunity of fish species.	(Aly et al., 2008b).
34.	*B. circulans*	Catla catle	*A. hydrophila*	Increased immunity of fish species.	(Bandyopadhy and Das 2009).
35.	*Vagococcus fluvialis*	Sea bass	*Vibrio anguillarum*	Increased survival.	(Sorroza et al., 2012).
36.	*Bacillus subtilis* and *Bacillus licheniformis*	Trout	*Y. ruckeri*	Increased survival.	(Safari et al., 2013).
37.	*B. subtilis*	Indian major carp	*A. hydrophila*	Control of Infection rate.	(Kumar et al., 2006).
		Rainbow trout	*Aeromonas*	Increase survival of fish species.	(Newaj-Fyzul et al., 2007).
		Channel catfish, striped catfish	*Edwardsiella ictaluri*	Reduced mortality rate.	(Ran et al., 2012).
		Red hybrid tilapia	*Streptococcus agalactiae*	Reduced mortality rate.	(Ng et al., 2014).
		Grouper	*Streptococcus* sp.	Increased survival rate.	(Liu et al., 2012).
38.	*Kocuria* sp.	Rainbow trout	*V. anguillarum* and *V. ordalii*	Reduced mortality rate.	(Sharifuzzaman and Austin 2010).

39. *Brochothrix thermosphacta*	Rainbow trout	*A. bestiarum*	Protection from skin infections.	(Ng et al., 2014).
40. *Rhodococcus* sp.	Rainbow trout	*V. anguillarum*	Batter protection against pathogens.	(Sharifuzzaman et al., 2011).
41. *Micrococcus luteus*	Nile tilapia	*A. hydrophila*.	Reduced mortality rate.	(Abd El-Rhman et al., 2009).
	Rainbow trout	*A. salmonicida*	Better survival.	(Sharifuzzaman et al., 2011)
42. *Clostridium butyricum*	Rainbow trout	Vibriosis	increase disease resistance.	(Pan et al., 2008b).
	Chinese drum	Vibriosis	Increased phagocytic activity, resistance to Vibriosis .	(Pan et al., 2008b).
43. *Kocuria* sp.	Rainbow trout	*V. anguillarum* and *V. ordalii*	Reduced mortality rate.	(Sharifuzzaman and Austin 2010).
44. *Brochothrix thermosphacta*	Rainbow trout	*A. bestiarum*	Protection from skin infections.	(Ng et al., 2014).
45. *Rhodococcus* sp.	Rainbow trout	*V. anguillarum*	Batter protection against pathogens.	(Sharifuzzaman et al., 2011).
46. *B. subtilis B. licheniformis*	Olive flounder	*S. iniae*	Higher survival rate.	(Cha et al., 2013).
47. *B. licheniformis*	Tilapia	*S. iniae*	Increase disease resistance.	(Han et al., 2015).
48. *B. amyloliquefaciens*	Nile tilapia	*Yersinia ruckeri, Clostridium perfringens type D*	Increased survival rate.	(Selim and Reda 2015).
Yeast				
49. *Debaryomyces hansenii*	Leopard grouper	*A. hydrophila*	Enhance disease resistance.	(Reyes-Becerril et al., 2011).
50. *Saccharomyces cerevisiae*	Tilapia	*A. hydrophila*	decreased death rate.	(Abdel-Tawwab et al., 2008).
51. *Saccharomyces cerevisiae* var. *boulardii*"	Rainbow trout"	*A. hydrophila*"	Enhance disease resistance."	(Quentel et al., 2005)."

Probiotics and prebiotics enhance the immune system of fish by increasing the cell production such as macrophages, neutrophils and lymphocytes. They also enhance the secretion of immunoglobulins and cytokines, which are important in immune defense and regulation. This means that the use of probiotics helps fish, to protect themselves from pathogens through the modulation of fish immune response. Some of probiotic bacteria produce antimicrobial compounds, such as bacteriocins, organic acids and H2O2 that prevent the growth of pathogenic bacteria in the gut (Hoseinifar et al., 2015).

Improved health status was given to histo-morphological changes in the fish gut by the prebiotic and probiotic treatment; for instance, Nile tilapia (Oreochromis niloticus) shows growth and resistance to pathogens and an improvement in physiological conditions by dietary supplementation with probiotics and prebiotics (Sîrbu et al., 2022).

Overview of Fish Immune System

Innate immune as well as adaptive immune components, classify the fish immune system, which together performs vital role in the defense against pathogens and securing fish from death in various aquatic environments. Fish immune components consist of physical barriers, cellular and humoral factors, hence making part of the innate immune system and serves as the first line of defense. (You et al., 2022). Macrophages, neutrophils and natural killer cells recognize and phagocytize pathogens, while other structures include complement proteins and antimicrobial peptides that neutralize and eliminate the invaders. More structures that are added are the mucosal-associated lymphoid tissues (MALT) in the gut and gills which have an important role in immune surveillance and response. (Bermudez-Brito et al., 2012).

In contrast, the adaptive immune system is much more specific, made up of lymphocytes B and T-cells and antibodies. B cells are responsible for producing antibodies against particular antigens, while T cells take a controlling function in immunity reactions and the killing of infected cells (Smith et al., 2019). For example, in jawless fish, the adaptive immune system is characterized by variable lymphocyte receptors (VLRs), while in jawed fish, it consists of major histocompatibility complex (MHC) molecules, enabling recognition and response to a wide array of pathogens (Plaza-Diaz et al., 2019). Also, the immune system of fish can be influenced by environmental factors, stressors and management practices. Thus, there is a need for the optimum husbandry practices to maintain immune function and health in aquaculture operations. (Mokhtar et al., 2023).

Probiotics and Prebiotics in Preventing and Treating Fish Diseases

Probiotics when applied in adequate quantities increase the phagocytic, lysozyme, complement, respiratory burst activity and cytokine expression in fish while stimulating the gut immune system with significant increases in the number of Ig (+) cells and acidophilic cells (Nayak, 2010). Prebiotics, on the other hand, have been shown to enhance growth, non-specific immunity, disease and stress resistance and antioxidant activities in fish species (Zhu et al., 2023). Probiotics and prebiotics in fish and shellfish act on the innate immune system, thereby increasing disease resistance and the overall health of the organisms (Akhter et al., 2015).

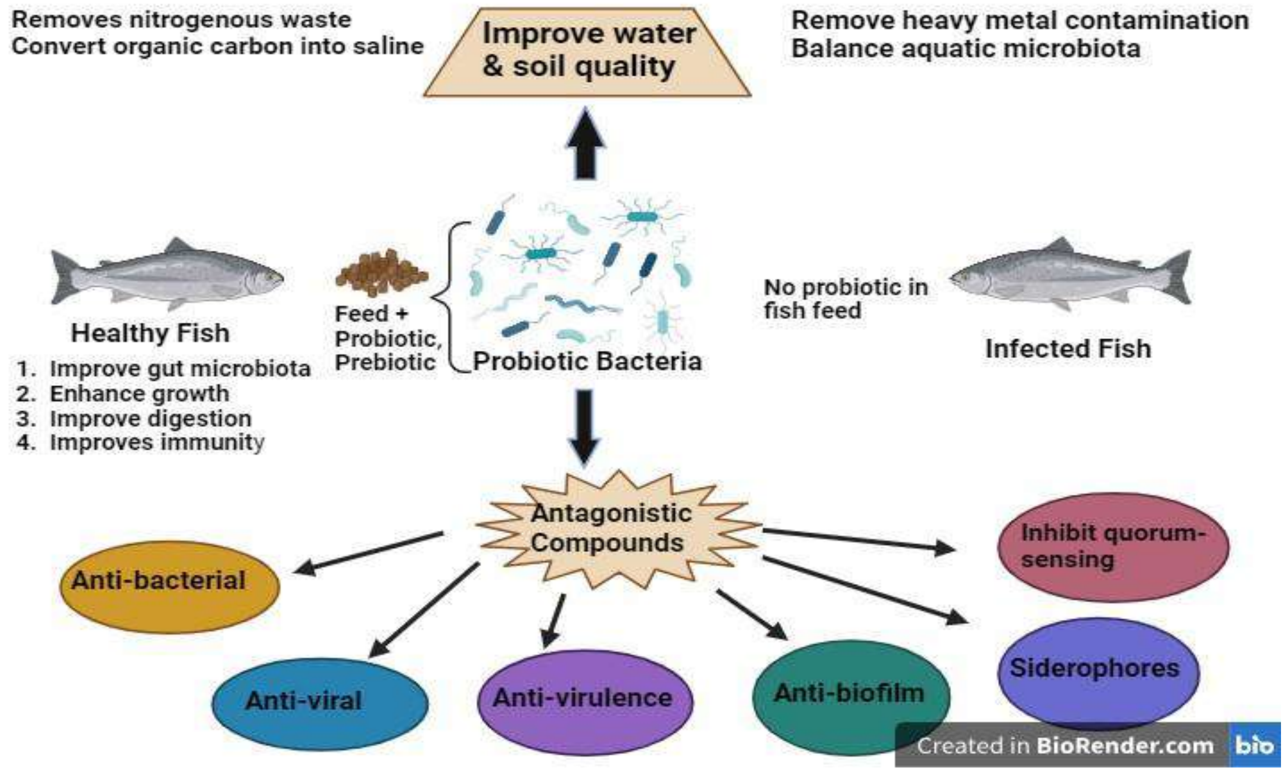

Fig 2: Diagrammatic representation of the role of probiotics and prebiotics in enhancing fish immunity

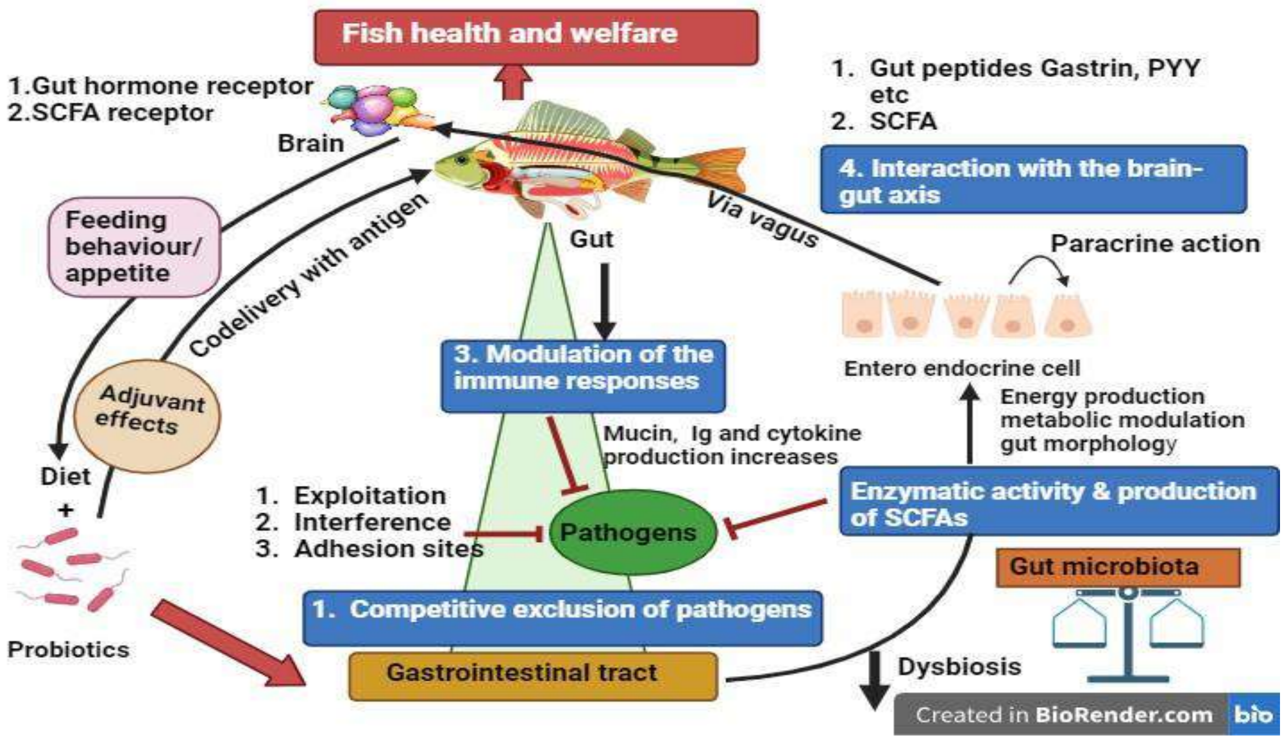

Fig 3: Probiotics and Prebiotics in Preventing and Treating Fish Diseases

Other methods being developed for the prevention and treatment of diseases in fish include the use of probiotics, prebiotics and synbiotics. Prebiotics stimulate gut microbiome, which enhances host immunity and the production of antibacterial substances to regulate bacterial, viral and parasitic diseases in the various species being used in aquaculture. Probiotics have been documented to reduce mortality in fish species such as the Atlantic salmon and rainbow trout (Hoseinifar et al., 2018). It improves growth performance, immune response and disease resistance in aquaculture species. (Wei et al., 2022).

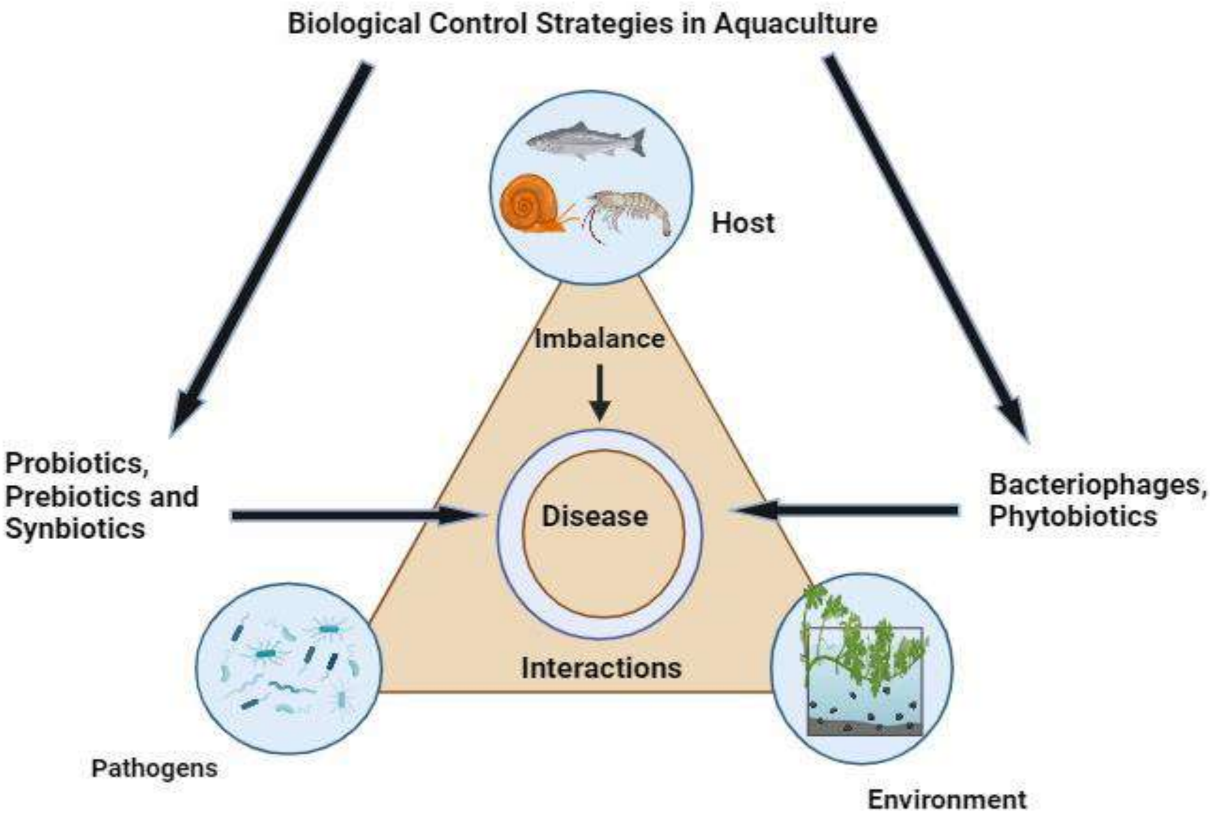

Fig. 4: Control strategies to control diseases through the use of Probiotics, Prebiotics and Synbiotics

Case Studies and Examples

Survival was enhanced in Rainbow trout by the use of the probiotic Micrococcus luteus. The application of probiotics has been shown to bring about lower mortalities in a variety of fish species (Wuertz et al., 2021). Prebiotic administration enhances the activity of probiotics, make them more resistant to reactive oxygen species (ROS) and enables them to pass through the gastrointestinal tract. In addition, growth and physiological conditions were increased in Nile tilapia exposed to a bacterial pathogen in the presence of probiotics and prebiotics. The potential of probiotics and prebiotics in disease control within aquaculture is increasingly recognized; as a result, numerous studies are currently being published with the use of these agents as antibiotic alternatives. (Hoseinifar et al., 2018).

Challenges and limitations of Probiotics and Prebiotics in Fish Health

Limitations of the use of probiotics and prebiotics in fish health are species-specific because of the differences in the gut microbiome of each species. Other challenges due to the use of probiotics in aquaculture include the persistence of probiotic strains in the digestive tract, resistance to acid and bile salts and interaction with host gut defenses (Wuertz et al., 2021). Other potential risks from the use of probiotics that should be taken into consideration are such as antibiotic resistance and risks of transferring genetic elements to other microorganisms in the gut of the fish. The general health status, along with further factors like the quality of the water, diet, stressors or other adverse situations, would condition the efficacy of the probiotics and prebiotics in prophylaxis and treatment of diseases in fish (Sîrbu et al., 2022). These highlight the limitations and need for more research on the interactions of probiotics and prebiotics with fish health and the possible risks and benefits in aquaculture practice. (Cruz et al., 2012).

Stability and Shelf-life of Probiotics and Prebiotics

Probiotic stability is affected by many factors, including packing, moisture and temperature. Most of the shelf-stable probiotics are meant to be used within one to two years (Butt and Volkoff 2019). Additionally, through un-opened blister packs, there is a protection from heat and humidity, which increases the shelf life of the probiotics. For example, Bacillus is one of those probiotic strains that are more heat- and environment-friendly and therefore more stable. The usual storage condition recommended by manufacturers is a cool, dry place away from direct sunlight to extend the shelf life of the probiotics. (Alvanou et al., 2023).

Freeze-dried probiotic products should be able to achieve low water activity levels for a long shelf life at room temperature. The probiotic supplements that require refrigeration are likely to have this indicated on their labels (Ringo et al., 2022). The composition and formulation of the probiotics are critical in the determination of their stability. By their prebiotic content, they are usually combined with other ingredients like fibers, sugars, or sugar alcohols which might influence their stability and shelf life. Manufacturers are in the process of developing formulations that will reduce the potential for contamination while retaining prebiotic activity upon storage (Alvanou et al., 2023).

Potential Risks and Side Effects of Probiotics and Prebiotics

Probiotics and prebiotics for fish have relatively few risks and side effects, but some considerations should be made:

Probiotics

Thus, the use of inappropriate probiotic strains for specific fish species causes negative effects (Hoseinifar et al., 2018). Probiotics could theoretically lead to systemic infection in some susceptible hosts, although the actual risk is generally low (Martínez et al., 2012).

Prebiotics

High levels of prebiotics may lead to some negative effects on aquatic animals, and some types of prebiotics enhance growth without elevating immunity. Probiotics and prebiotics in fish are safe and beneficial, but careful selection with proper dosage and monitoring for any potential side effects is necessary (Wee et al., 2022).

Conclusion

In conclusion, this chapter emphasized the importance of these supplements to improve fish gut health, immunity and disease resistance in aquaculture. From the data and research findings, it is obvious that probiotics and prebiotics have multiple effects on the fish's gut microbiota. This has a beneficial balancing effect, which improves the absorption of nutrients, digestion and general metabolic efficiency. These supplements, in addition, possess immunomodulatory properties that further improve the innate defense mechanisms of the fish, decrease susceptibility to infectious agents and increase resilience to diseases. All these combined approaches enhance production indices such as growth performance and feed utilization while supporting the goal of sustainable aquaculture through reduced reliance on antibiotics and chemical interventions. It is this order of thinking that leads us to modern aquaculture complexities of integrating probiotics and prebiotics as a promising avenue toward optimized fish health and welfare, assuring long-term viability and resiliency of aquaculture operations in the face of evolving challenges

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Chapter 27

Effective use of Probiotics and other Feed Additives in Veterinary Medicines

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ABSTRACT

The probiotics, prebiotics, organic acids, and enzymes are valuable feed additives in veterinary medicine, offering a range of benefits including promoting the gastrointestinal health, enhanced nutrient utilization, and disease prevention. With the effective use of probiotics and other feed additives, veterinary professionals and livestock producers can improve animal's immunity and health, and productivity. Their incorporation in balanced diets in conjunction with appropriate management practices improves health, and performance in the diverse animal species. Probiotics and other additives contribute equally well in the sustainability and performance of both livestock and agriculture when added in feed or fertilizers. Ultimately these compounds result in the promotion of this highly important economic and food sector of the nation. They stimulate the production of beneficial compounds such as short-chain fatty acids and immunoglobulins, which help in strengthening the animal's immune defense against pathogens. The probiotics and other feed additives play integral roles in veterinary medicine by promoting gut health, preventing diseases, improving nutrient utilization, enhancing immune function, reducing environmental impact, and enhancing production efficiency in animals.

KEYWORDS		
Probiotics, Feed additives, Organic acids, Enzymes and Veterinary Medicines	Received: 13-Jun-2024 Revised: 24-Jul-2024 Accepted: 19-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

The term "probiotic" comprises of two reek words "pro" and "bios" which means for life. Metchnikoff first introduced this term in 1908. As the name tells probiotics are living microorganisms. They improve the intestinal microbial composition with positive impacts on nutrient metabolism, absorption and utilization (Majidi-Mosleh et al., 2017). Hence their proper use whether mono or mixed strains confer useful health benefits to the animal (FAO/WHO, 2002). The probiotics possess peculiar but quite positive qualities. They are nonpathogenic, and multiply rapidly. They enhance the functions of the intestinal tract of an animal positively especially contributing to its health and wellbeing. *Lactobacillus bulgaricus, Enterococcus faecalis, Lactobacillus acidophilus, Lactobacillus lactis, Bifido bacterium spp., Lactobacillus plantarum, Lactobacillus casei, Lactobacillus helveticus, Lactobacillus salivarius, Enterococcus faecium, Streptococcus thermophilus, Escherichia coli* and some species of fungi like *Saccharomyces boulardii* and *Saccharomyces cerevisiae* are commonly used probiotics (Hossain et al., 2012).

Decades of research have indicated that the use of probiotics in farm animals is beneficial as it improves feed efficiency, weight gain, and immune response (Ezema, 2002). They primarily include beneficial bacteria such as *Lactobacilli, Bifidobacteria, and Enterococci*. The probiotics, prebiotics, and organic acids play a crucial role in maintaining optimal gut health in animals. Organic acids, such as formic acid, propionic acid, and butyric acid, exhibit antimicrobial properties and help in maintaining gut pH balance. In livestock production, organic acids are added to feed or water for controlling the growth of pathogenic bacteria and improving digestive health. They also enhance nutrient utilization and feed efficiency, leading to improved growth performance and production outcomes (Fig. 1).

In the digestive tract of animals, enzymes are biological catalysts that facilitate chemical reactions in the digestive system. In veterinary medicine, enzymes such as amylases, proteases, and lipases are used to break down complex nutrients in feed into simpler forms that are more easily absorbed by the animal. The probiotics help these enzymes in the digestion of nutrients provided to the animal in the food. The probiotics when introduced take over the control of intestinal microflora. Ultimately they manage the bacterial fauna responsible for proteolysis. Certain probiotics however, can release exo-enzymes. These enzymes support digestion of proteins in the animal's gut. It has also been observed that

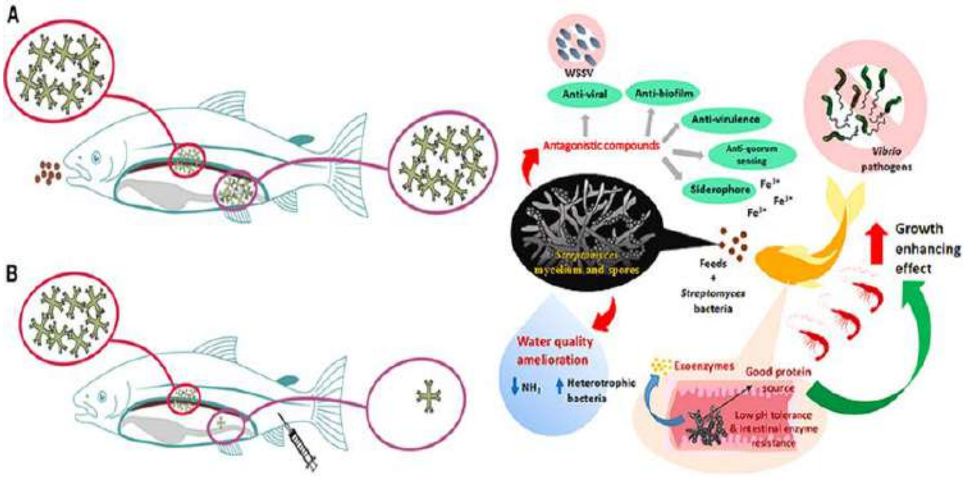

Fig. 1: Impacts of probiotics on feeding technology (Tan et al., 2016)

some can stimulate the activity of proteases and peptidases present in the same area. Main purpose of their application is the control of bacterial infections in animal farming. Most of these bacteria are pathogenic and cause variety of diseases and their in time and proper control is highly important for the economic functioning of these projects. Though they are extensively used with some limitations for disease control and growth promotion, their usage for breeding purposes has been banned due to the unknown reasons (Weathrall et al., 2006).

On introduction to animal gut they reduce the proliferation and colonization of the existing hidden harmful bacteria with efforts to totally wipe them out. In this scenario though they have lost their importance nonetheless their indiscriminate and excessive usage has intensified the problem and has inflicted harmful impact on consumer health. This happened mainly due to the resistance of target microorganisms to antibiotics and the residues of these antibiotics which they leave in food or in the organisms where they are applied on. Mechanisms of action include competitive exclusion of pathogenic bacteria, modulation of the gut microbiota, enhancement of mucosal barrier function, and stimulation of the immune system. The probiotics also help in the synthesis of vitamins and short-chain fatty acids, which contribute to the overall gut health and nutrient absorption. They help in balancing the gut microbiota by promoting the growth of beneficial bacteria and inhibiting the proliferation of harmful pathogens. As healthy gut microbiota is essential for proper digestion, absorption of nutrients, and overall immune function in animals hence probiotics successfully fulfill this requirement. In general, the use of probiotics, prebiotics, organic acids, enzymes, and other feed additives represents an important strategy for promoting gastrointestinal health in veterinary medicine because they enhance nutrient utilization, and improve overall animal health and welfare (Fig. 2). When used judiciously and in combination with appropriate management practices, these additives can contribute to sustainable and efficient animal production improving economy and ensuring food security for the continuously growing population(Gamage et al., 2023).

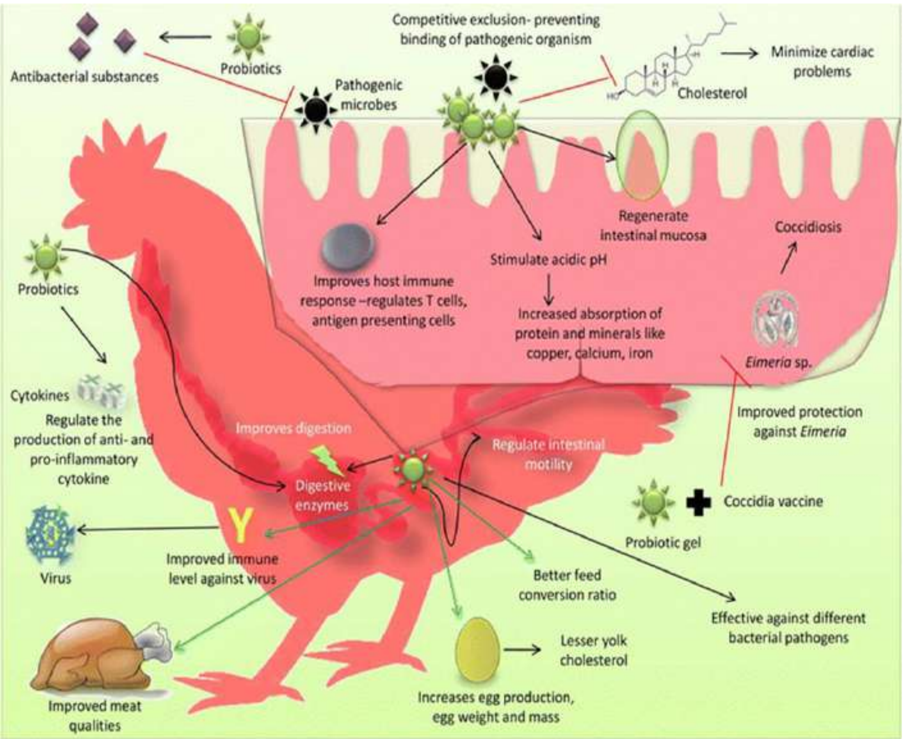

Fig. 2: Modes of action and beneficial activities of probiotics (Alagawany et al., 2018).

Mechanism of Action of Probiotics

The probiotics improve feeding behavior of animals with subsequent increase in production of meat, milk, and eggs (Rai et al., 2013). Hence they are commonly used as therapeutic agents in farm animals. Not only they increase the production capability of animals but they also decrease morbidity and mortality in animals. Their ability to inhibit a wide variety of pathogenic microorganisms imparts antibiotic qualities in them. It does not matter whether they come from the environment or introduced in feed their efficacy is equally well. Their successful use and positive outcome has enticed the food industrialist to use it in industry to enhance the efficacy of their product. Till so far there are two known mechanisms of action of probiotics. Either they produce inhibitory compounds to combat unwanted microorganisms or there might be cell-to-cell interactions through which they transfer effective compounds to take hold of pathogenicity and causative agents. Functioning of probiotics mainly depends on the production of antimicrobial compounds. These compounds are hydrogen peroxide, organic acids, biosurfactants, and bacteriocins. All these compounds either totally wipe out pathogenic organisms or at least inhibit their excessive proliferation keeping them in check and balance (Jonkers, 2016). The lactic acid and acetic acid produced during this process reduce the pH, creating a hostile environment for growth of these pathogenic organisms. In this way probiotics work at several options at the same time. They can totally exclude pathogenic bacteria, can modulate and regulate the gut microbiota, can stimulate and improve the immune system under observation, and the last but not the least they can enhance the function of mucosal barrier making it more stringent in the control of invading organisms. All these options facilitate the nutrient absorption, improvement in feed conversion and efficiency subsequently improving the health and growth of an organism (Fig. 3).

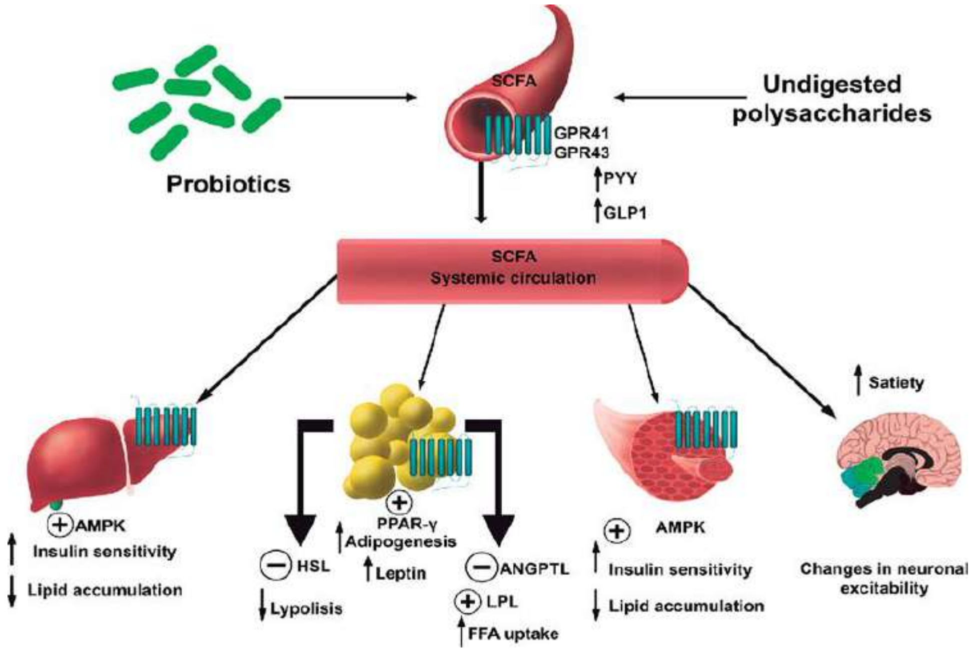

Fig. 3: Mechanism of action of probiotics (Plaza-Diaz et al., 2019)

Application of Probiotics in Animal Growth and Production

The animals rely on age, diet, genetics, and sex for appropriate growth. Above all is the feeding of an animal which plays a key role in the success of these factors ending up ultimately in the desired growth. Feed is of no use if not properly digested and absorbed. The probiotics can play a pivotal role in both above mentioned animal activities. When finishing pigs received probiotics (*Bacillus subtilis* and *Clostridium).* supplemented feed (Meng et al., 2010). they showed significant increase in growth. They attributed this growth to the added probiotics which enhanced the nutrient digestibility of animals in the digestive tract. Feed supplemented with Bacillus culture showed better nutrient absorption and growth. Studies were continued for 4 months. At the end pigs fed on probiotics supplemented diet showed 10% increase in protein utilization than those received feed without probiotics supplementation. Still studies however are needed to explore the exact mechanism of working of these probiotics in the animal gut. Nonetheless some studies have shown the possible relationship between the gut microbiome and the brain. This brain-gut-axis bring about neurological changes subsequently affecting the feeding behavior of farm animals (Kraimi et al., 2019).

Disease Prevention and Treatment

Probiotics, particularly, have been shown to be effective in preventing and treating gastrointestinal disorders in animals. They can help alleviate symptoms of diarrhea, enteritis, and other digestive disturbances by restoring microbial balance in the gut. Probiotics produce organic acids which exhibit antimicrobial properties that help control the growth of pathogenic bacteria in the gastrointestinal tract, reducing the risk of bacterial infections. Probiotics and prebiotics have been shown to modulate the immune system in animals, promoting both innate and adaptive immune responses (Fig. 4). They stimulate the production of beneficial compounds such as short-chain fatty acids and immunoglobulins, which help strengthen the animal's immune defenses against pathogens.

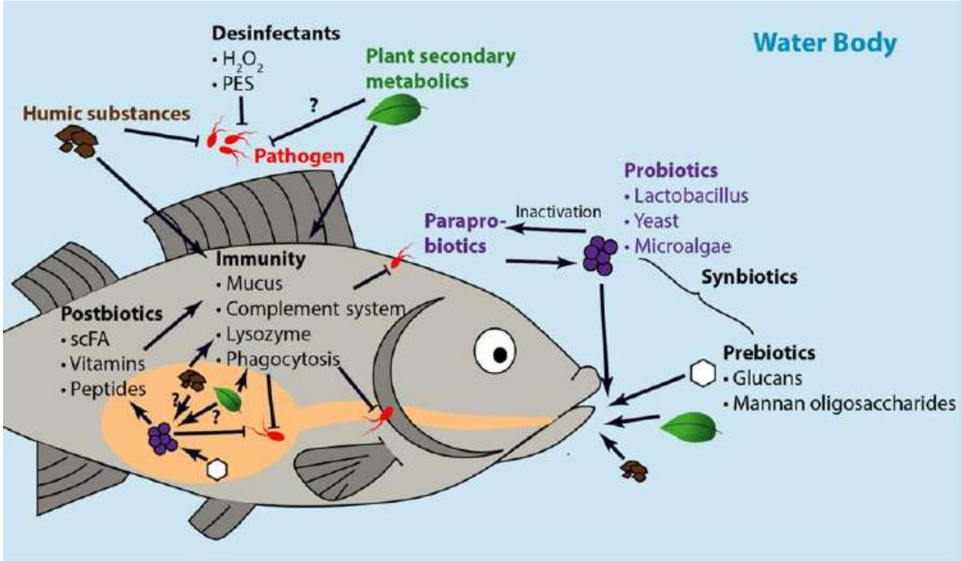

Fig. 4: Probiotics help in the production of healthy fish (with the courtesy of (Lieke et al., 2019).

Effect of Probiotics on the Quality of the Meat

Meat quality determines the purchasing power and consumer behavior. The most common traits are water holding capacity, meat color, and texture. These qualities vary during the life of an animal and are very much depend on method and time of harvesting, type of feed given, type of environment where animal is inhabiting, and processing method and conditions of the processing of the final product. Probiotics therefore influence the quality of meat in addition to growth increments (Bis-Souza, et al 2020). Probiotics supplemented meat is healthy, safe, good sensory qualities, and gives long shelf life to the product. Meat color is the most characteristics of the quality among others. It is the first perception of the consumer which leads to immediate purchase decision. From the color consumer can assess the overall freshness of the meat or the products developed from it. Color displays the methodology used during pre or postmortem handling of the meat. Case is however slightly different in carcass. It is mostly associated with post mortal handling and development of subsequent changes in it. Most important of them can be attributed to the changes in pH which becomes mostly apparent when animal is butchered (Matarneh, et al, 2017). After butchering, pH of the meat starts declining. It decreases from 7.2 to 5.6 means moving from neutrality towards acidity. This abnormal pH renders the meat defective. Meat becomes dry, dark, hard textured with pH of approximately >6. Contradictory to meat changes in carcass are more obvious and significant. When meat is still hot pH starts to decline at an accelerated pace. Meat becomes pale, soft textured and most often exudate releases from the met. The release of water from the meat carries several important proteins myoglobin for example. Therefore, among other attributes scientists has tried to explore extensive the role of probiotics on the meat. Mainly they focused on the meat color, and pH. Zheng et al. (2014). when fed chickens on the diet supplemented with *Enterococcus faecium* observed increase in pH of the pectoralis major muscle even after 45 minutes of their death. Similarly, when Meng et al. (2010) fed pigs on a probiotic supplemented diet they observed color changes in the pig meat. The meat became dark and/or red after death of the animal. How the probiotics tends to decline the pH towards acidic media is not yet fully clear. Nonetheless it is presumed that quality of microorganisms and methodology of their application might affect the changes in the pH after death of an animal. When feed with *Bacillus cereus* IP 5832 are supplemented in the diet of broilers, Ivanovic et al. (2012) observed pH increments in the meat. With the change in the bacterial strain pH however behaved differently. For example, application of *Streptococcus faecium cernelle* 68 in the feed decreased pH in the meat making it more acidic. Changes in pH and in the meat when it has probiotic touch are quite common and most often the authors have reported them in their research work. Similarly, to other researchers Pelicano et al. (2003) has the similar view point on this notion. They also report occurrence of changes in pH of the meat whenever diet received probiotics. Changes may vary from species to species used in the feed as well as the strains and the type of host used. This issue however can be resolved if any solution to stabilize pH changes can be sought. This development can give new boost to pork and poultry industry. Enzymes, such as amylases (carbohydrases), proteases, and lipases, aid in the breakdown of complex dietary components into simpler forms that are more easily absorbed and utilized by the animal. By enhancing nutrient digestibility and absorption, enzymes contribute to improve feed conversion efficiency and growth performance in animals. The incorporation of feed additives in the animal's diets can lead to improvements in the production efficiency and profitability for livestock producers. Enhanced growth performance, improved feed conversion ratios, and reduced incidence of diseases contribute to higher productivity and economic returns in animal production systems.

Use of Probiotic in Animal Breeding

Lactobacillus, *Streptococcus*, *Lactococcus*, and *Bifidobacterium* are most commonly used probiotics in humans and other animals although several others have shown potential as probiotics but literature is deficient to witness their efficacy (Hoseinifar et al., 2018). Modulation of the immune system of the host and improvement of physiological activities are the most common and dominant roles of probiotics. They attenuate virulence markers of the most common and prevalent pathogens and prevent spoilage of meat, inflammatory diseases, and control infectious diseases. In addition to bacteria, several species of yeast have the same role. *Saccharomyces cerevisiae*, and fungal strains (*Aspergillus oryzae*) are the most common species used as probiotics however their effectiveness is slightly different from the bacterial probiotics. Bacterial probiotics are more effective in pigs, chickens, and young calves. Yeast probiotics do well in adult ruminants (Markowiak and Śliżewska, 2018) (Fig. 5).

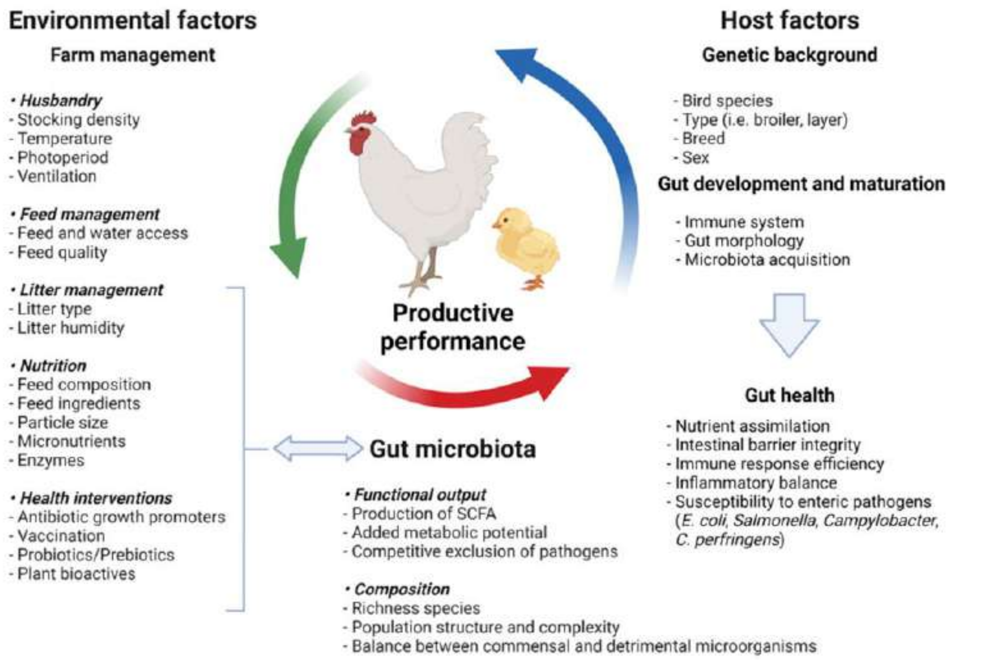

Fig. 5: Effect of probiotics on breeding and reproduction of poultry (Shehata et al., 2022)

Residues of Antibiotics in Environment and Food and their Effect on the Life Around

Use of antibiotics leaves sufficient residues in agriculture, livestock, and /or human beings. The antibiotic residues are pharmacologically active metabolic products which are harmful. The release of these metabolites after treatment with antibiotics is fully proven AMCRA (2020). Teratogenicity, allergic reactions, potential carcinogenicity, mutagenicity, and disruption of normal flora are the common ailments which they cause. It is however very much dependent on the half-life of the metabolite which varies among different antibiotics due to structure of the molecule present in them. For example, oxytetracycline degrades much slower than Tylosin. That is why oxytetracycline is hard to detect after 45 days while Tylosin remains in calves manure even after 5 months of treatment. In addition to these hazards they also impact various fermentation processes. Their presence inhibits the growth of probiotics in cheese, yogurt, and fermented meat processing. In this way these residues drastically affect the quality and quantity of these products having strong bearing on the economic viability of the project (Beyene, 2016). For the alleviation of this problem and control of such menaces, probiotics are an important and reliable alternative. They are equally effective in agriculture, livestock, and /or human beings strengthening health and reliable growth.

Commonly used Probiotics

Lactobacillus

They are Gram-positive bacteria. This group is very heterogonous and wide and has about one hundred different species. They produce lactic acid during their physiological function. They are normally present in mammals. Their inclusion is very common in both dairy and non-dairy foods intended for human use (Mbarga et al., 2019). When introduced into the animal body they enhance its immune function. This is an outcome of healthy microbial balance, and proper digestion in the gut. Overall all the species of genus *Lactobacillus* are beneficial. When fed to fish they strengthen the immune system with considerable reduction in mortality. Similarly, they improve piglet's performance when added to their diet (Huang et al., 2004). In addition to the above attributes they produce more and quality eggs in poultry with the reduction in *Salmonella* contamination. The production of digestive enzymes is their attribute.*Lactobacillus* strains commonly produce phytase, lipase, amylase, and proteases which facilitate digestion of the nutrients taken in the body of an animal (Kim et al., 2007). However, exceptions are there (Vesterlund et al., 2007). All species in the genus *Lactobacillus* are not beneficial. Two of them named *Lactobacillus casei* and *Lactobacillus rhamnosus* cause variety of infections in animal body where present. Nonetheless generally they are safe for livestock and human beings.

Bifidobacterium

The *Bifidobacteria* are found in abundance in the human and animal gut. Their presence has the capability to maintain the microbial balance in the gut. They proliferate quite rapidly and suppress the pathogenic bacteria alleviating the chances of infection in the animal. They are extensively used as probiotics in the pharmaceutical formulations and directly in human beings or in the preparation of their food. The host specificity however has been observed in several species of this genus. They produce short chain fatty acids, and help in the absorption of nutrients after digestion in the animal gut. In addition to aforementioned attributes they ferment dietary fibers making them more prone to digestion. This ensures that they are quite safe (Afonso et al., 2013) in their application whether it be livestock, agriculture, or human beings. The *Bifidobacterium pseudolongum* have shown promising results on piglets. Their supplementation improved food conversion ratio (FCR). No variation however was observed in feed intake, final weight, and weight gain. Both *Thermophilum,* and *Bifidobacterium longum*, have been tested in poultry infected with *Eimeria*. They reduced the intensity of coccidiosis in broiler chickens. They also protected chickens from the infection of *Salmonella* and *Listeria* species *in-vitro*. However, they did equally well *in-vivo*. They effectively controlled the infection of *E. coli* in chickens. This bacterium has an anti-Campylobacter activity in the chickens when present. In general, these bacteria are safe as probiotics with minimum hazards and have positive impacts on the animals whether it is their health or growth and/or breeding activity.

Bacillus

The *Bacillus* is a Gram-positive ubiquitous bacterium. They are heterotrophic, and facultative in their feeding behavior. They can thrive equally well in aerobic and anaerobic environments. For successful breeding in fish and poultry *Bacillus subtilis* are used as feed supplement. Kumar et al. (2006) fed Indian major carp, *Labeo rohita* on *B. subtilis* at 1.5 × 107 CFU/g supplemented. This fish showed sufficient resistance when exposed to *A. hydrophila*, pathogenic bacteria. Similarly, groupers, *Epinephelus coioides,* showed resistance against *Streptococcus spp.* when reared on *B. subtilis* supplemented diet at the dosages of 104, 106, and 108 CFU/g for 14 and 28 days. Based on this information, researchers think it as an excellent immunomodulatory agent in the animals. Hence it can be an effective biological player for the control of infectious diseases in fishes (Hoseinifar et al., 2018). In addition to the above *Bacillus licheniformis* also possess probiotic potential. When post weaning piglets (3-10 days) were fed on diet supplemented with *Bacillus licheniformis,* they effectively controlled diarrhea in these animals. This shows that they have the capability to control and eliminate pathogenic bacteria like enterotoxic strains of *E. coli* observed in piglets. Not all the species of this genus are useful and beneficial. Some like *Bacillus cereus* produce and contain endotoxins and emetic toxins which produce toxicity in animals and may cause morbidity and mortality in animals. Nonetheless other species are quite safe and can be used in animal production systems without any harm. When used they not only promote the growth of animals but also serve as an alternative to antibiotics.

Enterococcus

The *Enterococcus* is though universally present in animal and human gut but it is not considered safe for use as probiotics. Despite its potential risks (Araújo and Célia, 2013) *Enterococcus faecium strains* have been used in pigs and poultry both as an alternate to antibiotics and as feed additives Pollmann et al. (2005) microencapsulated *Enterococcus faecium* SF68 (NCIMB 10415) @ 9×109 CF/g supplemented to *Chlamydia* infected pig diet. On the termination pigs were challenged to *Chlamydia* infection. These probiotics reduced severity and frequency of infections. In turkey *E. faecium* has exerted stimulatory effect specifically on *Lactobacillus*. It displayed improvements in intestinal morphology and FCR *of* the bird. When broilers received these probiotics in diet they positively manipulated the cecal microflora. Pigs, fish, and poultry have shown similar positive responses when fed on *E. faecium* supplemented diet. However, the use of *Enterococcus* as probiotics hampered the efficacy of antibiotics and resisted their actions. Unlike above contributions Heikens et al. (2007) reported its pathogenicity because this species caused infections in endocardium and urogenital tract when supplemented with feed of the animal. Further this species has produced gelatinase, aggregation substances, and β-hemolysin in their media which are Therefore it is advisable use only those strains which display beneficial effects on the test organisms.

Lactococcus

The *Lactococcus* are proven and well tested probiotics. They have been used successfully in fish. For example, *Lactococcus lactis* when used in the fish resisted the pathogenicity of variety of harmful bacteria. Manufacture of fermented dairy products owes them. When the brown trout was fed on a *L. lactis* supplemented feed it improved its immune system and protected it from the onset of furunculosis (Balcázar et al., 2009). Similar results have been found in olive flounder (Heo et al., 2013). When this fish was fed on a *L. Lactis* (108 CFU/g) supplemented feed these bacteria increased the concentration of blood respiratory burst activities, lysozyme concentration, antiprotease, and serum super oxidase activity. Moreover, it gave better resistance to the fish when exposed to *Streptococcus iniae*. Like genus *Enterococcus* its performance is quite contradictory. Some strains of this genus have performed negatively. In some instances, *L. lactis* is considered useful probiotics, but *L. garvieae* performed negatively and induced infections to the consumer animal. Therefore, its utilization should be with care and should not be applied prior to well testing and harnessing its positive and negative effects. If used carelessly it can cause disease which can lead to mortality of the animal. We will face losses instead of benefits.

Saccharomyces

The *Saccharomyces* is budding yeast. The *S. cerevisiae* is the best-known species and is a part of the gut microbiota. The *S. cerevisiae* is sensitive to immunoglobulin G (IgG) concentration in colostrum. It gives similar response to plasma IgG of piglets. In these places it maintains health and proper working of the pig intestine. Stimulation of intestinal working improves the growth of pigs (LeMieux et al., 2010). In Nile Tilapia *S. cerevisiae* strengthened immunity, antioxidant activity, hematological parameters, growth and in combating the infectious effects of pathogenic fungus *A. flavus*. Feeding of *S. cerevisiae* developed a stronger immune system in gilthead seabream too. Gaggìa et al. (2010) suggested that when *Saccharomyces carlsbergensis* was supplemented in feed of various animals it improved digestive capabilities and ultimately growth of the organism (Fig. 6).

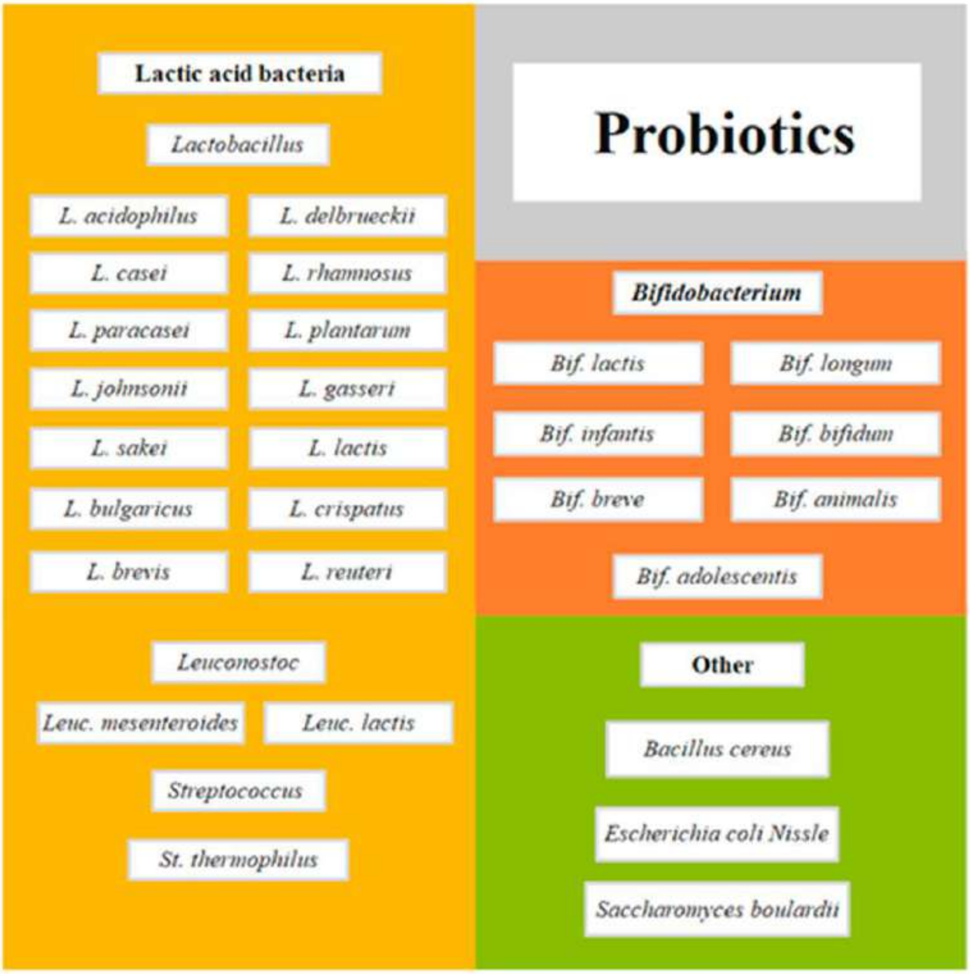

Fig. 6: Types of bacteria used as probiotics (Vera-Santander et al., 2023)

Sources of Probiotics in Aquaculture

In the aquaculture members of Gram-positive, yeasts, and microalgae have been tested for their potentials as probiotics in different shellfish and finfish species (Van Doan et al., 2021; Amenyogbe et al., 2020). Till so far the availability of probiotics does not suffice the requirements of the aquaculture industry. The probiotics used for fish come from different sources. They are collected either from rearing water, gastrointestinal tracts of fishes, and shellfishes, bottom sediments of pond water, from terrestrial animals, and/or from fermented food products (Shefat, 2018). As the purpose is to establish a specific relationship between existing microbiota and the added probiotics it is therefore important that the hosts associated microorganisms are preferred as the potential probiotics.

Selection Criteria of Probiotics in Aquaculture

Several methods are used for the selection of probiotics for aquaculture. The potential bacteria which can be used as probiotics are screened through several steps of *in-vitro* and *in-vivo* assays to ascertain their utility in aquaculture systems (Alonso et al., 2019). These strains are primarily screened for pathogenicity and their safety in applications. Some authors have characterized these bacteria as essential or favorable (Merrifield et al., 2010). The most prevalent criteria used for the selection of appropriate bacteria to be used as probiotics are the following;

1: These bacteria are non-pathogenic to the host.
2: They are resistant to high bile concentrations (>2.5%) and high pH.
3: They are capable to grow within the intestine mucous of the host.
4: They do not possess plasmid-encoded antibiotic resistance genes.
5: They are properly registered with the relevant organization for use as probiotics.
6: They promote the growth when supplanted with feed, their reproduction matches the intestinal temperature of the host, and they have short generation time and lag period.
7: They are antagonistically against at least one of the economically important pathogens.
8: They have the potential to secrete extracellular digestive enzymes, and /or vitamins.

9: They are autochthonous to the culturing environment or to the host where they inhabit.

10: They can live successfully under normal storage conditions, under industrial processes, and do not impart any unacceptable flavor or taste to the product at the same time having required growth potential with immunity inducing effect against various pathogenic bacteria.

Till so far none of the bacterial strains fulfill this criterion. Achievement of these criteria will pave the way towards appropriate selection of the probiotics to be used in aquaculture (Binda et al., 2020). Studies for the selection of appropriate strains of bacteria are underway and should be continued in future till the accomplishment of the selection of the suitable bacteria which can work successfully in aquaculture meeting the requirements of this sector in totality.

Conclusion

This review concludes that probiotics influence positively on animals, food processing, food products. They improve gut microbiota of an animal. They suppress harmful bacteria and promote useful microbiota. Its feed intake, growth and breeding efficiency is improved. However, more research is needed to standardize their dosage, selection of suitable animal, and provision of appropriate environment for their maximum output

Recommendation

The probiotics have displayed positive and encouraging effects on health and growth of terrestrial and aquatic animals hence their use is highly recommended. It is also suggested to continue working on the characterization of specific strains, standardize the optimal dosage of application in variety of livestock, and always try to explore the development of interactions between probiotics and the gut microbiota. This can help in the formulation of more effective probiotic mixtures to be used in animal feeds used in framing systems and food industries.

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Chapter 28

Strain-specific Probiotics: Linking Probiotic Strains with Health and Disease

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ABSTRACT

Probiotics are beneficial live microorganisms present in the food and are not harmful to humans. Several microbial strains act as probiotics, which belong to the genera, *Lactobacillus, Bifidobacteria, Saccharomyces, Bacillus, Streptococcus,* and *Enterococcus*. Probiotics may benefit human health via enhancing intestinal barrier function, generating neurotransmitters, immunomodulating the host's body, and competitively excluding pathogens. In the 1940s, microbiologists were focused on identifying pathogenic microbes and their underlying mechanisms involved in disease pathogenesis. It was later on in 1950s-1980s, when beneficial microbes were point of interest for researchers and they aimed at isolating these strains from the living organisms and the surrounding environment, which later on took attention owing to their potential role in addressing physical health diseases and mental disorders. Probiotics have been found effective in improving physical health during diseases like diabetes, cancer, Alzheimer's disease, and mental health situations by overcoming depression, and anxiety. Researchers have developed the most recent techniques that allow probiotics to tolerate GI stressors and severe processing conditions with relative ease. However, further studies are required for the specification and usage of probiotics as useful strains. This chapter highlights the latest developments about the health advantages of probiotics and their growing uses in the treatment of diseases.

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INTRODUCTION

The probiotic concept is based on the idea that human physiology depends on the commensal microbiota and the advantageous changes to its composition may assist in preserving health and lowering the risk of disease (Latif et al., 2023). The basic concept of probiotics includes all microbial species imparting health benefits as shown in research trials (Zaib et al., 2024). Louis Pasteur revealed the microorganisms responsible for fermentation at the beginning of the 20th century (Brogren, 2024), while E. Metchnikoff associated the frequent use of fermented dairy products, such as yogurt, with longer life spans among Bulgarian rural populations. Metchnikoff found lactobacilli fulfilling the criteria to be called probiotic bacteria because they may improve health and prolong the aging process (Renuka et al., 2023).

Probiotic studies typically use single strains, which are sometimes used as yogurt that include *Lactobacillus delbrueckii subspecies Bulgaricus* and *Streptococcus thermophilus* (Castro et al., 2023). The effectiveness of probiotic strain mixtures is less well understood, particularly if combining strains leads to lower efficiency because of reciprocal inhibition between the component bacteria or to additive or even synergistic benefits in terms of bioactivity (L. V. J. D. d. McFarland & sciences, 2021). Probiotics are recognized to have many health benefits, but it's still important to understand the processes behind how they interact with immune cells to promote immunomodulatory effects (Beterams et al., 2021). Probiotics may benefit to human health by enhancing intestinal barrier function, generating neurotransmitters, immunomodulating the host's body, and competitively excluding pathogens (Mazziotta et al., 2023).

For medicinal and dietary purposes, probiotic microorganisms are essential and helpful (Sharma et al., 2023). Microbial food supplements called probiotics change the Gut microbiota. A few RCTs or randomized controlled studies have examined how probiotic therapies affect T2DM patients' glycemic control (Li et al., 2023). Nutritional factors have been found critical in the treatment of cancer, as shown by the Association of Modifiable Health's finding that at least 50% of all cancers may have dietary origin (Dasari et al., 2017). Therefore, scientists interested in developing natural medications have been interested in a variety of food elements and natural health products (Noor et al., 2023). Probiotics and their impact on the Gut-Brain axis (GBA) have been found very beneficial and are being used for treating various conditions like Alzheimer's disease. Probiotics have an impact on the immune system and reduce inflammation. It has been discovered that they are important in the area of food-based anti-Alzheimer disease methods (Anand et al., 2023). According to (Azadeh et al., 2023), Probiotics use may help to reduce depression symptoms. Recent researches showed that probiotics can act as antimicrobial agents that can kill or damage the pathogens in the human body along with improving the Gut Microbiota (Fijan, 2023).

What are Probiotics?

Probiotics are living non-pathogenic microorganisms found in food that are good for human health (Saarela et al., 2020). The most common element in the age of functional foods is probiotics, whether they are found in food products or medicine. Probiotics have long been seen as an essential element and an attractive target due to their potential health benefits (Fig. 1) (Sanz et al., 2016). Werner Kollath originated the term "probiotic" in 1953. It is derived from the Latin word pro and the Greek word βιο, which means "for life." Kollath characterized probiotics as living organisms that have vital roles in enhancing health outcomes (Gasbarrini et al., 2016). Lilly and Stillwell originally used this word in 1965 to refer to "substances secreted by one organism which stimulate the growth of another". Probiotics are more precisely described as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" by Fuller in 1992 (McFarland, 2015). The present history of probiotics begins in the early 1900s with the groundbreaking research of Russian scientist Elie Metchnikoff, who worked at the Pasteur Institute in Paris. While Louis Pasteur discovered the microorganisms that cause fermentation, Metchnikoff initially made an effort to determine whether these microbes could have any negative effect on human health (Diplock et al., 1999). Additionally, according to Metchnikoff, "it is possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes because of the intestinal microbes' dependence on food." This phrase provides a comprehensive explanation of the "probiotic concept"(Gasbarrini et al., 2016). According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), these are "live microbes which confer health benefits on host organisms when administered in adequate quantities"(Munir et al., 2022).

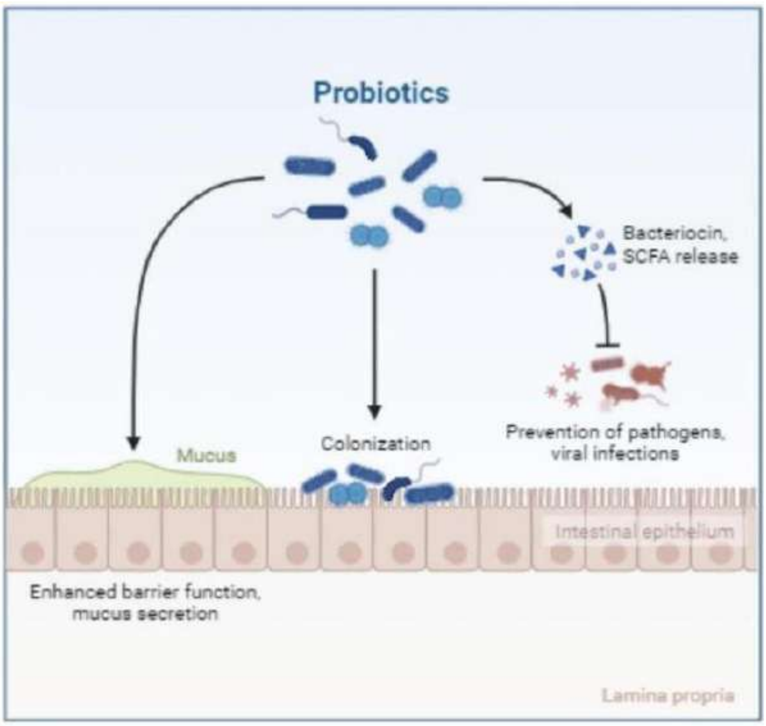

Fig. 1: Effect of probiotics in the prevention of infectious diseases

General Properties of Probiotics

The properties that a perfect probiotic preparation needs to possess are enlisted (Table 1) (Harmsen et al., 2000). A dosage of five billion colony-forming units ($5x10^9$ CFU/day) has been suggested, to be taken for at least five days, to get a sufficient level of health advantages (Gronlund, Lehtonen, & Eerola). The microbes normally recommended in probiotic preparations are generally recognized as safe (GRAS), have decreased intestinal permeability, produce lactic acid, are resistant to bile, pancreatic juice, and hydrochloric acid, are anti-carcinogenic, and should activate the immune system. They should also be able to withstand the acidic and alkaline environment in the stomach and duodenum (Vimala & Kumar, 2006). The viable cell count of a microbe in a probiotic product during the production and shelf-life period is influenced by pH, titratable acidity, molecular oxygen levels, redox potential, hydrogen peroxide, flavoring compounds, and packaging materials (Mortazavian et al., 2015). In probiotic preparations, either single or multistrain cultures of living microbes have been used (D'souza et al., 2002).

Table 1: Properties for any microorganism to be called a Probiotic.

1.	They ought to be able to communicate with or interact with the immune cells connected to the gastrointestinal tract.
2.	They ought to be derived from humans.
3.	It must not be pathogenic.
4.	Processing resistance.
5.	Need to be able to affect the local metabolism.

Probiotic Strains

Probiotic strains are living bacteria added to food to improve health. Several *Lactobacillus* species, *Bifdobacterium sp.*, *Saccharomyces boulardii*, and other microbes have been engaged in imparting health benefits (Ljungh & Wadström, 2006). The majority of probiotic bacteria, including *Lactobacillus sp.*, *Bifdobacterium sp.*, and *Enterococcus sp.*, are members of the Lactic Acid Bacteria (LAB) group (Fig 2) (Klein et al., 1998). In addition other bacterial species including *Bacillus* (Senesi et al., 2001) and *Clostridium butyricum* (Takahashi et al., 2004), the yeast *Saccharomyces boulardii* have also been the focus of many studies (Table 2) (Elmer, Martin, Horner, Mcfarland, & Levy, 1999). Up till now, *Lactobacillus rhamnosus GG* (LGG) was the first probiotic to attract significant clinical attention.[12] Because the *Lactobacillus* strain that the dairy industry had previously used for fermentation could not colonize the gut, *Lactobacillus rhamnosus* strain *GG* was identified in 1985 via the development of a list of optimal probiotic characteristics (Vimala & Kumar, 2006).

Table 2: Microbial strains used as Probiotics (Luise et al., 2022; Pudgar et al., 2021; Sharma et al., 2021)

Sr.no	Genera	Species reported for probiotic characteristics
1.	*Lactobacillus spp*	*Lactobacillus rhamnosus Lactobacillus casei* *Lactobacillus plantarum Lactobacillus reuteri* *Lactobacillus fermentum Lactobacillus salivarius* *Lactobacillus paracasei Lactobacillus gasseri* *Lactobacillus brevis Lactobacillus helveticus* *Lactobacillus johnsonii*
2.	*Bifidobacterium spp*	*Bifidobacterium bifidum* Bifidobacterium longum *Bifidobacterium infantis Bifidobacterium breve* *Bifidobacterium adolescentis Bifidobacterium angulatum* *Bifidobacterium catenulatum Bifidobacterium dentium* *Bifidobacterium pseudocatenulatum*
3.	*Saccharomyces spp*	*Saccharomyces chevalieri Saccharomyces dairenensis* *Saccharomyces ellipsoideus Saccharomyces martiniae* *Saccharomyces monacensis Saccharomyces norbensis* *Saccharomyces paradoxus*
4.	*Streptococcus spp*	*Streptococcus thermophiles*
5.	*Enterococcus spp*	*Enterococcus faecium Enterococcus faecalis*
6.	*Bacillus spp*	*Bacillus subtilis Bacillus cereus* *Bacillus velezensis*

Single-strain vs Multi-strain Probiotics

Mono-strain probiotics include just one strain of a certain species, while multi-strain use many strains of the same or nearly related species (Timmerman et al., 2004). Since the gut microbiome is complex and comprises more than 400 species, it has been hypothesized that using a variety of probiotic strains may be more effective in restoring the microbiome once dysbiosis has arisen (Ouwehand et al., 2000). However, evidence-based efficacy supports the use of single-strain probiotics. Synergistic effects of several strains in the combination (increased adherence, higher pathogen inhibition) may be one advantage of multi-strain mixes (Timmerman et al., 2004). Different strains may potentially have different pathways of action, resulting in a broader coverage (Medina et al., 2007). For example, *B. longum W11* stimulates the growth of T-helper cells, but *B. longum NCIMB8809* does not (Mileti et al., 2009). A potential drawback of multi-strain mixtures might be reduced effectiveness owing to antagonistic intra-strain suppression by various probiotic strains (Chapman et al., 2012). A multistrain probiotic may have more effectiveness and consistency than a mono-strain probiotic. Colonization of an ecosystem offering a habitat for over 400 species in conjunction with individually selected host factors is expected to be more effective with multiple-strain probiotic preparations (Klaenhammer & Kullen, 1999) (Table 3).

Table 3: Single strain and Multi strain probiotics

	Single Strain Probiotics	Multi Strain Probiotics
1.	*Bacillus coagulans*	*Bacillus coagulans GBI-30*
2.	*Bacillus subtilis*	*Bacillus subtilis DE111*
3.	*Lactobacillus rhamnosus LCR35*	*Lactobacillus rhamnosus*

4.	*Lactobacillus pentous/plantarum*	*Lactobacillus pentous/plantarum complex*

Mechanism of Action

Several mechanisms (Kumar Bajaj et al., 2015; Walker, 2008) have been proposed for the activity of probiotics (Macfarlane & Cummings, 1999) (Fig. 3). Despite outstanding achievements in the field of probiotics, a fundamental breakthrough is still awaited particularly in documenting their mode of action (Latif et al., 2023). Probiotics may have a good effect on the human host via primary mechanisms which include: their role in removing pathogens by competitive exclusion criteria, betterment in the intestinal barrier functioning, immunomodulating the host's systems, and producing neurotransmitters (Plaza-Diaz et al., 2019). Probiotics contend with infectious agents for food and receptor-binding sites, making it harder for them to survive in the gut (Kumar Bajaj et al., 2015). Probiotics function as antimicrobials by creating short-chain fatty acids (SCFA), organic acids, hydrogen peroxide (Ahire et al., 2021), and bacteriocins (Fantinato et al., 2019), which reduce infective microorganisms in the gut. Furthermore, probiotics lead to more mucin production in the intestine (Chang et al., 2021), maintain the levels of tight junction proteins such as occludin and claudin-1, and modulate the gut immune response (Bu et al., 2022; Ma et al., 2022). Probiotics also influence the innate as well as adaptive immune responses by altering dendritic cells (DC), macrophages, and B and T lymphocytes. Probiotics also stimulate the generation of anti-inflammatory cytokines, interact with intestinal epithelial cells, and attract macrophages and mononuclear cells (Petruzziello, Saviano, & Ojetti, 2023). Furthermore, probiotics may generate neurotransmitters in the stomach via the gut-brain axis. Specific probiotic stains may alter serotonin, gamma-aminobutyric acid (GABA), and dopamine levels, influencing mood, behavior, gut motility, and stress-related pathways (Gangaraju et al., 2022; Sajedi et al., 2021; Srivastav et al., 2019). Probiotics have been shown to provide several health benefits, including more effective digestion, suppression of harmful bacteria in the gastrointestinal tract (Sanap et al., 2019), lowering blood pressure and blood sugar (Suez, Zmora, Segal, & Elinav, 2019), enhancing intestinal health (Reid et al., 2019), lowering serum cholesterol, breaking down toxins (Singh & Natraj, 2021), generating cofactors and vitamins (Nasr, 2018), immune system upregulation, anti-inflammatory properties (Abid et al., 2022), and protection against tumors and cancers (Idrees et al., 2022). These mechanisms have been the subject of numerous paradigms (Ferreira et al., 2022; Plaza-Diaz et al., 2019; Reque & Brandelli, 2022).

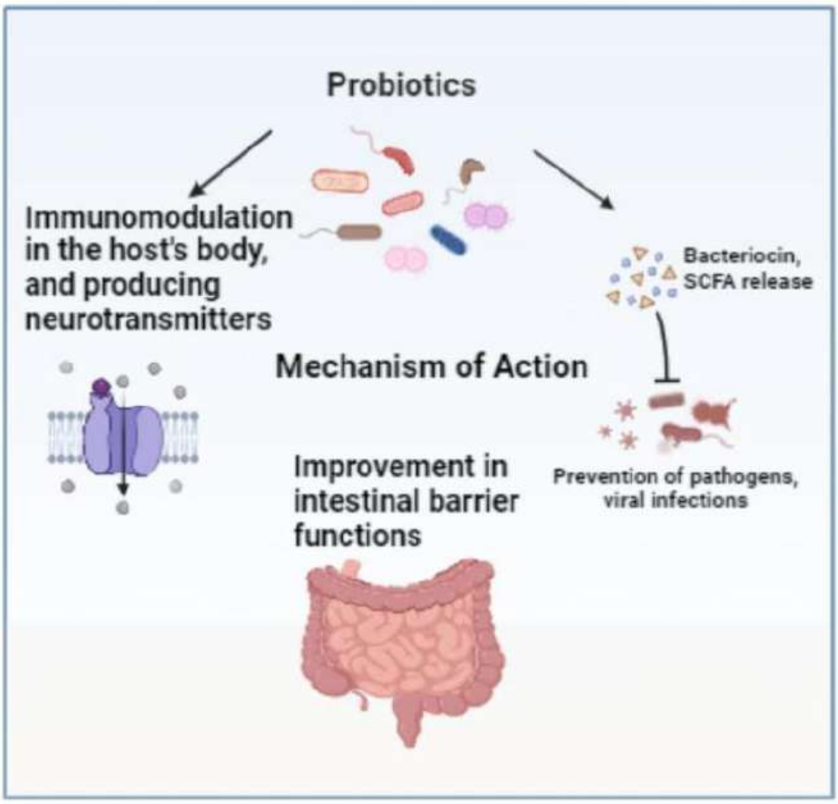

Fig. 3.: Mechanism of Action of Probiotics

Broader Efficacy of Multi-strain Probiotics

Multi-strain probiotics blend numerous strains, each with its characteristics and effects, their mechanisms of action are complex and multidimensional. Because many bacterial strains interact synergistically, multistrain probiotics may provide a wider variety of advantages than single-strain probiotics (Grumet et al., 2020). These are the main pathways by which probiotics of many strains work. Comparing multi-strain probiotics to single strains, the former may occupy more gastrointestinal tract niches. By more successfully competing for resources and attachment sites on the mucosal surfaces, this varied colonization may stop the proliferation of harmful bacteria (Valdez-Baez et al., 2022). The overall efficacy may be increased by aggregating many strains. As an example, while one strain may increase the functionality of the intestinal barrier, another strain may change the response of the immune system (Kwoji et al., 2021). The strains are combined to ensure that the gut is kept healthy and that immune responses are stepped up. Different probiotic strains create various bioactive compounds like bacteriocins and short-chain fatty acids (Chugh & Kamal-Eldin, 2020; Maldonado Galdeano et al., 2019). The diverse group of beneficial compounds in a multi-strain composition can potentially promote gut health, heighten the immune system's activity, and impede bacterial growth (Ouwehand et al., 2018). In comparison to single strains, multi-strain probiotic has a greater capacity to alter the overall composition of the gut microbiota (McFarland, 2021). They restore balance to the microbiota after perturbations like antibiotic treatment, dietary changes, or diseases. Different strains interact with different parts of the immune system (Duan et al., 2022). For example, some species of bacteria may increase the production of anti-inflammatory cytokines, while others may stimulate immune cells such as macrophages and natural killer cells. As a result, the immune response becomes both fairer and more effective. (Rizzello et al., 2011; Srivastav et al., 2019). Simultaneously, multi-strain probiotics may impact numerous metabolic pathways or processes, increasing throughput and productivity. They improve digestion and increase nutrient absorption, in addition to enhancing detoxification. They also improve the conversion of lipids and carbohydrates, which means they could potentially help with conditions such as diabetes and obesity (Puvanasundram et al., 2021). Multi-strain probiotics are effective in reducing systemic and local inflammation when several strains exhibit a synergistic effect (Giacchi et al., 2016). This should be pursued, especially for inflammatory conditions within the gastrointestinal system as seen in cases of Crohn's disease and ulcerative colitis (Kumar et al., 2016).

Probiotics in Physical and Mental Health

Diabetes

Diabetes has become a serious health concern across the globe, and it is associated with high blood glucose levels. Diabetes seems to be prevalent, with an estimated 463.0 million persons aged 20 to 79 years old, and the figure is anticipated to rise to 578.4 million by 2030 (Huang et al., 2018). Type 2 diabetes (T2D) is the most common form of diabetes, accounting for around 90% of cases. T2D may lead to a variety of problems, including cardiovascular, eye, renal, nerve, and vascular illnesses (Association, 2014). T2D and its consequences may have an impact on people's quality of life while also increasing treatment costs. As a consequence, diabetes prevention is critical, particularly for high-risk individuals, via screening, lifestyle changes, and nutritional supplements (Wang et al., 2021). Prior research highlighted the impact of gut microbiota in the progression of insulin resistance and diabetes (Gurung et al., 2020; He & Shi, 2017). The total number of bacteria associated with short-chain fatty acids (SCFAs) was shown to be decreased in T2D patients (Qin et al., 2012). Gut microbiota disruption may reduce SCFA synthesis, promote inflammation, impair insulin secretion and sensitivity, and cause insulin resistance (Aw & Fukuda, 2018). It has been proposed that oral probiotic delivery may be a useful strategy for modifying the gut microbiota in those at risk of diabetes (Aw & Fukuda, 2018; Barengolts, 2016). *L acidophilus*, *L. casei*, *L. rhamnosus*, *L. bulgaricus, B. breve, B. longum, B. infantis, B. lactis, Streptococcus thermophilus*, and *Bacillus coagulans* (*L. sporogenes*) are probiotic strains that have been linked to the regulation of blood sugar levels (Yao et al., 2017).

Cancer

Numerous *in vitro* research results have demonstrated the positive effects of probiotics in influencing the growth and death of cancer cells (Śliżewska et al., 2020). Probiotics have several anticancer mechanisms, which include lowering intestinal pH, inhibiting enzymes that may produce potentially carcinogenic substances, altering metabolic activity, binding and degrading carcinogens, immunomodulation in reducing chronic inflammation, and positive regulation of intestinal vegetation (Fig. 4) (Reis, da Conceição, and Peluzio, 2019; Molska and Reguła, 2019). Certain microbial strains are utilized to cure cancer, such as subspecies of *Propionibacterium sp.* (*freudenreichii), Bifidobacteria, Lactobacilli*, and *Streptococcus sp. (salivarius*) (Lu, et al., 2021). These may be used either on their own or in conjunction with antiviral drugs (Zhang et al., 2019). In combination, probiotics and TGF-β receptor blockers may enhance the antitumor immune response, hence inhibiting the development of tumors (Shi et al., 2019). By modifying gut microbiota and reducing carcinogen levels, lactobacilli may reduce the risk of cancer (Ling et al., 1994). Consequences of antitumor medications can include gastrointestinal distress. Radiochemotherapy instantly destroys intestinal cells (Osterlund et al., 2007). Because the stress response it causes destroys the intestinal mucosal barrier (Linn et al., 2019). Probiotics generated from *Lactobacillus* and *Bifidobacterium* can biologically prevent the development of pathogenic bacteria (Zhao et al., 2017).

Alzheimer's Disease

Alzheimer's disease (AD) is a neurological disorder that progresses over time and is responsible for 80% of dementia cases globally, especially in older adults over 60 years (DeTure & Dickson, 2019). A major global health concern in the future, AD is expected to affect over 131 million people by the year 2050, according to the world AD projection made in 2016 (Prince et al., 2016). The exact pathophysiology of AD is yet unknown. On the other hand, increasing evidence indicates that gut microbiota plays a role in AD neuropathology. Numerous mechanisms exist for the gut microbiota to interact with AD pathogenesis (Rutsch, Kantsjö, & Ronchi, 2020). According to a clinical investigation, the gut microbiota of AD patients has altered in terms of bacterial abundance and microbial diversity, with higher levels of Bacteroidetes and lower levels of Firmicutes and *Bifidobacterium* (Rinninella et al., 2019). Three primary mechanisms by which probiotics affect brain function include immunological modulation, endocrine pathways, and neuronal control (Psichas et al., 2015). The primary metabolites resulting from gut microbiota fermentation, small chain fatty acids (SCFAs), upregulate anti-inflammatory mediators and decrease pro-inflammatory mediators (Vijay & Morris, 2014). Probiotics work through endocrine pathways to trigger the production of cortisol, a powerful anti-inflammatory hormone, from the adrenal glands by activating the hypothalamic-pituitary-adrenal (HPA) axis. Probiotics also boost the intestine's enteroendocrine L-cells' (EECs) synthesis of peptide YY (PYY) and glucagon-like-peptide-1 (GLP-1) (Yano et al., 2015). Additionally, probiotics emit specific neurotransmitters like GABA (GLU) or regulate the release of neurotransmitters like serotonin (5-HT) through enterochromaffin cells (EC). Together, these neurotransmitters and neuroactive metabolites inhibit neuronal death by exerting neuroprotective effects (Naomi et al., 2022). Most commonly, *Bifidobacterium* and *Lactobacillus* species are used as an effective psychobiotics (Dinan et al., 2013; Zhu et al., 2021).

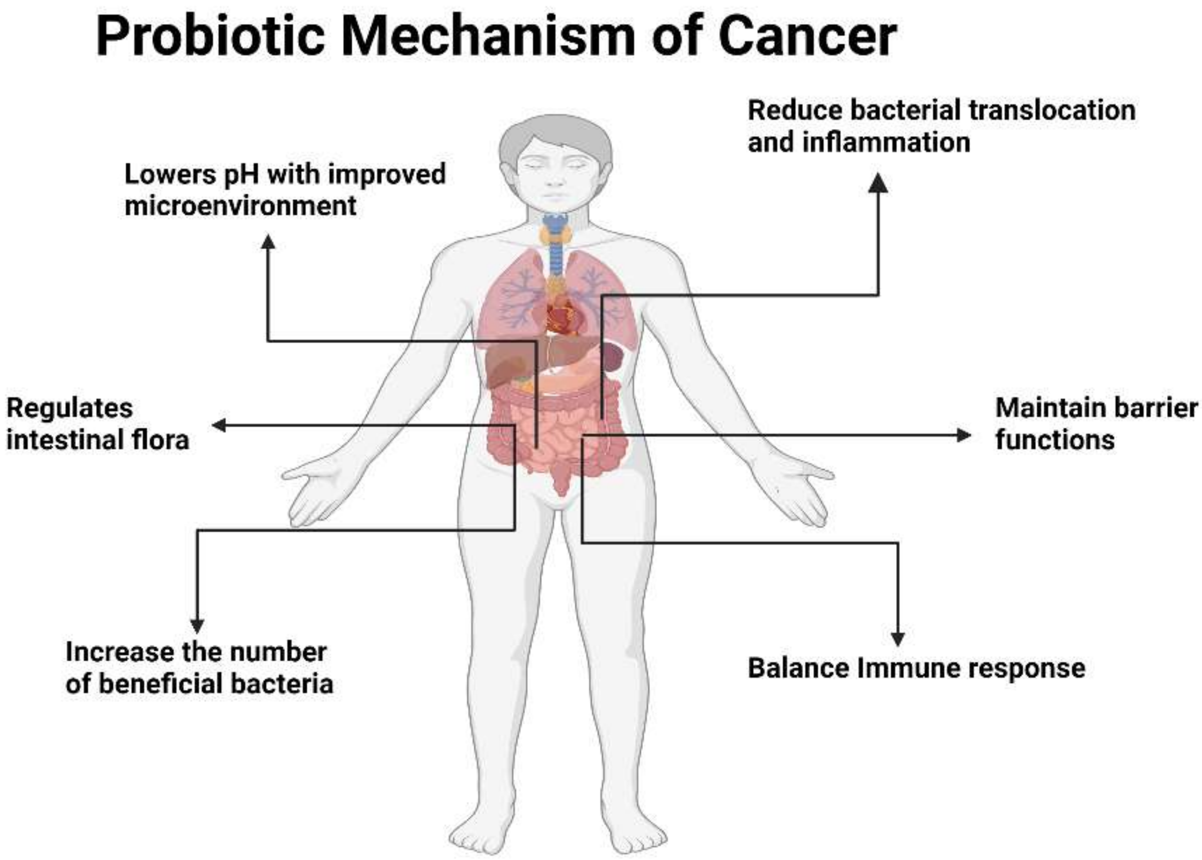

Fig. 4: Related Mechanism of Probiotics in Cancer

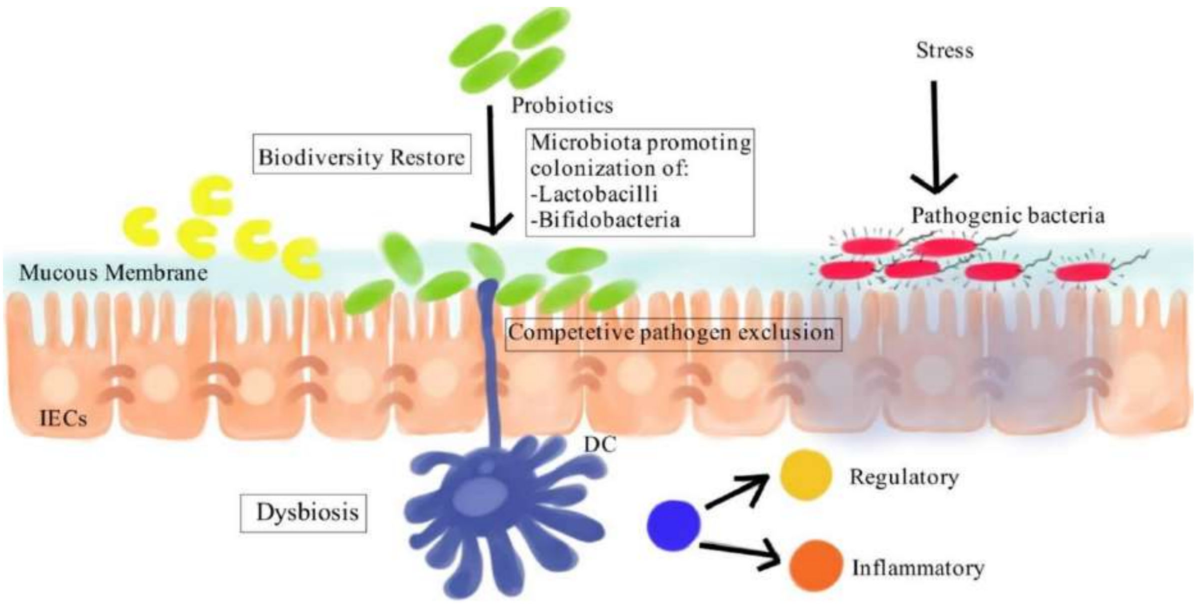

Fig. 5: Probiotics help to restore microbiota in stress and depression

Depression

Depression and anxiety are intricate and diverse psychiatric disorders, representing significant contributors to global disability (Psychiatry, 2022). Depression is a serious mental issue affecting a significant number of people globally. Symptoms include feelings of hopelessness, grief, loss of interest, poor appetite, and sleep disturbance (Thapar et al., 2022). Pervasive feelings of worry and fear, accompanied by noticeable alteration in behavior are referred to as anxiety (Chorpita & Barlow, 2018). According to the World Health Organization, around 4.4% of the world's population suffers from depression, and anxiety disorder affects more than 260 million (a, 2023). The global burden of disease, injuries, and Risk Factors Study (GBD) underscored the profound impact of mental health issues with depressive and anxiety disorders emerging as the two most disabling conditions. Both were prominently ranked among the 25 leading causes of global burden (Vos et al., 2020).

Common antidepressants and anxiolytics primarily target neurotransmitters in the brain to alleviate symptoms (Radosavljevic et al., 2023). Probiotics have gained growing attention due to their crucial role in mood regulation. Probiotics can affect mood and host health by regulating the microbial-gut-brain axis (Lou, Liu, & Liu, 2023). Scientists have identified the "Gut-brain axis," a communication pathway between the gastrointestinal tract and central nervous system (Chaudhry et al., 2023; Pan et al., 2023). This connection is influenced by various factors like genes, age, sex, diet, and stress. The microbe in our gut, particularly *Bifidobacterium* and *Lactobacillus*, play a crucial role in maintaining gut health and impacting symptoms of depression and anxiety (Kumar et al., 2023; Samiappan & Dhailappan, 2024; Xiong et al., 2023). Probiotics can positively influence the Central nervous system by regulating important neurotransmitters associated with depression and anxiety (Mudaliar et al., 2024). Chronic treatment with specific probiotics, such as *Bifibacterium infantis* and *Lactobacilli* has shown significant improvement in patients with major depressive disorder (Ribera et al., 2024). *Lactobacillus rhamnosus* was identified as a potential analytics (Matin & Dadkhah, 2024) (Fig 5).

Probiotics as an Anti-microbial Agent

Probiotic antibacterial effects may be determined using a variety of *in vitro* and *in vivo* techniques. Various variations of the spot-on lawn test, the agar well diffusion assay (AWDA), co-culturing techniques, the use of cell lines, and other techniques are examples of *in vitro* procedures (Fijan, 2023). Since the *in vivo* techniques use animal models, research is being done to find alternatives to animal research in accordance with the EU directive 2010/63/EU and its consolidated text EUR-Lex—02010L0063-20190626 from 2019 to preserve animals (Gjerris et al., 2023). As members of the Lactobacillaceae class, lactic acid bacteria, or LAB, are the most significant probiotics that have been shown to benefit the human gastrointestinal system (Santacroce et al., 2019). Probiotics can also generate a wide range of compounds that bear resemblance to antibiotics: Bacteriocins and antibiotics (Fijan, 2023) (Fig. 6).

Challenges of Probiotics as Medicines in Clinical Uses

Probiotics have made significant progress in therapeutic applications, but it still has several drawbacks and difficulties. To reach its full therapeutic potential and gain broader acceptance, these obstacles must be overcome.

Probiotic strain specificity is important because different bacterial strains can have quite different effects on how effective probiotics are. It takes more investigation and assessment to determine which strain is most suited for a given disease. The absence of uniformity in probiotic strain, dosage, and formulation creates difficulties when comparing research findings and choosing the most effective therapeutic strategy. The success of probiotic products depends on quality control, yet inconsistent production methods and storage environments might degrade product quality and change clinical results. The fact that probiotics are classified differently in different countries as dietary supplements, food additives, or medications, resulting in different regulatory standards and licensing procedures, raises regulatory problems.

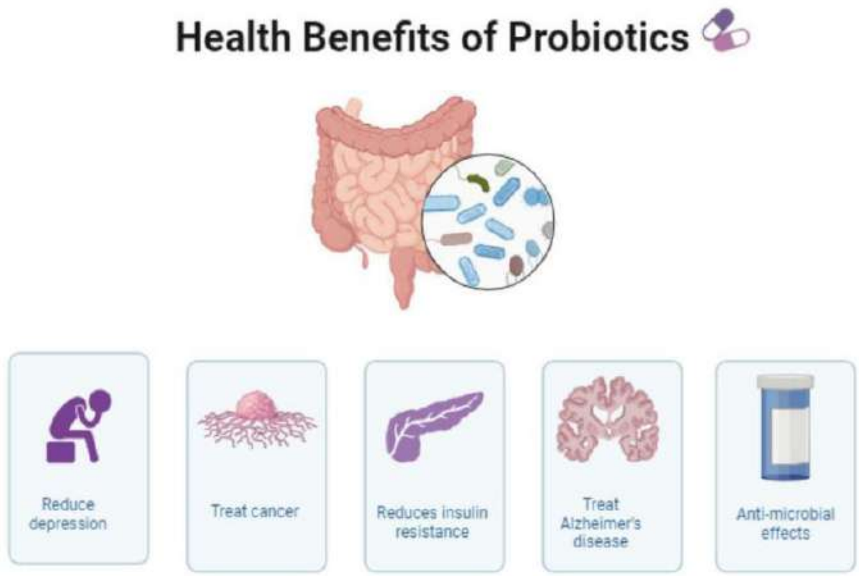

Fig. 6: Health benefits of Probiotics in physical and mental diseases

Impregnability of Probiotics

Even though probiotic strains are usually accepted as safe, this isn't always the case because of side effects, substandard probiotic supplements, and antibiotic resistance that can spread. Regarding the side effects of probiotics, it is important to note that while many studies have demonstrated the positive advantages of using probiotics as a health booster, relatively few have addressed the potential risks to the health of patients and healthy consumers that they may offer. Probiotic safety is a crucial factor to consider, particularly for immunocompromised people, critically sick patients, children, and those with central venous catheters, since these individuals may be more susceptible to infections or other problems. Probiotics may interact with other drugs in ways that affect how well a patient responds to therapy, therefore it's critical to recognize and handle these interactions in clinical practice. Taste, cost, and convenience are a few examples of the elements that might make patient compliance difficult. Individual diversity caused by variations in nutrition, genetics, gut flora, and other variables calls for a tailored treatment that will need more time and study to fully comprehend. Some short-term probiotic treatment trials have failed to detect significant changes in the gut microbiota, and specific individuals or diseases might demand an extended period of treatment for effective therapeutic effects. Changes in the gut microbiota may only be temporary with short-term probiotic interventions. Furthermore, the great majority of research on the effects of probiotics on health via the gut-brain axis has been done on animal models, and there remain unanswered questions about how these microorganisms interact with the human body and how to treat certain disorders.

Future Directions and Limitations

Probiotics have become increasingly well-known and acknowledged for their potential health advantages, but there are still several obstacles that need to be overcome before researchers and business owners can fully realize the benefits of probiotics. Probiotic bacterial strains can affect the human body in many ways. Determining which strains work best for a given set of medical issues and comprehending how they work is essential. Subsequent investigations ought to concentrate on clarifying the impacts of distinct strains and creating customized probiotic therapies. Consequently, it might not be accurate to extrapolate the benefits of probiotics to every strain within a species. Probiotic efficacy can also differ from person to person. What is effective for one person may not be effective for another, and it can be difficult to predict a person's particular reaction to probiotic treatment. Since probiotics are live bacteria, it is crucial to preserve their viability during manufacturing, storage, and ingestion. Putting in place strong quality control procedures and uniform manufacturing standards for probiotics is essential to ensure reliable and efficient products. Even though probiotics have shown several health benefits, further investigation is needed to determine the precise mechanisms and interactions that probiotics and the host have. This information will help with the development of focused probiotic treatments.

Conclusions

Probiotics are live and non-pathogenic bacteria that exert beneficial effects on human health even in case of diseases. Different strains of probiotics, either single strains or multi-strains have positive effects on health and disease. Several mechanisms of action have been proposed for probiotics as they are useful in many diseases like diabetes, cancer, Alzheimer's disease, Depression, and anxiety. Probiotics have antimicrobial properties; they can be used in many infectious diseases. For this conventional remedy to show to be a useful tool for medical therapy, it is crucial to carefully choose the probiotic agent, standardize its dose, and have a good understanding of its positive benefits over and above the harmful consequences. In relation to probiotic side effects, it's crucial to remember that although much research has shown the benefits of using probiotics as a health enhancer, only a small number have examined the possible concerns they may pose to patients' and healthy consumers' health. Probiotics have advanced a long way in terms of medicinal uses, but there are still a lot of challenges and disadvantages. More research is required to pinpoint the processes and interactions that probiotics and the host have.

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Chapter 29

Encapsulated Probiotics Exploring the Role in Functional Food Industry

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ABSTRACT

Generally speaking, it is a mechanical or physicochemical process whereby bacterial cells are ensnared in encapsulating materials with varying characteristics that can lessen or prevent the encapsulated microorganisms' harm or cellular losses, particularly, in contrast to the anticipated positive effect on the host. Probiotics have gained popularity for their numerous health benefits, but their sensitivity to environmental factors and poor survival rates during processing and storage hinder their effectiveness. Encapsulation technology offers a solution by protecting probiotics from adverse conditions and enhancing their delivery to the target site. This chapter reviews the benefits and methods of encapsulating probiotics, including spray drying, emulsification, micro fluidization, and coacervation. The advantages of encapsulation, such as improved stability, controlled release, and enhanced functionality, are discussed. The chapter also highlights the importance of selecting suitable encapsulation materials and techniques to ensure the survival and viability of probiotics. By encapsulating probiotics, their therapeutic potential can be unlocked, leading to the development of innovative functional foods and supplements that promote gut health and overall well-being.

KEYWORDS		
Probiotics, Food industry, Encapsulation of probiotics	Received: 12-Jun-2024 Revised: 15-Jul-2024 Accepted: 22-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

The health benefits of probiotics, which are live microbial supplements, are well-known. Among other health benefits, the usage of probiotics has been connected to enhanced gut flora composition and greater disease resistance. In recent years, probiotic-based food products have grown in popularity. Bifidobacterium has been associated mostly with the human digestive system and has been used as probiotics over the years (Sharifi Rad et al., 2020).Foods containing probiotics were first discovered in cheeses and milks made by lactic acid bacterial and fungal fermentation, as well as in leavened bread fermented by yeast (Suvarna and Boby, 2005). Furthermore, it is commonly recognized that fermented foods are healthy. Probiotic-rich milk was suggested as a potential treatment for gastrointestinal disorders by Hippocrates and other ancient physicians. Additionally, fermented milk products have been proposed as a treatment for gastroenteritis by the Roman historian Plinius. Viable nonpathogenic microorganisms were frequently employed in the treatment of intestinal illnesses in order to modify or replace the intestinal microbiota. Nissle 1917 is one of the rare Escherichia coli strains that is not laboratory produced. It wasn't until the 1960s that the term "probiotic" was used to describe substances produced by bacteria that promote the growth of others (Beswick and Mullins, 1964). Although little has been discovered about how probiotics act in the gut, more research should be done to determine the role they play in human health as well as the safety of using them. Because there is no scientific understanding or categorization study of the probiotics found in fermented food, people are unaware of their full nutritious potential. Our above concerns can be resolved with the aid of a probiotic database derived from fermented foods. By adding vitamins, proteins, essential fatty acids, and necessary amino acids to dietary substrates, fermentation can enhance the food's nutritional value and digestibility. More specifically, fermentation may connect process energetics and product quality to the variety of the fermenting microbial community and their characteristics. Probiotic fermented foods have gained popularity in recent years, which has sparked creativity and accelerated the creation of new products globally. Foods containing probiotic bacteria are being added more frequently in an effort to maintain the microbial balance in the gastrointestinal tract and enhance gut health. Some significant bacteria are Bacillus, which is linked to the fermentation of legumes, and Acetobacter, which produces acetic acid and is involved in the fermentation of fruits and vegetables. Furthermore, we offer some details regarding the bacteriocins that are generated by bacteria in foods that have undergone fermentation. Because yeast produces the enzymes that lead to desired biochemical reactions, it is essential to the food business. This is demonstrated by how ethanol is produced in beer and wine as well as how bread rises. As such, it is advantageous for the industrial development of probiotics. We have gained a better understanding of probiotics and their active ingredients by studying the biological data of probiotics in various fermented meals. Furthermore, PBDB can be used to understand the traits and roles of distinct microorganisms in a variety of fermented foods. Even though no study has completely examined the probiotics in fermented foods using an integrated database, this effort is crucial to the advancement of the medical field (Zhao et al., 2019).

History of Probiotics

The term "probiotic" was first used in 1953 to describe "active substances that are essential for a healthy development of life" by German scientist Werner Kollath. It is derived from the Latin pro and the Greek βιοσ, which means "for life." This word was first used in a different context in 1965 by Lilly and Stillwell to describe "substances secreted by one organism which stimulate the growth of another." More accurately, Fuller (1992) defined probiotics as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”(Gasbarrini et al., 2016). Elie Metchnikoff discovered around 1900 that eating living microbes (Lactobacillus bulgaricus) in fermented milk or yogurt improved certain GIT properties. Today, the Food and Agricultural Organization of the United Nations and the World Health Organization define probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." The International Association for Scientific Prebiotics and Probiotics has reviewed and approved this definition.

Commonly used Probiotic Microorganisms

Numerous bacteria belonging to various genera and species may possess probiotic qualities. However, over time, probiotics have been made from the genera Lactobacillus and Bifidobacterium (Fijan, 2014). Mainly because these are the most common bacteria in the human digestive tract and are regarded as GRAS (generally recognized as safe) (Brodmann et al., 2017). Specifically, the dairy industry often uses the probiotic strain Lactobacillus spp. Lactobacilli are rod-shaped, gram-positive, non-spore-forming microorganisms that often inhabit an anaerobic environment. Despite this, they are acid- and aero-tolerant, fermentative, and selective.

Health Benefits of Probiotics

Functional foods have a role in improving human health that goes beyond nutrient content. Functional foods are used to promote health and balance out minor physiological problems that healthy hosts may encounter. They fall between nutrients, which provide basic physiological functions, and medications, which treat diseases. Apart from the well-known functional ingredients like vitamins, minerals, and micronutrients, probiotics are a part of the new wave of active ingredients that also includes phytonutrients, lipids, and prebiotics.

Probiotic bacteria enhance the equilibrium of the gut microbiota and strengthen the body's defenses against infections, which is helpful to human health. Probiotics are also said to have anti-bacterial, anti-carcinogenic, blood cholesterol-lowering, immune system-stimulating, and vitamin-synthesizing properties. Probiotics have several main advantages, including the reduction or elimination of conditions like constipation, diarrhea, and colon irritation (Rehaiem et al., 2014). Several studies have demonstrated their beneficial effects on gastrointestinal infections, antimicrobial activity, lactose metabolism enhancement, serum cholesterol reduction, immune system stimulation, antimutagenic, anti-carcinogenic, anti-diarrheal, and reduction of Helicobacter pylori infection through the addition of specific strains to food products (Pereira et al., 2011).

Encapsulation

Encapsulation is a useful method for improving the way that living cells and bioactive compounds are transported into food products. It requires covering active ingredients in a carrier medium. It is also possible to encapsulate proteins and lipids. The most commonly utilized encapsulation technique in the food sector is spray drying since it's a continuous, adaptable, and most importantly, economical procedure. Most encapsulates are produced by spray-drying; the remainder are made via freeze-drying, melt extrusion, melt injection, and spray-chilling (Nedovic et al., 2011). During the manufacturing process and storage of food, non-encapsulated probiotic microbes may be subjected to high temperatures, low pH, high osmotic pressure, and high oxygen levels (Kailasapathy,2006). Research into surrounding probiotics in a physical barrier has been conducted by numerous investigators. Encapsulation is a process that involves the incorporation of protective elements into miniature capsules that can be released under specified conditions at a regulated rate.

Encapsulation Components

Typically, encapsulated particles are made up of two parts: the covering material/shell and the core. The active components to be coated, known as core materials, can be in any of three physical states: liquid, solid, or gas. Using the solid-phase separation approach, solids are disseminated in a polymeric solution and then polymer precipitation occurs (Duduković et al., 2002). Furthermore, depending on their core solubility, solids can dissolve into suitable liquids. If the core dissolves in an organic solvent, the coating materials and the core are also dissolved in the solvent. The organic solvent is then emulsified and evaporated, using the single-emulsion solvent evaporation procedure, to produce Nano-precipitation (Yu et al., 2018). The solid is dissolved in water and then emulsified in the case of a water-soluble core. While gas cores can be adsorbed on an inert material and then enclosed as a solid core, liquid cores can be emulsified. The inert polymeric material that coats the core materials to the appropriate thickness is known as the coating material or shell. These materials should offer desired qualities including stability, strength, flexibility, impermeability, and non-hygroscopicity and be compatible and non-reactive with the core material. Naturally occurring polymers like polysaccharides are among the frequently utilized coating polymers. Matrix and vesicular encapsulated particles fall into two primary categories according to the dispersion of the core substance. Systems with a physically and evenly distributed active component, or core, are called matrix systems. On the other hand, vesicular systems, also known as capsules, have the core material contained within a hollow surrounded by a polymer membrane (Duduković et al., 2002). Different terminology can be used to refer to vesicular and matrix systems based on their composition shapes, coating materials, and production procedures.

Benefits of Encapsulation

Enhancing stability in finished products and during processing is one of the main justifications for encapsulating active substances. Less evaporation and degradation of volatile actives, including scent, is another advantage of encapsulation. Encapsulation is also employed to cover up unpleasant food-related sensations, such the astringency and bitter taste of polyphenols. Preventing reactions with other ingredients, such oxygen or water, in food products is another reason to use encapsulation. Apart from the aforementioned uses, encapsulation can also be employed in food processing applications, such the fermentation and metabolite synthesis processes, to immobilize cells or enzymes. The need to identify practical solutions that offer great production while also ensuring that the finished food items are of a satisfactory quality is growing (Livney, 2010).

Methods used in Microencapsulation

This study highlights the key principles behind probiotics' ability to withstand stress and describes novel techniques to probiotic microencapsulation. Additionally, a study of current in vivo and in vitro models is done in order to evaluate the efficacy of probiotic administration techniques. Probiotics must be encapsulated in order to maintain their viability both in storage and in the human gut, which increases the likelihood of colonization.

These solutions work by protecting the probiotics from harmful environmental elements and enhancing their mucoadhesive properties. Usually, the probiotics are coated or embedded with food-grade materials such as lipids or biopolymers. To improve their chances of life, other components like nutrition or defense compounds are occasionally encapsulated. The importance of having suitable in vitro and in vivo models to evaluate the efficacy of probiotic administration techniques is also emphasized. Encapsulation is a commonly used method for creating customized products in the food industries and specialized food production, food processing sectors (Yao et al., 2020).It involves covering a functionally linked central substance in an inert material matrix for protection. The material that will be enclosed is referred to as "core" or "active material". It also goes by the title’s payload, internal phase, and fills. On the other hand, the material that surrounds the active ingredient is called the shell, carrier material, matrix, coating material, wall, capsule, and membrane. Microencapsulation technology has attracted more attention in industrial applications due to its ability to protect unstable bioactive components, provide designed food products additional functional qualities, and distribute active chemicals at controlled rates to specified areas. Encapsulation strategies have therefore been researched extensively for a very long time. The optimum process depends on a number of factors, including the type of the active substance, the characteristics of the shell material, and the attributes that the finished product must have based on its intended use. Several encapsulation techniques may be used in delivery system design.

There are Four Factors that Guide the Design

The physical and chemical characteristics of the substance that need to be encapsulated; the mode of distribution, which influences the choice of wall material; the capsule's dimensions; and, finally, the encapsulation process. Encapsulation helps to screen the contents from the external environment, prolong and facilitate the process of storage or transit, and protect the inside of the item. The application of encapsulation in commerce was initially recorded in 1957 (Kłosowska et al., 2023). Encapsulation has now become more common in several industries, such as food, lipids, essential oils, agriculture (pesticides), cosmetics and fragrances, and nutritional supplements (fish oil and vitamins). In these fields, knowledge has also continued to advance. There are many different ways to encapsulate information. The ideal approach relies on a number of variables, such as the intended use, the size of the encapsulates, the chemical makeup, cost, and availability of the coating, as well as the contained core substance. Blends of organic fragrance compounds, such as artificially produced naturally occurring substances like essential oils or resins with a natural equivalent, make up scent compositions and tastes. Encapsulation changes the material's structure to make it solid from a liquid or gas, immobilizes the active material by encasing it, shields the core from the damaging effects of the environment, releases the core material gradually to increase its exposure to the active material, and facilitates functionality, among other things (Kliszcz et al., 2021).

Advantages to Taste and Fragrance Encapsulation

Taste and aroma encapsulation has following benefits:

1. Extended toughness
2. Greater stability in the final product, which undergoes a structural change from liquid to solid and has improved dispersibility, fluidity, and dosage precision.
3. The time of exposure to taste or odor is prolonged by the controlled and progressive release of fragrance components.
4. Masking taste and aroma
5. Protection from external factors, severing highly volatile and chemically unstable components from their surroundings, safeguarding against ultraviolet radiation, deterioration processes, heat, oxidation, and dryness.
6. Safety increases when volatile substances become less flammable.

Proper Process of Encapsulation

a) Physico-mechanical Methods

Spray Drying

The basic idea behind spray drying is to emulsify and dissolve the core material in an aqueous solution of the carrier substance. The combination is then atomized in a heated chamber, where the active particle is coated, and smaller water molecules evaporate. This method finds application in the culinary, pharmaceutical, and cosmetic sectors, as well as in the manufacturing of milk powder. Because strongly flammable scent ingredients evaporate more quickly than water, it's important to use the right carrier to prevent losing volatile fragrance compounds while letting water evaporate. When selecting a suitable carrier, the following aspects should be considered:

- flavor and odor release beneath the appropriate conditions
- low cost and accessibility
- taste impartiality
- Stability
- good solubility in water
- and good emulsifying characteristics

Emulsification

One popular way for co-encapsulating probiotics is this procedure. This procedure is divided into two stages: the dispersed phase, which contains the suspension of the cell polymer, and the continuous phase, which is composed of oil (mineral or vegetable oil) or organic solution. The mixture is homogenized with the help of surfactants to create the emulsion. When a cross-linking agent is applied or the water-soluble polymer is cooled, the particles are created inside the oil phase. After that, the microbeads are either filtered or centrifuged. The agitation speed, surfactant concentration, rate of cross-linking agent addition, and water to oil ratio—which can vary from 25μm to 2mm—all affect the size of the beads. Emulsifiers reduce surface tension, which makes microspheres smaller and increases the stability of emulsions (Chandramouli et al., 2004). This approach is simpler to scale up, produces microcapsules with a lower diameter, and increases probiotic life. The main drawbacks are that it creates microcapsules with varying sizes and shapes and necessitates the use of a second polymer solution for the extra coating on the cel. Milk protein is used in emulsification with gelification to encapsulate probiotics since it has gelation capabilities and serves as a natural probiotic carrier (Misra et al., 2021).

Micro Fluidization Method

Using the previously described technique, microcapsules are produced using the Microfluidic device, which has micro channels that permit laminar fluid flow and the creation of double emulsions. This apparatus can produce consistent microcapsule sizes and ensure process repeatability.

Pan Coating

This is a technique to microencapsulate solid particles that are greater in diameter than 600μm. This apparatus has perforations all around it and a revolving disk that spins in the opposite direction of the drum's motion (Kłosowska et al., 2023). The cores are provided into the disc's center, where they are transformed into a coating material layer. Holes are then filled with an encapsulating material solution. When the combined mass of the shell and core reaches a certain threshold, centrifugal force exceeds the forces that hold the hole together membrane, causing the microcapsules to be discharged external to the person who receives. Heat-treated air is used to remove the solvent after the shells have undergone chemical or physical curing. This method has high production efficiency and the advantages of being rapid, efficient, and manageably simple to utilize.

Physicochemical Methods

Coacervation Method

Microencapsulation seems to originate from a physicochemical process called coacervation. Two varieties of coacervation are available, based on how the technique is conducted out: basic and sophisticated (Rutz et al., 2017). Simple coacervation comes in one type only of polymer, and there is also an extremely hydrophilic substance present in the colloidal solution. In sophisticated coacervation, two or more polymers can be used. Anionic and cationic polymers need to be able to communicate with each other underwater in order to become complex coacervate, a liquid phase rich in polymers. In this case, the most typically used cationic polymer is gelatin. Gelatin with several synthetic and natural water-soluble polymers combines to form complex coacervates suitable for encapsulation. These coacervates take place in balance with a transparent liquid at the surface. Phases in a colloidal or polymeric solution separate during the coacervation process, and more than two liquid stages are created Consequently of the solution environment being carefully changed. This includes modifying the solubility, temperature, ionic strength, pH, and adding salt or a polymer with a countercharge. The coacervation process yields an equilibrium phase and a colloid-rich coacervate phase.

Chemical Methods

Polymerization

Among the chemical methods for making encapsulates is the polymerization procedure, which encompasses both in situ interfacial polymerization. The previous technique polymerization produces encapsulates by polymerizing monomers at the interface between the dispersing (ethyl alcohol, glycerol, chloroform, water) and scatter (vegetable oils, animals fats and synthetic oils) stages. The dispersion stages are combined with the encapsulating monomer and the suspended or dissolved active component until an o/w emulsion is formed. The most widely utilized monomers have multifunctional organic acid chlorides and multifunctional isocyanates. Furthermore, to the components mentioned above, vinyl acetate, methyl methacrylate and a mixture of vinylbenzene styrene or diamines are also used. As monomers permeate between the phases, the process generates an oil-insoluble polymeric membrane (polyurea, poly-nylon, or polyurethane). On the other hand, unlike interfacial polymerization, in situ polymerization doesn't call for the inclusion of additional reactive substances. When the resulting polymer first forms, it has a low molecular weight, but it gradually becomes larger. After that, the core material is covered with this polymer to form a solid capsule shell.

Encapsulation Efficiency

Capture efficiency (EE) is the most important parameter for assessing the performance of the encapsulation process. EE determines the proportion of bioactive material trapped inside the inert core during the encapsulation process. This can be calculated by dividing the mass of the core material used in the formulation by the mass of the core material that is entirely contained within the wall material. Simply said, the bioactive is more stable throughout the encapsulation process when almost all of it is completely entrapped inside the shell matrix, which is when the best encapsulation efficiency happens. Put differently, the technique provides high-level protection for the bioactive core. It is better to encapsulate PUFA-rich oil and trap almost all of the oil within the shell matrix, leaving very little oil on the surface of the shell matrix. Any oil that remains on the surface of the shell remains outside of the shell matrix. We call this surface fat or free fat. The surface oil rapidly undergoes oxidative degradation as soon as the capsules come into contact with ambient air. Elevated surface oil so frequently correlates with microcapsule off-flavor and worsens consumer approval of the finished product. Many emulsion formulations have far more surface oil than the recommended 0.1% (w/w) concentration for microcapsule preservation (Kaushik et al., 2015).

Conclusion

Encapsulation of probiotics is a crucial technology to enhance their stability, viability, and functionality in food products. Various encapsulation methods, including spray drying, emulsification, micro fluidization, pan coating, and coacervation, have been developed to protect probiotics from environmental stresses and ensure their delivery to the target site. The choice of encapsulation method depends on the type of probiotic, desired release profile, and food application. Encapsulation materials, such as polysaccharides, proteins, and lipids, play a vital role in determining the properties of the encapsulated probiotics. The benefits of encapsulated probiotics include improved gut health, enhanced immune system function, and increased tolerance to stress conditions. Future research should focus on developing more efficient and cost-effective encapsulation methods, exploring new encapsulation materials, and investigating the impact of encapsulated probiotics on human health. By advancing encapsulation technology, we can unlock the full potential of probiotics and create innovative food products that promote human well-being.

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Chapter 30

Harnessing the Power of Probiotics and Prebiotics: A Comprehensive Guide to Gut Health and Beyond

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ABSTRACT

In today's world, maintaining good gut health is crucial for achieving optimal well-being. Probiotics and prebiotics offer a transformative potential for achieving this goal. Probiotics are live microorganisms that provide numerous health benefits when consumed in adequate amounts, while prebiotics are non-digestible fibers that stimulate the growth and activity of beneficial bacteria in the gut. Together, they play a vital role in shaping the gut microbiome and influencing various physiological processes beyond digestion. This comprehensive guide explores the fundamental principles of probiotics and prebiotics, highlighting their symbiotic relationship in fostering a balanced gut microbiota. By modulating immune responses, enhancing nutrient absorption, and protecting against harmful pathogens, probiotics and prebiotics can promote gut health and beyond. The guide also delves into the diverse applications of probiotics and prebiotics in managing GI disorders such as irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD). Additionally, they have emerging roles in improving mental health, immune function, and metabolic regulation, making them a valuable tool for achieving holistic well-being. Moreover, innovative applications of probiotics and prebiotics in areas like dermatology, oral health, and women's health underscore their potential for personalized interventions based on individual microbiome profiles. By combining cutting-edge research with practical insights, this guide equips readers with a comprehensive understanding of harnessing the power of probiotics and prebiotics for optimizing gut health and promoting holistic well-being. It serves as a valuable resource for healthcare professionals, researchers, and individuals seeking to leverage microbial therapies for improved health outcomes and enhanced quality of life.

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Probiotics, Prebiotics, Gut health, Microbiome	Received: 10-Jun-2024 Revised: 21-Jul-2024 Accepted: 18-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Nowadays, nutritional and healthcare practitioners typically endorse the advantageous effects of probiotics on human well-being. Probiotic treatment has been shown to be an effective therapy for maintaining and repairing the gut environment. Consumption of healthy living microorganisms (lactic acid bacteria) may enhance the makeup of the healthy colonic microbiota. This combination may improve bacteria's ability to survive in the upper gastrointestinal (GI) tract, hence increasing their impact. Furthermore, their effects could be cumulative or synergistic (Jain et al., 2014).

Probiotics are living microbes that improve human and animal health by competing with intestinal pathogens (Fioramonti et al., 2003). Furthermore, they improve digestive capacity, lower gut pH, and boost mucus immunity. Because of these features, probiotic bacteria create an unfavorable environment for the growth of enteric pathogenic bacteria, preventing them from colonizing the intestine. Probiotic bacteria lower the host's inflammatory reactions by strengthening the intestinal permeability barrier, stabilizing the gut's microbial ecosystem, destroying antigens, and altering their immunity and antigenicity (Fig. 1). Consequently, the host's gut microbiota becomes more resilient and stable to population disturbances (Ozen and Dinleyici, 2015).

Fig. 1: Health Benefits of Probiotics

History of Probiotics

Metchnikoff (1907) hypothesized that Bulgarian peasants' longevity was due to their heavy consumption of fermented milk. Following Tissier's (1900) studies on the role of bifidobacteria in newborn guts, Metchnikoff (1907) recommended modifying the gut microbiota and replacing harmful bacteria with helpful ones. He believed that the positive benefits of fermented milk stemmed from the implantation and growth of *Lactobacillus bulgaricus* in the colon. At the close of World War I, many children suffered from digestive ailments. Encouraged by Metchnikoff's findings, Isaac Carasso established a yoghurt production facility in Barcelona in 1919, employing Pasteur Institute fermentations. The products were only available with a prescription in Barcelona pharmacies. Later, Rettger and Cheplin (1921) established that implanting *Lactobacillus acidophilus* in people' GI tracts could help alleviate constipation, diarrhea and other intestinal disorders. This bacterium originated in the human intestine, and it was believed that native bacteria were more likely to have the intended effect in the gut. Meanwhile, in 1917, an *Escherichia coli* strain was found that efficiently cured acute infectious intestinal disorders such as shigellosis and salmonellosis (Helene et al., 2014).

Probiotics were later defined as "monocultures or mixed cultures of microbes used on animals and humans, which benefit the host by enhancing the properties of indigenous microflora". In 1998, Guarner and Schaafsma hypothesized that probiotics are live bacteria that, when consumed in adequate quantities, improve the health of the host. They have been defined as "microbial cells introduced through a particular way that reach the GI tract and stay alive with an objective of improving health" (Islam, 2016). The notion of probiotics was expanded to encompass "live microbial supplements that are beneficial to the host by enhancing its microbial balance" in the same year, thanks to studies on the suppression of infections with probiotics (Calatayud and Suárez, 2017).

Mechanism of Action of Probiotics

Currently, it is known that probiotics have an antimicrobial effect by altering the microbiota in the intestines, secreting antibacterial substances (bacteriocins or organic acids), competing with pathogenic bacteria to prevent their adherence to the intestinal tract, competing for nutrients required for pathogenic bacteria survival, and producing antitoxin effects. In addition, probiotics can modulate allergy reactions, the immunological system, and tumor formation in mammals. Probiotics are so useful when administered at the appropriate concentration and viability (Marteau et al., 2001).

Some lactic acid bacterial (LAB) strains can serve as probiotics for both humans and animals. In general, lactic acid bacteria (*Streptococcus*, *Lactobacillus*, and *Bifidobacterium*) probiotic capabilities include survival in the host GI tract, adherence to the host's intestinal epithelium, and the prevention of dangerous microbe invasion or ingrowth in the animal intestine, such as *Salmonella* spp. and *Escherichia coli*. For lactic acid bacteria cells that have survived bile and gastric conditions, the ability to adhere to host intestinal epithelium may provide a competitive advantage and is critical for bacterial persistence in the human GI tract. This trait may influence the competitive exclusion of dangerous bacteria. Furthermore, it has been hypothesized that specific LAB (lactic acid bacteria) strains may have extra favorable health effects, such as increasing human immunity.

The idea that some probiotic strains can boost the innate immune response is supported by studies using *Lacticaseibacillus casei* injected into mice, which revealed a significant increase in the activity of natural killer cells from mesenteric node cells but not from Peyer's patch cells or spleen cells. Spleen cells in mice treated with various probiotic strains produced much more gamma interferon and had significantly higher proliferative responses to mitogens such as concanavalin A (a T-cell mitogen) and lipopolysaccharide (a B-cell mitogen). Some LAB strains of vegetable origin, isolated from plant material and various fermented meals, have been shown to exhibit immune-boosting, anticancer, and antibacterial activities (Kumar and Ghosh, 2012).

The Role of Food Matrices in Probiotic

Food matrixes serve as carriers for probiotic microorganisms in human and animal bodies. These matrixes are utilized to transport probiotics through the GI tract and promote colonization. Yogurt and fermented milk are the finest food carriers for delivering probiotic microorganisms to both humans and animals. To get the most out of probiotics, these items should be consumed on a daily basis. Occasional usage of the probiotic will not provide full advantages. Furthermore, the physiochemical properties of yoghurt and fermented milk make it the greatest food carrier matrix for the delivery of probiotic strains to the body when compared to ice cream and cheese carriers (Srinu et al., 2013).

The survival of probiotic strains in the GI tracts of animals and humans is a difficult process that requires nutrition availability, interaction with bacteria in the GIT tract, adhesion qualities, diet type, co-aggregation and auto-aggregation properties. In many ecological settings, aggregation between bacteria from the same strains (auto aggregation) is more essential than aggregation between genetically different bacterial strains (co aggregation). To accomplish their tasks, aggregating bacteria either build a sufficient mass-forming biofilm or cling to their hosts' mucosal membranes. In the context of probiotics, bacteria that can aggregate with other bacteria in the same habitat have an advantage over bacteria that cannot combine.

Additionally, another mechanism of colonization and adherence involves the binding of the surface. The bacteria adhere to the layer of mucus membrane that covers the epithelial cells of the GI system. Some bacteria strains produce particular adherence proteins such as collagen-binding proteins, fibronectin, mucus-binding protein (which enhances adhesion to mucus), and elongation factor. Bacteria rely heavily on the production of these proteins to colonize the gut (Srinu et al., 2013).

Nutritional Benefits, Production, and Potential Prebiotics

Prebiotics, a diverse array of nutrients susceptible to degradation by gut microbiota, have garnered substantial attention in recent years due to their potential impact on human health. These compounds serve as vital nourishment for the intestinal microbiota, yielding short-chain fatty acids upon breakdown, which subsequently enter the bloodstream, exerting effects not only on the GI system but also on distant organs. Among the noteworthy prebiotics, fructo-oligosaccharides and galacto-oligosaccharides stand out for their beneficial effects on human health. While these compounds occur naturally in limited quantities in various foods, concerted efforts by scientists are underway to upscale their production on an industrial scale. Given their demonstrated health benefits, safety profile, and logistical advantages in production and storage vis-à-vis probiotics, prebiotics emerge as compelling candidates for enhancing human health either as standalone interventions or in conjunction with probiotics. This review comprehensively explores diverse facets of prebiotics, underscoring their pivotal role in promoting human well-being (Davari et al.,2019).

Importance of Prebiotics

Prebiotics and probiotics constitute pivotal components in augmenting human nutrition and bolstering overall health. Prebiotics, characterized as compounds resistant to host digestion but fermentable by probiotics, serve as essential substrates fueling the proliferation and metabolic activity of beneficial gut bacteria, thereby fortifying bodily health. The clinical utility of probiotics has been well-documented in the prevention and management of a spectrum of conditions, spanning intestinal, respiratory, and urogenital infections, allergic reactions, inflammatory bowel diseases, and irritable bowel syndrome, among others. Yet, despite their demonstrable efficacy, a comprehensive synthesis elucidating the diverse types, mechanisms of action, and the intricate interplay between prebiotics and probiotics remains scarce. Thus, our endeavor encapsulates a meticulous compilation delineating the myriad varieties of prebiotics and probiotics, their unique modes of action, and the intricate mechanisms whereby prebiotics foster the proliferation of probiotics within the

GI milieu. We envisage that this review will furnish novel insights underpinning the future application and refinement of prebiotics and probiotics in clinical practice and health promotion initiatives (You et al., 2022).

Furthermore, the exploration of bioactive from food sources with prebiotic properties delves into their multifaceted application beyond mere nutritional supplementation. In recent years, there has been a burgeoning interest in leveraging prebiotics not only for their health-promoting effects but also for their functional attributes in food manufacturing. These compounds, with their capacity to modulate the gut microbiota and foster the growth of beneficial bacteria, hold immense potential in revolutionizing the landscape of functional food formulations. From enhancing the shelf life of perishable goods to fortifying the nutritional profile of processed foods, prebiotics offer a versatile solution to address both consumer demand for healthier dietary options and industry imperatives for product innovation.

Moreover, the incorporation of prebiotics into various food matrices presents a promising avenue for meeting evolving consumer preferences. As individuals increasingly prioritize health-conscious choices, the integration of prebiotics into everyday food staples serves as a strategic approach for bridging the gap between nutritional fortification and culinary enjoyment. Whether infused into savory snacks, incorporated into breakfast cereals, or blended into indulgent desserts, prebiotics have the potential to confer both functional benefits and sensory appeal to a diverse array of food products. This symbiotic relationship between prebiotics and food innovation underscores the integral role of these compounds in shaping the future of the food industry and advancing the paradigm of personalized nutrition.

In light of these developments, ongoing research endeavors continue to unravel the intricate interplay between prebiotics, probiotics, and host health, paving the way for novel therapeutic interventions and dietary strategies. As scientific understanding deepens and technological advancements accelerate, the potential applications of prebiotics in food and nutrition are poised to expand exponentially. From targeted formulations tailored to address specific health conditions to customized dietary regimens optimized for individual well-being, the integration of prebiotics into the culinary landscape heralds a new era of precision nutrition and holistic wellness (Al-Sheraji et al., 2013).

Probiotics and Prebiotics for Specific Populations

Probiotics during Pregnancy and Infancy

As probiotics have been shown to have many beneficial effects on health, their consumption, and widespread acceptance have increased throughout the world. Probiotics have been demonstrated to promote the well-being of the gut and GI system during pregnancy and in non-pregnant people, aiding alleviate or avoid conditions like necrotizing enterocolitis, irritable bowel syndrome, diarrhea associated with *Clostridium difficile*, and abdominal discomfort and bloating (Ritchie and Romanuk, 2012).

Probiotics may also protect against depressive symptoms following childbirth, mastitis, bowel movements, gestational diabetes (GD), and the proliferation of Group B *Streptococcus* bacteria during pregnancy. Several probiotic products can modify vaginal and breastmilk bacterial composition, subsequently, they are being employed to avoid the reappearance of bacteria-related vaginosis while preserving the gut health of newborns by regulating the overall composition of their gut microbiota (Sheyholislami and Connor, 2021). The administration of probiotic strains during pregnancy has also been suggested as an effective way of enhancing immunological development in the baby, thus preventing the probability of immune deficiencies and strengthening the immunity of the host (Sanz, 2011).

Probiotics in Athletes and Physically Active Individuals

Dietary intake is essential for athletes since it contributes to training, performance, and post-exercise recuperation. An adult with a healthy gut is distinguished by a significant amount of bacterial abundance. Gut microbiome populations exhibiting a significant level of diversity in microbial communities have been demonstrated to confer many positive health outcomes in adults. *Bacteroidetes* and *Firmicutes* bacteria dominate the gut microbiome, followed by *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*. The gut microbiome is also composed of a fungal community, comprising species of *Candida* and *Saccharomyces*, viruses (mainly bacteriophages), as well as members of the archaeal domain (Wosinska et al., 2019). The microbes in the gut can impose benefits utilizing metabolites such as SCFAs and neurotransmitters, which may modify the tissues of the mucosal membrane locally or reach the circulatory system and affect extra-intestinal tissues. Exercise promotes cardiovascular health, strength of muscles, and metabolism of glucose, immune system performance, and mental well-being. In comparison with lethargic individuals, athletes, and other physically active people have shown greater fecal diversity of microorganisms and more health-associated bacterial genera, including *Akkermansia*, *Veillonella*, and *Prevotella* (Clarke et al., 2014; Petersen et al., 2017).

The prominent beneficial effects of probiotics on gut health and immune system activity could benefit athletes with endurance training, who work out and compete at high intensities and frequently confront physiological issues associated with GI and immunological health before and following competition. Thus, probiotic food supplements could potentially boost the performance of athletes by improving the amount of healthy competition and training days as well as enhancing stamina (Marttinen et al., 2020). The International Society of Sports Nutrition (ISSN) recently published a policy declaration on probiotics, which indicates that probiotics exhibit strain-specific impacts on athletes (Jäger et al., 2019).

Probiotics and their Efficacy in Improving Oral Health

The oral cavity is an intricate environment containing diverse microbes. There is an abundance of metabolic activity occurring in the mouth cavity. Probiotics participate in both direct and indirect activities. The benefits of direct encounters are numerous. Probiotics promote greater oral hygiene. Probiotics aid the binding of microbes in the mouth with proteins and produce biofilms. They combat the formation of plaque and its intricate ecosystem by disrupting and interfering with the adhesion of bacterial cells. Probiotics generate substances that suppress oral pathogenic microorganisms, which harm oral hygiene (El-Nezami et al., 2006). Probiotics' indirect interactions, on the contrary, are beneficial for the process of eliminating hazardous microbes while maintaining normal conditions. Probiotics modify and regulate immune system activity on both local and non-immunologic defense routes. Probiotics have the potential to modulate porosity and generate communities in the microbiota of the oral cavity with less harmful species (Reddy et al., 2011).

Probiotics and Dental Caries

Numerous studies investigated the influence of probiotics on dental caries using various test strains. Lactobacillus rhamnosus GG and *L. casei* have demonstrated the ability to hinder the development of these oral *streptococci*. Definite drop in *S. mutans* counts following 2-week ingestion of yogurt containing *L. reuteri* (Burton et al., 2005).

Probiotics and Periodontal Disease

Periodontics, previously thought of as a sensitive very tiny subject matter, has undergone a phase of transformation in which it investigates and analyzes the body's functions at the biomolecular stage. Changing the fundamentals of life is a prudent and precise technique to address faults. There were substantial changes in methods of therapy from indiscriminate to more specialized approaches. Currently, therapy methods involve modifying the ecological makeup of niches to convert pathogenic plaques to a biofilm of commensalisms. Probiotics are live microbes that, when provided in suitable proportions, have an advantageous impact on the well-being of the recipient. Probiotic nano soldiers are microorganisms that serve a substantial part in minimizing, adjusting, or postponing dental conditions (Chatterjee et al., 2011).

The research conducted on *Streptococcus oralis* and *Streptococcus uberis*, beneficial bacterial species, appears to be useful in inhibiting the spread of bacteria that cause diseases. The mere existence of *S. oralis* and *S. uberis* has been demonstrated to be a reliable indicator of healthy gingiva. Grudianov et al reported that in comparison with Tantum Verde mouthwash, particular strains of *L. reuteri* significantly decreased gingivitis and plaque, as well as lowered the number of *S. mutans* (Burton et al., 2005; Çaglar et al., 2005).

Impact of Gut Health on Autoimmune Diseases

Autoimmune disorders, including systemic lupus erythematosus, autoimmune hepatitis, rheumatoid arthritis, and systemic sclerosis are progressive and possibly fatal inflammatory conditions. The gut microbiome can play a crucial role in initiating and promoting autoimmune responses, alongside environmental and physical factors. In vulnerable people, dysbiosis of the gut, mouth, and skin microbiome has been related to auto-inflammation and tissue damage. Thus, alterations in the human microbiome may be a substantial attributing element in autoimmune diseases, because modifications to the composition of bacteria can trigger inflammation and decrease immunological tolerance.

The diversity and stability of the intestinal microbiome not only assist in absorbing nutrients but also direct the mucosal immune system; therefore, an imbalance can result in different ADs. Probiotics, which are live bacteria, have favorable effects on the host and, when given in sufficient quantities, may successfully avoid or manage immune-triggered disorders (Khan and Wang, 2020). Animal models and clinical experiments have shown that probiotics are useful in a variety of autoimmune diseases (Zamani et al., 2016).

Impact of Gut Microbiome on Mood Disorders

Disorders such as anxiety and depression impact approximately 10% of the worldwide population annually. Research suggests that gut microbial communities, which consist of trillions of bacteria, viruses, archaea, and fungi, communicate with the host's neurological system. This biochemical communication network, the gut-brain axis, influences cognitive abilities and moods through neuronal, metabolic processes, hormonal, and immune-driven pathways. The microbiota in the gut is a significant regulator inside the gut-brain axis: different kinds of bacteria influence the generation of neurotransmitters and their precursors (e.g., serotonin, GABA, tryptophan). They can express and stimulate crucial metabolites and proteins that contribute to neuropeptides and gut hormone manufacturing, like shorter-chain fatty acids (SCFAs; e.g., *Faecalibacterium prausnitzii* and *Clostridium leptum*) and brain-derived neurotrophic factor. Recent research has examined the gut microbiota's impact on anxiety and depression, as GI bacteria play a role in bidirectional communication between the gut and brain (Simpson et al., 2021; Venegas et al., 2019).

Modulating the MGB axis, vital in central nervous system functioning, could improve mental well-being. Probiotics (living, helpful bacteria), prebiotics (nondigestible dietary compounds), and synbiotics (probiotics and prebiotics together) have all been employed to modify the intestinal microbiome composition. Research on animals has demonstrated that probiotic products, particularly those containing *Bifidobacterium* and *Lactobacillus*, can improve anxiety and depression symptoms. Chronic therapy with *Bifidobacterium infantis* alleviated immunological changes, depressive-like conduct, and recovered noradrenaline levels in the brainstem in a model of early-life stress (Malan-Muller et al., 2018).

Probiotics in Dietetics Practice

The worldwide population has grown more cognizant of the association underlying health and food consumption. This prompted more investigation into identifying food and nutrition components with particular consumer advantages. With these attempts, probiotic supplements have emerged in marketplaces and have been recognized as health-promoting foods. Such foods comprise phytochemicals, dietary fiber, structural lipids, bioactive peptides, polyunsaturated fatty acids, etc. Probiotic organisms need an approach to reaching their intended site where they act in an active form, such as the human digestive tract. The means of transport is typically a dietary product containing these live microorganisms. The scientific literature indicates that ingesting probiotic organisms at a level of 10^9-10^{11} cfu/day may minimize the frequency and extent of various digestive disorders (Zubillaga et al., 2001).

Currently, nearly all different probiotic-rich foods are dairy-based goods such as yogurt, fermented milk, and cheese. Cheddar cheese has been demonstrated to be a great medium for the administration of several probiotic bacteria, particularly *L. paracasei*. Aside from milk-based probiotic items, efforts are underway to produce non-milk probiotic goods, particularly for the management of intolerance to lactose and cholesterol control, which is a limitation of milk-based products. Probiotics have demonstrated their efficacy in combating certain illnesses as well as improving different aspects of health. They assist in alleviating nutritional deficiency, intolerance to lactose, digestion of calcium, and congestion (Agrawal, 2005).

Current Trends, Functionalities, and Prospects of Probiotics

Recent breakthroughs in the comprehension of the microbiome in the gut and its relationship to the host have shown that gut commensals serve crucial functions in the well-being of humans. The term "probiotic" refers to an organism that has health-promoting features; therefore, numerous studies are now discovering and presenting members of the gut microbiome as well as commensals as next-generation probiotics. Lactic acid bacteria (LAB), which include *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus*, *Enterococcus*, and *Bifidobacterium*, are among the most frequently used probiotic organisms in dietary supplements and foods. *Bifidobacterium* is not categorized as LAB, but it represents an essential probiotic bacterium that performs carbohydrate fermentation, which generates lactic acid, thus few microbiologists classify it as LAB.

Research indicates that non-LAB bacteria may also function as valuable probiotics. This category contains microorganisms like *Clostridium butyricum*, a spore-forming organism, as well as some commensal organisms which include *Akkermansia*, *Faecalibacterium*, *Bacteroidetes*, and even a specific strain of *Clostridium*, *Bacillus*, *Escherichia coli*, and *E. coli* Nissle. *Akkermansia*, *Faecalibacterium*, and *Bacteroides* are examples of “next-generation probiotics” (NGP), which are naturally present in the gut microbiota. NGP has been researched in vivo for the medical management of inflammatory, chronic, and metabolic syndromes. These microorganisms successfully modulate the immune system, minimize mucosal inflammation, and avoid colon cancer and inflammatory bowel disease. Yet, there are considerable challenges with viable intestine administration since they are oxygen-sensitive and require new growth and preservation techniques.

Additionally, the requirement for effective GI delivery of probiotics spurred researchers to take into account utilizing *Bacillus* spp. and *Clostridium* spp. as naturally encapsulated members of the intestinal microbiome due to the production of spores. The spores are extremely resistant to acidity, heat, and chemical substances, so they may thrive in adverse settings such as digestive fluids and high-temperature processing in bakery items. They also contribute to the well-being of humans by regulating the body's immune system and releasing valuable substances (Jafari et al., 2023). Microorganisms in the human microbiome form symbiotic relationships with both their hosts and one another. Even so, it has been now generally proposed that probiotics should be employed alongside a diversity of indigenous gut microbiota species. Probiotic products can thus be generated from various kinds of bacteria, notably commensal microbes, which is one of the most cutting-edge approaches to this product category.

There is currently an organized attempt across Europe to investigate any gaps in understanding regarding the therapeutic efficacy and molecular characteristics of microbiota employing probiotics, prebiotics, and synbiotics. All of these investigations have boosted human health and lowered disease prevalence (Mattila-Sandholm et al., 2002). If probiotics are to be a practical and efficient substitute for antimicrobial agents and chemotherapy treatments, additional study is needed to discover LAB strains with high probiotic effects, as well as strategies to ensure optimal effectiveness of probiotics during ingestion. Antibiotic resistance is a significant global public health issue. Therefore, natural alternatives are growing increasingly enticing (Agrawal, 2005).

Probiotics have been successfully shown in clinical investigations to suppress pathogens, suggesting their effectiveness in reducing the spread of enteropathogens (Drago et al., 1997; Hudault et al., 1997). Furthermore, the findings obtained highlight the possible benefits of probiotics in decreasing bacterial infections of the urogenital tract (Reid et al., 1998). There is a requirement to examine the practical application of probiotics as substitutes for antibiotics in livestock and poultry. Probiotics are currently in high demand due to widespread health issues resulting from viral diseases such as MERS and COVID-19, a growing older population, and easy accessibility to health knowledge.

Next-generation probiotics have emerged for both overall health and specific intestinal functions. In the future, tailored probiotics will be developed by assembling various intestinal microbiota compositions from each individual. Microbiome investigations can be undertaken in conjunction with biological technologies such as computational biology and biomaterial engineering. Furthermore, multi-omics network evaluation can be employed to investigate the functional interactions of gut microbes in a given environment and create a database (Jeon et al., 2022). Therefore, research concerning probiotic benefits employing diverse biotechnological advances will be enhanced to assist individuals in strengthening their health.

Conclusion

Probiotics are one of the most significant beneficial nutrients. They represent over 65% of the worldwide nutritious food category. The scientific knowledge of prebiotics as well as probiotics continues to advance. Contemporary worldwide study endeavors have led to significant improvements in our knowledge regarding the importance of GI commensals in their distinctive symbiotic relationship with humans. Probiotics are intended to deliver further benefits that will compensate for, replace, or supplement the intestinal microbiota, thus impacting the host's health directly or indirectly through "cross-talk" with the microbes in the gut and/or the host. Prebiotics have the purpose of enhancing the endogenous microbiota by specifically triggering the groups considered to be necessary for eubiosis.

The significance of the intestinal microbiota in both wellness and illness has grown more apparent and there is an expanding amount of research on the therapeutic value of probiotics and prebiotics in digestive tract conditions, in particular for the management of infectious GI (GI) in children, and in minimizing adverse reactions of antibiotic therapy for *H. pylori*. Among the most persuasive instances demonstrating the significance of the microbes in the gut comes from innovative research and studies in patients with antibiotic-resistant *Clostridium difficile* infection, where transplantation of fecal microbiota is quite successful in eliminating illness and its associated signs and symptoms. More studies into gut microbes undoubtedly bring about an improved knowledge of the effects of prebiotics and probiotics on human beings.

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Chapter 31

Benefits of using Yeast *Saccharomyces cerevisiae* as a Probiotic for the Growth of Nile Tilapia

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ABSTRACT

Global aquaculture production increased by more than 5% between 2001 and 2018. The aquaculture industry's primary goals are to improve the growth or survival performance, feed efficiency, and resilience of aquatic species. Tilapia is the second most produced species in the world. The Nile tilapia grows quickly and often has great survival rates when given artificial feeding from hatching. Thus, offering a balanced diet with food additives could be a useful technique to reduce losses at this point and offer extra advantages in later stages. Probiotics are among the most widely used supplements in aquaculture. Probiotics are live microorganisms that provide health advantages to fish, including improved food consumption, modification of gut microbiota, increase of immunological responses, and pathogen antagonism. Out of the several probiotics in aquaculture, the yeast Saccharomyces cerevisiae is the most frequent single-cell protein found in fish feeds, either whole or in a hydrolyzed extract. Adding S. cerevisiae with other probiotics can have varying effects on the gut microbiota, depending on their type, quantity, and interaction, and has several benefits such as improved digestion. S. cerevisiae promotes innate immunity, making it an eco-friendly alternative to antibiotics for disease control. Juveniles, using baker's yeast S. cerevisiae as a pro-health element in the feed of farmed Oreochromis niloticus juveniles can improve growth performance, immunity, and stress tolerance.

KEYWORDS		
Saccharomyces cerevisiae, Yeast, Probiotic, Oreochromis niloticus Growth	Received: 08-Jun-2024 Revised: 12-Jul-2024 Accepted: 14-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

As compared to other agricultural food production industries, aquaculture is the one that is expanding the fastest. According to data from the State of The World Fisheries and Aquaculture (SOFIA), global aquaculture production increased by more than 5% between 2001 and 2018, reaching over 82.1 million tons of fish in the year 2018 (Agboola et al., 2021). In many nations, aquaculture has grown to be a significant economic sector (Balcazar et al., 2006). This is mostly caused by large-scale production facilities, where aquatic animals are subjected to stressful circumstances, disease problems, an insufficient nutrient balance in artificial diets, and degradation of environmental factors. It has been observed that physiological stress is one of the key causes of aquatic organism's sickness, poor growth, and death in aquaculture (El Haroun et al., 2006). Currently, the aquaculture industry's primary goals are to improve the growth or survival performance, feed efficiency, and resilience of aquatic species. These strategies will have a favorable impact on production costs (Lara-Flores et al., 2010)

Due to its advantageous qualities, including good consumer acceptance, rusticity, quick growth, and high-quality meat (Meurer et al., 2003), tilapia is the second most produced species in the world (Van Hai, 2015). Filipetto et al. (2015) state that adequate nutrition can guarantee fish growth and health maintenance (Kim et al., 2003) during the embryonic stage when they grow at faster rates (Hayashi et al., 2002). After carp, the Nile tilapia (*Oreochromis niloticus*) is the most popular variety of tilapia in aquaculture. The Nile tilapia, (*O. Niloticus*) grow quickly and often have great survival rates when given artificial feeding from hatching (El-Sayed, 2006). Thus, offering a balanced diet with food additives could be a useful technique to reduce losses at this point and offer extra advantages in later stages (Hayashi et al., 2002). The explanation for adding chemicals, sometimes referred to as functional feeds, to tilapia diets (Ribeiro et al., 2012). This helps the animal's immune response, improvement, and progress during the larvae and fattening stages, as well as the quality of the water (Iwashita et al., 2014).

Probiotics are among the most widely used supplements in aquaculture. Probiotics are live bacteria that, when provided in sufficient quantities, might benefit the host and perhaps minimize illness incidence (Reid et al., 2003). Over the last decennary, probiotics have gained great interest as a technique for improving healthiness and performance in aquaculture. Probiotics are living microorganisms that provide health advantages to the host when provided through diet or rearing water. Probiotics have a variety of advantages, including better food consumption, modification of gut microbiota, increase of immunological responses, and pathogen antagonism. Probiotics have a variety of impacts, including the production of enzymes in the digestive tract, which improves its structure and functionality. In this way, when fish consume probiotics, it not only modifies its intestinal microbial composition but also improves the immune system and as a result, the organism's health state (Bombardelli et al., 2010) and growth performance (Dharmaraj and Dhevendaran, 2010).

Yeasts and their processing products are among the most often utilized probiotics in fish nutrition due to their biosafety, ease of inclusion into diets, and beneficial effects on growth performance (Hisano et al., 2008). Yeast contains glycoproteins, β-glucan, chitin (a minor component), and nucleic acids, which have immunostimulatory capabilities (Ortuno et al., 2002). Out of several probiotics used in aquaculture, the yeast *S. cerevisiae* is the most frequent single-cell protein found in fish feeds, either whole or in a hydrolyzed extract (Nayak, 2010).

S. cerevisiae yeast is derived from the bread sector and includes various immunostimulants, including mannan-oligosaccharides, beta-glucan, chitin, and nucleic acids (Gopalakannan and Arul, 2010). This yeast is abundant in proteins, amino acids, B-complex vitamins, fatty acids, minerals, and enzymes. Studies found that supplementing animals with this yeast strain improved their growth performance (Schwarz et al., 2010) and intestinal health status (Carvalho et al., 2011). Fish species' development, immunology, and disease resistance were all enhanced when S. cerevisiae was added as a probiotic (Dawood et al., 2019). The growth, immunology, and stress resistance of different fish species were not adversely affected by the acceptance of fermented plant protein including *S. cerevisiae* by aquatic animals (Hassaan et al., 2015).

Benefits of Yeast *Saccharomyces cerevisiae*

Numerous outstanding reviews of Saccharomyces cerevisiae have been published; some of them have described the organism's morphology, physiology, and genetics (Delorme-Axford et al., 2015). As a "model organism," yeast has been the focus of research for several metabolic pathways (Wloch-Salamon et al., 2017). Yeast's potential as a source of protein in prepared foods was discussed in several editions (Mahdy et al., 2022). In other studies, Dawood and Koshio (2020) highlighted the role that yeast plays in the manufacture of fermbiotics, which serve as dietary supplements for sustainable aquafarming. Previous studies on yeast as probiotics for aquafarming have been conducted by Mahdy et al. (2022), for rainbow trout (*Oncorhynchus mykiss*) (Huyben et al., 2017), rohu (*Labeo rohita*) (Jahan et al., 2021), cichlid (*Cichlasoma Trimaculatum*), Nile Tilapia (*Oreochromis niloticus*) (Abass et al., 2018) and seabream (*Sparus Murata*) (Ortuno et al., 2002). Khanjani et al. (2022) noted that several research studies also examined yeast as a prebiotic. Nonetheless, there hasn't been a complete integration of the many studies on *S. cerevisiae* yeast used in aquaculture. Thus, the purpose of this review is to incorporate the probiotic benefit of S. cerevisiae for Nile tilapia development.

Saccharomyces Cerevisiae as a Probiotic

Probiotics have a variety of strategies to alter the gut microbiota. As per Vargas-Albores et al., (2021), probiotics can settle in the mucous membrane of the digestive system and produce helpful chemicals and chemical signals for the host, compete for adherence and exclusion, and block pathogen colonization. In this sense, the gut flora of fish and arthropods has been effectively modulated by brewer's yeast (*Saccharomyces cerevisiae*) as a probiotic, improving the host's intestinal functioning (Mahdy et al., 2022). These microbes are provided as water probiotics, which are yeast directly added to water and infect internal or outer surfaces by ingestion (drinking), or as feed (gut probiotics), which are yeast supplements that are prepared, encapsulated, and incorporated in living food. Alternatively, by removing organic debris, pollutant toxins and infectious agents *S. cerevisiae* can improve the water's quality by inhibiting the growth of dangerous bacteria through nutrient competition (Yilmaz et al., 2022).

Studies on fish recently have shown the benefits of giving *S. cerevisiae* by diet. Yeast-supplemented diets improve blood biochemistry, development, feed efficiency, chance of survival, and innate immune responses in olive flounder (*Paralichthys olivaceus*) infected with *Uronema marinum* (Harikrishnan et al., 2011).

Saccharomyces Cerevisiae as Synbiotics

Synbiotics are a combination of probiotics and prebiotics that work together to improve growth, gastrointestinal microbes, immune response, digestibility, and disease resistance in farmed fish and crustaceans. Symbiotics benefit microbial populations in the human digestive system by promoting their development and survival (Abdel-Latif et al., 2022). Synbiotics use probiotics for their positive effects on the host, while prebiotics stimulate the endurance and growth of lucrative microbes (Butt et al., 2021). Synbiotics boost the defense system by promoting the development of helpful bacteria on the mucus membrane of the digestive system while inhibiting the production of harmful germs that fight for substratum and attachment sites (Huynh et al., 2017).

Leukocytes are regarded as the defense system's greatest achievement. Research on immature Common Carp (*Cyprinus carpio*) found that oligofructan combined with *S. cerevisiae* enhanced Leukocytes. Moreover, the impact is dependent on the quantity and kind of prebiotics administered to the experimental diet (Abdulrahman and Ahmed, 2016). The combination of Mannon oligosaccharides (MOS) yeast extract and a whole yeast cell as a probiotic improves *O. niloticus's* growth performance and inherent resistance to infections (Abu-Elala et al., 2013).

Saccharomyces Cerevisiae as a Dietary Supplement

Due to the addition of *S. cerevisiae* to feed, several farmed fish and shrimp produced more and had higher feed efficiency. According to several publications, compared to non-probiotic-fed species, fish and crustaceans-fed probiotics exhibited superior growth performance, physiological state, and immunological indices. According to Rohani et al. (2022), dietary yeast produces polyamines that aid in the growth of the fish hatchlings' intestinal lining. Prior to their first feeding, Padeniya et al. (2022) found that the probiotic improves the larvae's nutrient assimilation of yolk. These advancements might boost the catalytic activity of the enzymes, which would subsequently improve the condition of the fish in response to probiotics. Additionally, the fry tilapia (*O. niloticus*) treated with yeast as nutritional supplements showed the maximum feed consumption, indicating that the nutrients were utilized more effectively (Lara-Flores et al., 2003). Similarly, dietary yeast usage may increase the activities of digestive enzymes in crustaceans, improving their nutrition, health, and digestion (Zhao et al., 2017).

***Saccharomyces cerevisiae* Improves Digestion**

In additional studies, Jahan et al. (2021) provided proof that the intestinal quantitative analysis such as villi breadth, villi length, villi wall thickness, villi breadth, and crypt depth of farmed fish was enhanced by dietary brewer's yeast. These intestinal measurements are thought to be crucial indicators of a healthy gut, indicating that *S. cerevisiae* may be able to enhance nutritional absorption, digestion, and assimilation by modifying the morphometry of the gut. Additionally, the authors suggested probiotic yeast *S. cerevisiae* for fish after improving the microbiota in intestines (yeast count, lactic acid bacteria, and total viability) (Jahan et al., 2021). As digestive enzymes are vital to the process of breaking down essential nutrients, the communities of microbes in fish guts play a crucial role in maintaining their availability (Rohani et al., 2022).

It has been shown that including *S. cerevisiae* in the diet can help accelerate the development of the digestive system and enhance food digestibility by activating digestive enzymes and enhancing intestinal mucosa integrity and villi density (Dimitroglou et al., 2008).

***Saccharomyces cerevisiae* is a Disease Resistant**

Furthermore, it was suggested that probiotics provide health advantages to fish raised in culture. Probiotics can, therefore, improve an individual's ability to resist illness or produce compounds that stop harmful organisms from spreading disease. These substances can prevent the growth of harmful bacteria in fish culture's gut and on their exterior surfaces. In this way, feeding fish and crabs supplements containing *S. cerevisiae* has an impact on their immune systems (Mahdy et al., 2022). Yeast supply in the diet has been shown to have a significant impact on immune system cells, including monocytes, phagocytic cells, leukocytes, macrophages, polymorphonuclear and natural killer (NK) cells, which improve immunity. Additionally, probiotics stimulate fish production of immunoglobulins and the growth of B and T lymphocytes. The reason for this might be that yeast's cell wall contains β-glucan, which attaches itself to receptors on phagocytic cells. These receptors aid in the release of signal molecules that, in turn, boost white blood cell production, improve immunological function, and increase resistance to illness (Rohani et al., 2022).

***Saccharomyces cerevisiae* as an Immunostimulant**

In many fish species, chitin cell wall (poly (1-4)-β-N-acetyl-D-glucosamine) is a highly effective immunostimulant of *S. cerevisiae* that initiates the non-specific cellular and antibody-mediated immunity of fish to fight infections (Abu-Elala et al., 2013). The high degree of chitin stability makes it a more favorable candidate for dietary integration than other compounds that are more soluble and susceptible to variations in the physical parameters as well as chemical parameters of the growth medium (Esteban et al., 2001). The immune system's masterpiece is regarded as white blood cells. Research on juvenile *Cyprinus carpio* (Common Carp) shows that the combination of fructooligosaccharides and *S. cerevisiae* boosted white blood cell counts (Abdulrahman and Ahmed, 2016).

Challenge with *Yersinia ruckeri*, in juvenile Rainbow Trout, a supplemented meal with *S. cerevisiae* treated with beta-mercaptoethanol was superior to n-3 highly unsaturated fatty acid and whole cell yeast enriched yeast as a growth booster and immune system (Tukmechi et al., 2011).

***Saccharomyces cerevisiae* as a Stress-free Agent**

Multiple REDOX (Oxidation-Reduction) mechanism-related enzymes are expressed by *S. cerevisiae*. As glutathione transferases, glutaredoxins, thioredoxins, or glutathione peroxidases safeguard various cell macromolecules primarily proteins from oxidative damage. They are therefore essential for maintaining cell functioning and signaling (Herrero et al., 2008).

***Saccharomyces cerevisiae* as a Growth Promoter**

S. cerevisiae, popularly referred to as baker's yeast, is thought to promote growth and has the potential to be employed as an immunostimulant. Bread yeast is rich in fat, protein, vitamins, and minerals, among other nutrients (Babo et al., 2013). Furthermore, this baker's yeast (*S. cerevisiae*) can create recombinant proteins and biochemical compounds of single-cell proteins (Darafsh et al., 2020). This will result in a favorable reaction to the absorption of nutrients, which will boost catfish growth. Probiotics are known to accelerate development and regenerate the tissue that is already present in the gut, which is the digestive tract (Gatesoupe, 2007). Baker's yeast can stimulate fish appetite because it includes nucleotides that take the form of purines and pyrimidines. Fish may benefit from this. One probiotic that can boost the immune system, provide an enzyme for food digestion, and improve feed digestibility is bread yeast. Fish meal can be substituted with Baker's yeast in the feed mix (El-Boshy et al., 2010).

Numerous studies have demonstrated that adding baker's yeast (*S. cerevisiae*) to fish feed can boost the immune system and accelerate fish growth. Numerous studies demonstrate that adding bread yeast to feed up to 5-10 g/kg can accelerate the development of a variety of fish species. Furthermore, adding probiotic mannan Oligosaccharides and baker's yeast to the meal had a positive effect on the development of the catfish. Pomfret grows at a faster pace the more baker's yeast it receives (Djauhari and Monalisa, 2019).

Conclusion

This revision integrates existing knowledge on the advantages of *S. cerevisiae* as a probiotic and opens new avenues for further research. Research in fish and crustaceans has shown that *Saccharomyces cerevisiae* and its components enhance development, digestive system, anatomy, and immunological responses, resulting in better survival rates and health management. However, its application at a wide-scale aquaculture is still emerging. More research to determine the optimal dosage and frequency of using yeast saccharomyces cerevisiae as probiotics in various fish and crustacean species is needed. Only a few studies have explored the possible advantages of combining *S. cerevisiae* with other probiotics (mostly bacteria). When adding *S. cerevisiae* as a probiotic to food, it's important to examine the method (encapsulated pellets, dry or in water) and its proportion (% of dried or wet food) as the effects may differ according to surrounding circumstances, the species, eating habits, reproductive status, sex, age, and developmental stage as these variables can alter the gut flora. Adding *S. cerevisiae* in combination with other probiotics can have varying effects on the gut microbiota, depending on their type, quantity, and interaction. *S. cerevisiae* promotes innate immunity, making it an eco-friendly alternative to antibiotics for disease control. Further study is required to determine the effectiveness of yeast in preventing and treating various infections in crustaceans and fish. This review suggests that using baker's yeast *S. cerevisiae* as a pro-health element in the diets of farmed *Oreochromis niloticus* juveniles can improve growth performance, immunity, and stress tolerance.

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Chapter 32

Role of Prebiotics in Early Childhood Development

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ABSTRACT

A relatively new field of research is the relationship between colorectal cancer (CRC) and the gut microbiota. This is a novel approach: using gut microbiota modification to reverse microbial dysbiosis and possibly prevent or treat colorectal cancer. In this context, a number of approaches have been used, such as the use of probiotics, prebiotics, postbiotics, antibiotics, and fecal microbiota transplantation (FMT). It is crucial to recognize the risks and controversies associated with these strategies, which may cause clinical complications, despite their promising results in improving gut barrier function, correcting microbiota composition, modulating the natural immune system, avoiding pathogen colonization, and exerting specific cytotoxicity against tumor cells. Therefore, a thorough assessment of the risk-benefit ratio and patient selection is essential for the translation of bench to bedside research. The creation of tailored microbiome therapy is essential for effective clinical treatment because it takes into account the unique response that every person has to gut microbiota intervention.

KEYWORDS		
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INTRODUCTION

An additional 1.8 million cases and 881,000 deaths from colorectal cancer (CRC) were reported globally in 2018, making it the second most prevalent cause of death from cancer and the third most frequently diagnosed malignancy. There is still an increasing tendency in CRC incidence and mortality rates in many developing countries, despite the fact that colonoscopy screening and treatment breakthroughs have reduced the disease's incidence and mortality in highly industrialized nations. A complex interplay between genetic, epigenetic, and environmental variables leads to CRC. The most important environmental factors that have been found to contribute to the onset and progression of colorectal cancer (CRC) are food carcinogen ingestion, physical inactivity, and cigarette smoking (Sestito et al., 2020).

The study of the gut microbiome has become essential to understanding how the environment affects colorectal cancer. The gut microbiota is a diverse collection of bacteria, fungus, viruses, and protozoa that makes up about 100 trillion microbial cells. The development of high throughput microbiome sequencing technology has made it feasible to thoroughly profile the microbial makeup without the need for techniques that depend on culture. This discovery makes it possible for researchers to connect the host physiology, metabolism, immunity, and gut microbiome to the development of cancer (Paiva et al., 2020).

Frequently called the "forgotten organ," preserving the equilibrium of the gut microbiota is essential to the host's general well-being.A growing body of studies in recent years has suggested a relationship between gut dysbiosis of microorganisms and colorectal cancer (CRC) (Manzoor et al., 2022). Enrichment of specific bacterial species in the gut, such as enterotoxigenic *Bacteroides fragilis, Peptostreptococcus anaerobius,* and *Fusobacterium nucleatum*, has been shown to promote tumor growth, induce inflammation, damage DNA, and evade immune responses, all of which are associated with colorectal carcinogenesis (Pujari and Banerjee, 2021).

In contrast, people with colorectal cancer have been reported to have a decrease in numerous bacteria, primarily probiotics *like Bifidobacterium animalis, Streptococcus thermophilus, and Lachnospiraceae* species (Fiocchi et al., 2022).

These microorganisms are thought to provide protection against colorectal cancer. The important influence of commensal microorganisms on cancer patients' prognosis has also been demonstrated by recent studies. Shorter survival has been linked in large-cohort patient studies to the prevalence of the well-known pro-tumorigenic gut bacterium Fusobacterium nucleatum (Ke et al., 2021). Its function in triggering autophagy, which eventually results in treatment failure or disease recurrence, has been identified through functional investigations involving CRC patients(D'Argenio and Sarnataro, 2021).

As the understanding of the gut microbiome's role in carcinogenesis and treatment outcomes grows, restoring homeostasis through gastrointestinal microbiota modification has emerged as a promising technique for treating and preventing colorectal cancer. In this review, we will discuss various strategies to gut microbiota modulation, including proposed mechanisms of action and utilization of probiotics, prebiotics, postbiotics, antibacterial agents, and fecal microbiota transplantation (FMT) (Franco-Robles et al., 2020).

In addition, even though these techniques are thought to have high safety profiles, we discuss the inherent dangers and controversy surrounding them. Lastly, we go over the most recent developments in transferring these tactics from the lab to clinical settings as well as their potential therapeutic applications in the treatment of colorectal cancer.

Strategies of Gut Microbiota Modulation

Probiotics

Probiotics are live microorganisms that, when administered in appropriate quantities, benefit their host's health. Probiotics were initially proposed by Nobel winner Élie Metchnikoff in the early twentieth century (Jiang et al., 2020).

They were defined as substances that alter the gut microflora's composition by substituting beneficial germs for "putrefactive" bacteria. Probiotics have the capacity to alter the host's physiology and metabolism in addition to modulating the microbiota, as our knowledge of them has grown (Bodke and Jogdand, 2022).

Colonization Resistance against Pathogenic Bacteria

Probiotics are thought to colonize the host tissue and inhibit the growth of harmful bacteria, restoring microbial dysbiosis and maintaining a balanced intestinal microbiota. Probiotics have been proven in several studies to decrease the colonization of pathogens including Staphylococcus aureus and Clostridium difficile, hence supporting their use in the prevention of intestinal infections. Colonization resistance is attained by probiotics and other commensal microbiota through aggregating to prevent pathogen colonization, competing for resources, or sticking to the surface of mucus or epithelial cells (Sivamaruthi et al., 2020).

Probiotics limit pathogen growth by generating metabolites like lactic and acetic acid or bacteriocins, which have antibacterial activity and lower the luminal pH, in addition to their direct interactions (Eslami et al., 2020).

Fengycins, a fungicide lipopeptide produced by the probiotic species of Bacillus, have been found in a recent study to effectively decolonize *Staphylococcus aureus* by disrupting the bacterial communication mechanism known as quorum sensing.

Consuming probiotics helps lower the likelihood of intestinal infections and the inflammation that follows, which may stop the development of colorectal cancer (CRC) and lessen problems in those who have already been diagnosed with the disease. This is achieved by preventing pathogenic invasion (Tegegne and Kebede, 2022).

Immunomodulation of Mucosa

Probiotics have an immunomodulatory impact on the gut and can reduce colonic inflammation or improve immunity surveillance, depending on the strain's specific actions. Certain probiotic strains of *Bifidobacterium infantis and Bifidobacterium breve*, by binding to toll-like receptors (TLRs) and triggering retinoid acid metabolism, can activate intestinal dendritic cells (DCs), induce the expression of Foxp3+ regulatory T cells (Treg) and type 1 regulatory T cells (Tr1), and release IL-10 (Su et al., 2020).

Lactobacillus rhamnosus GG and *Lactobacillus acidophilus* are two probiotic bacteria that reduce Th17 cell production and IL23 and IL17 secretion by blocking STAT3 and NF-kB activation. Additionally, they induce a change in macrophage phenotype from immunosuppressive M2 to pro-inflammatory M1 (Johnson et al., 2021).

On the other hand, probiotics can also have an apparently paradoxical effect by making inflammation worse. Both the elimination of pathogenic organisms and the enhancement of vaccine response have been linked to probiotic-mediated immune response activation (Saturio et al., 2021).

It involves a rise in natural killer cell activity and phagocytosis capacity. On the other hand, its potential role in enhancing antitumor immunity has received more attention lately. Lactobacillus casei BL23, a pro-inflammatory probiotic strain, has been demonstrated to exhibit beneficial effects against cancer in CRC mice models treated with dimethylhydrazine (DMH) (Navarro-Tapia et al., 2020).

When pro-inflammatory cytokines (MCP-1, TNF-a) are downregulated in intestinal content and IL-10 is overexpressed, a Th17/Treg mixed-type immune system reaction develops.

Splenocyte research showed a decrease in Treg levels and an increase in Th17 population across the body. These findings indicate that the modulation of anticancer immunity is complex, possibly via the IL-2 communication route. Another probiotic strain, Lactobacillus acidophilus NCFM, has been found to inhibit tumor formation in animal models with CT-26 implants. The anticancer impact is thought to be caused by the downregulation of MHC class I in tumor cells, which activates T cells to identify them and harm, as well as the decreased expression of CXCR4, which is associated with the growth of micro metastases (Anee et al., 2021).

Notably, a lot of work has recently been done to identify the specific cell-surface components—Lipoteichoic acid, exopolysaccharides, and S-layer proteins—that are responsible for the immunomodulatory effect. Probiotics can be genetically modified or protein deleted to have an anti-inflammatory profile rather than a pro-inflammatory one. For example, elimination of the immunostimulatory protein lipoteichoic acid in Lactobacillus acidophilus reduces the expression of inflammatory substances, lowering colonic inflammation and CRC polyposis (Ding et al., 2020).

In light of these findings, a novel strategy to accomplish the desired immunomodulatory impact has been suggested, probiotic engineering.

Improving the Purpose of the Intestinal Barrier

In CRC, increased tight junction permeability or gut barrier dysfunction have frequently been noted. Endotoxemia and microbial translocation, both of which are aided by a leaky gut, lead to cachexia. The decrease of tight junction protein in colorectal cancer (CRC) is also linked to increased metastasis and epithelial-mesenchymal transition (EMT). Probiotic strains that have been shown to increase gut barrier function include *Lactobacillus rhamnosus, Lactobacillus plantarum,* and *Escherichia coli*. These strains accomplish this by increasing or normalizing the expression of tight junction proteins (claudin-1, occludin, ZO-1, and ZO-2), stimulating mucin synthesis, lowering inflammation, and facilitating epithelial restoration. Probiotics have the potential to benefit patients with colorectal cancer by restoring epithelial integrity (Alam et al., 2022).

Utilization of Probiotics in Medical Situations?

Probiotics are typically considered to be harmless and tolerated in healthy people; nevertheless, the safety of probiotics has been called into doubt in patients with underlying medical disorders. One of the most serious concerns is probiotic translocation, which is the introduction of live microbes into extraintestinal regions, resulting in systemic or regional illnesses (Pino et al., 2020).

Although healthy people can also experience bacterial translocation, in these situations the germs are usually eradicated and confined in the mesenteric lymph nodes beneath the shield of a functioning immune system, thus there is no adverse effect. Individuals who share clinical features with cancer patients, such as compromised immunity or a compromised intestinal barrier, might not be able to survive this physiological defense, leaving them vulnerable.

It is yet unknown if taking probiotics raises the risk of contracting infectious issues, but multiple meta-analyses of cancer patients have demonstrated that the frequency of these potentially lethal side effects is rare. Although there is insufficient data to fully justify a probiotic ban for people with cancer, additional clinical research are needed to evaluate the therapeutic effects of probiotics and assess the benefits and drawbacks for persons who are more susceptible to infections (Tripathy et al., 2021).

Transmission of Resistant Genes

Another conceivable worry related to prolonged use of probiotics is the possibility of horizontal gene transfer (HGT) transferring genes that are resistant to antibiotics. Bacteria can acquire resistance genes and improve their ability to withstand selection pressure through horizontal gene transfer (HGT) between species (such as that generated by antibiotic therapy). Interestingly, because the human gastrointestinal tract is a densely populated niche, it is thought to represent a substantial reservoir for the transmission of resistant to antibiotics characteristics to bacteria that grow nearby.

For instance, metagenomic research showed that the use of antibiotics exacerbates a common group of tetracycline-resistant genes (TcR) in the gut microbiota. This discovery implies that the gut microbiota is where HGT takes place (Wong and Yu, 2023).

Probiotics, on the other hand, are supposed to provide a variety of health advantages; yet little research has been conducted on resistance to antibiotics in bacteria that are not pathogenic due to their uncertain therapeutic usefulness.

When examining the genes causing antibiotic resistance in probiotics, it is important to distinguish between innate and acquired resistance).Furthermore, the latter category should be separated into non-transmissible resistance (such as spontaneous mutations on chromosome genes) and transmissible resistance (such as resistant gene sequences found on plasmids or transposons that are easily propagated via HGT) (Sánchez-Alcoholado et al., 2020).

There are further concerns about the final resistance type in probiotic-mediated transfer of genes. Antibiotic-resistant genes have been found in the mobile genetic components that make up probiotic strains. Lactobacillus plantarum, Lactococcus lactis, and Streptococcus thermophilus include the drfA gene, which encodes trimethoprim and vancomycin resistance.

One interesting example of a widely dispersed resistance gene is tet (W), which is chromosomally positioned but may still be transferable due to a flanking nucleic acid that sits between its targeting and transposase-encoding sequences (Lamichhane et al., 2020).

Preclinical research has indicated that mobile genetic components termed plasmids or transposons can transfer resistance genes from probiotics to pathogenic bacteria prevalent in the gut microbiome.

It has been demonstrated that the two generally known transferable genes, ermB and tetM, transmit from probiotics like Lactobacillus or Streptococcus to possible pathogens like Listeria monocytogenes and Enterococcus faecalis, adding novel resistance components to these harmful bacteria. Tetracycline and macrolide resistance are encoded by these genes, respectively (Kvakova et al., 2022).

Probiotics

According to Gibson and Roberfroid's 1995 definition, prebiotics are indigestible food components that promote the proliferation and/or activity of particular bacteria in the gut, hence improving the well-being of the host. However, recent research has revealed that prebiotics have far more applications, leading to the term "the substrate that is specifically used by host microbes conveying a health advantage" being altered in a 2017 expert consensus report. The term "indigestible food ingredients" refers only to traditional prebiotics based on fiber and carbohydrates; however, in the last ten years, it has been established that other compounds, such as polyunsaturated fatty acids (PUFAs) and polyphenols, may also have prebiotic capability (Wu and Lui, 2022).

Changes in the Makeup of the Gut Microbiota

Prebiotics were first discovered by evaluating their stimulation on different probiotics, which at the time were restricted to Lactobacillus and Bifidobacterium species, using culture-based models. Nonetheless, the range has greatly expanded as a result of recent advancements in high-throughput sequencing technology. Numerous clinical studies have indicated an increase in the predominance of different potential probiotics, such as *Faecalibacterium, Akkermansia, Ruminococcus,* and *Rosebura* species, following prebiotic administration.

The gut's selective enrichment of probiotics has been related to immune response modulation and pathogen protection, as was previously indicated. Prebiotic treatment has been demonstrated in multiple human investigations to reduce inflammatory response in persons with chronic intestinal inflammation and to minimize pathogen colonization (Kaźmierczak-Siedlecka et al., 2020).

Generation of Metabolites from Fermentation

Colonic probiotics degrade prebiotics to create short-chain fatty acids (SCFAs) such butyrate, propionate, and acetate. The liver and muscle convert propionate and acetate into gluconeogenesis and energy, respectively. Butyrate is mainly consumed up by colonocytes and serves as the principal power substrate.

Butyrate, an inhibitor of histone deacetylase, may help colorectal cancer patients by promoting apoptosis, reducing inflammation, controlling stress caused by oxidative damage, and improving epithelial barrier integrity. New research reveals that propionate and acetate may lower inflammation in the colon and protect against intestinal disease, although their effect on CRC and intestinal inflammation is unknown (Shamekhi et al., 2020).

Prebiotics' Immediate Impact

Prebiotics can directly affect the gut in addition to beginning fermentation and increasing probiotic development. Research has focused on the antiadhesive properties of materials to combat infections. To prevent pathogen colonization, prebiotic oligosaccharides could communicate with bacterial receptors that imitate glycoconjugates present in microvillus.

Prebiotics have been shown to directly impact gene expression in intestinal cells. A study found that only prebiotics with low DP can increase IFN-γ and IL-10 production in CD4+ T cells. This suggests that the prebiotic is immediately absorbed into the intestine and then adjusts the digestive reaction. However, would these mechanisms benefit every subject taking prebiotics in the same way? Right now, it seems like the response is no. The latest study has revealed a highly complicated phenomenon: prebiotic medicines can help different people differently and, more astonishingly, may sometimes harm the host (Shrifteylik et al., 2023).

Variability between Individuals in the Host Reaction

Belcheva et al.'s groundbreaking discovery sparked worries about the potential drawbacks of butyrate and prebiotic supplements. The study's APCMin/+; Msh2-/Mice were given a low-carbohydrate diet or wide-spectrum antibacteria; the development of polyps in the little colon and intestine were seen to be decreased in both treatment groups. Based on later 16S rRNA sequencing, the butyrate-producing bacteria that have become less frequent are *Ruminococcaceae, Lachnospiraceae,* and *Clostridiaceae*. The liquid chromatography-tandem mass spectrometry (LC/MS/MS) testing revealed a consistent and considerable decrease in butyrate production.

Taken together, these findings appear to suggest that butyrate generated from microorganisms is an oncogenic metabolite, and that reducing it inhibits the growth of tumors. As a validation tool, butyrate was given to the antibiotic-treated APCMin/+; Msh2-/- mice. Notable is the fact that butyrate treatment has led to an increase in epithelial cell production, the development of polyps, and finally the establishment of tumors. These results seem to contradict the results of numerous other analyses. But it is crucial to consider the differences in the genetic background of the host, since these could explain differences in the phenotype of the tumor, oncogenic pathways, and consequently the responsiveness to specific treatments. Thus, whereas somatic genetic background plays a significant role in determining individual variation, Prebiotic/butyrate supplementation may not always be associated with improved host well-being.

First, the researchers investigated if inulin may assist mice lacking the Toll-like receptor 5 (TLR5) experience a less severe episode of metabolic disorder. Surprisingly, though, a prolonged inulin-enriched diet raises cholestasis and necroinflammation, which subsequently lead to hepatocellular carcinoma (HCC) and improves metabolic dysfunction. Similar results have not been observed with cellulose, an insoluble, non-fermentable fiber, but rather with other soluble fibers as pectin and fructooligosaccharide (Kan et al., 2024). Following further investigation, it was shown that Clostridium species, especially Clostridium cluster XIVa, which is believed to be the primary source of butyrate and the other bile acids that cause cancer, were present in greater abundance in mice with HCC.

The occurrence of HCC was lowered in TLR5 deletion mice by lowering the quantity of bacteria that produce butyrate. Moreover, frequent addition of inulin to drinking water resulted in inflammation and hepatic fibrosis but did not stimulate the development of tumor.

These all indicate the possibility that butyrate production and prebiotic fermentation are not the main causes of HCC development, although they do play a part. Furthermore, Belcheva et al. found that such cancer risk only occurs in a specific background of genes, confirming the idea of interindividual variance in reaction to prebiotic therapy (Pandey et al., 2023).

Afterbiotics

The term "postbiotics" refers to resolvable metabolites and byproducts produced by the gut microbiota that have psychological impacts on the host. The most well-known postbiotic is SCFA, which is generated during probiotic fermenting. The beneficial effect of certain probiotic strains is caused by the prepared medium, also known as culture supernatants, rather than the actual bacteria (Song et al., 2023).

Therefore, in some situations, using postbiotics may be a safer yet equally effective strategy as opposed to ingesting live bacteria. Postbiotic isolation and characterization is an established field, although it has attracted a lot of interest recently.

Intestinal Epithelium Defense

Numerous postbiotics are believed to restore the integrity of the intestinal barrier and lessen colonic inflammation. Research has shown that the soluble protein p40, which is obtained from *Lactobacillus rhamnosus* GG, breaks down the intestinal barrier, enhances immunoglobulin A synthesis occurs by the transactivation of the receptor for epidermal growth factor (EGFR). Directed distribution of hydrogel-coating p40 (to prevent p40 from breakdown) can help reduce and treat intestinal damage and inflammation and trigger a defensive immune response

By cell-free supernatant, a number of additional probiotic strains, like Bifidobacterium breve, Lactobacillus rhamnosus GG, Lactobacillus acidophilus, Lactobacillus casei, and Lactobacillus casei, have also been demonstrated to lessen inflammation or protect the integrity of the gut barrier. The trustworthy The primary causes of this effect are the identity of the postbiotics, and the molecular pathways involved, both of which are poorly understood.

Targeted Cytotoxicity for Tumors

Some postbiotics, such as lactate dehydrogenase or various unidentified Lactobacillus species, have been demonstrated to induce death or inhibit invasion in CRC cell lines.

However, the lack of confirmation in in vivo models significantly limits the majority of these studies. The *Lactobacillus casei* ATCC334 supernatant was discovered to have a significant tumoricidal effect in a recent investigation. Ferrichrome has been found to be the molecule that triggers apoptosis through the JNK-DDTI3 signaling pathway.

The separated postbiotic demonstrated superior anticancer activity compared to conventional CRC treatments, with no impact on normal intestinal epithelial cells. This suggests that postbiotics could have therapeutic implications. The subject of postbiotics research is still relatively unknown, despite rapid advancement. Scientists face challenges in identifying the molecule responsible for therapeutic activity and determining its safety profile in preclinical and clinical contexts because of the vast array and variety of metabolites that have been reported.

As postbiotics become more established, we can expect to see more safety information.

Antibiotics Destroying Harmful Bacteria

Disrupting the gut microbe population can lead to reduced function of the intestinal barrier, swelling, carcinogenesis, and tumor development. Antibiotics can lower gut microbiota and restore dysbiosis, making them a viable study option. Figure 3 illustrates a cancer treatment and preventive method. In vivo models are frequently utilized to investigate the role of gut microbiota in cancer and other inflammatory diseases using antibiotics.

They are often administered via gavage or drinking water. Multiple studies have indicated that antibiotic-mediated microbiome depletion reduces the risk of colon cancer. The elimination of carcinogenic Bacteroides fragilis and other bacteria that cause mucin breakdown, inflammation, and DNA methylation is likely to be the primary reason for this protective effect. Antibiotics can reduce tumor development, invasion, and proliferation.

Metronidazole treatment reduces Fusobacterium colonization and suppresses CRC growth in mice with CRC xenografts, suggesting antibiotics may be effective for treating Fusobacterium-enriched CRC mice with and without neutrophil removal had distinct microbiota compositions in another investigation on the involvement of neutrophils in colon cancer. Antibiotic therapy, on the other hand, reduced bacterial load in the tumor and prevented tumor invasion.

Antibiotic-induced gut microbiota reduction has been linked to an antitumor immune response and reduced growth of tumor in mouse models, leading to its promotion as an immunotherapeutic technique. Antibiotics, which drastically alter the gut flora, have sparked discussion about their effectiveness in cancer treatment.

Although gut microbiome depletion can slow cancer progression, recent research suggests that antibiotics can worsen microbial dysbiosis and reduce the effectiveness of immunotherapy.

Efficacy of Immunotherapy Compromised

The pharmacological foundations of immunotherapy involve altering innate immunity and consequently inducing an anticancer immune response. Therefore, the tumor microenvironment plays a critical role in determining how effectively a treatment works. The gut flora has played a critical role in assessing the reaction to medication since it reduces the immunity of the host. Nevertheless, if these commensal bacteria are completely eliminated by antibiotics without selection, the anticancer immunity might be jeopardized.

Many studies have shown how some GIT bacteria, such as Bacteroides thetaiotaomicron, Bifidobacterium species, Bacteroides fragilis, Akkermansia muciniphila, and Alistipes shaii, respond to immunotherapy. As a result, using antibiotics to destroy the microbiota reduces their efficiency and encourages resistance to therapy. For example, Vétizou et al. demonstrated that imipenem alone or an antibiotic cocktail consisting of ampicillin, streptomycin, and colistin could eliminate the inhibition of CTLA-4 in mouse models of sarcoma, melanoma, and colorectal cancer (CRC).

Antibiotic-mediated microbiota deficiency may worsen treatment toxicity, which in a medical setting

may need treatment suspension or dosage decrease. According to a recent study, Bifidobacterium helps to lower autoimmune toxicities without reducing the therapeutic efficacy. On the other hand, giving vancomycin as a pretreatment to animals with colitis while they are receiving anti-CTLA-4 medication causes a more deadly and severe kind of intestinal inflammation.

Clinical observations verify these preclinical findings. A retrospective analysis found that concurrent utilization of immunotherapy and antibiotics is connected with a higher risk of disease progression, as well as reduce overall survival (OS) and progression-free survival (PFS), despite a lack of prospective research in this field. Another study found that based on programmed cell death protein 1 (PD1), the use of antibiotics is a predictor of resistance to immunotherapy.

In a similar vein, individuals who started immunotherapy 30 days before starting antibiotics were more likely to acquire primary resistance and typically had a lower survival duration.

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Chapter 33

Potential of Probiotics against Necrotic Enteritis in Commercial Broilers

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ABSTRACT

Necrotic enteritis (NE) is an economically significant intestinal disease of poultry caused by toxigenic strains of the *Clostridium perfringens* (*C. perfringens*) type A, C, and G. The worldwide effort to restrict the use of antibiotic growth promoters (AGPs) in livestock has resultantly caused a rise in the occurrence of NE in chickens, particularly in the broiler flocks. Among various non-antibiotic interventions for NE management studied so far, probiotics have provided a potential solution. This chapter highlights studies that evaluate the influence of different probiotic strains on the proliferation of *C. perfringens* and the incidence of NE. Various probiotic strains derived from bacterial genera including *Lactobacillus, Bacillus, Enterococcus, Bacteroides*, and some yeast species have been studied in chickens to assess their effectiveness in preventing the occurrence of NE. Probiotics can improve gut health by modulating microbial balance, tight-junction protein expression, and decreasing inflammatory cytokines. In conclusion, these characteristics indicate that probiotics may be a suitable replacement for AGPs in reducing NE. Hence, further investigation is required to ascertain the effectiveness of probiotics in preventing NE in commercial broiler farms.

KEYWORDS		
Necrotic enteritis; *Clostridium perfringens*; Broiler chickens; Probiotics	Received: 18-Jun-2024 Revised: 16-Jul-2024 Accepted: 28-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Enteric diseases pose a significant threat to the poultry industry as they result in reduced bird well-being, decreased production, elevated mortality rates, and an increased likelihood of contamination in chicken products meant for human consumption. Various pathogenic organisms, including bacteria, parasites, viruses, and other infectious and non-infectious agents, have been recognized as sources of enteric diseases, either alone or in combination. Gastrointestinal issues include dysbacteriosis, malabsorption syndrome, moist droppings, diarrhea, colibacillosis, coccidiosis, and necrotic enteritis (Hafez, 2011).

Necrotic enteritis (NE), which was first documented in 1961, is a significant enteric disease of poultry. The disease is caused by a bacterium; *Clostridium perfringens* toxinotypes A, C, and G (Abd El-Hack et al., 2022). There are seven toxinotypes (A–G) of *C. perfringens* based on whether or not six major toxins are present. *Clostridium perfringens* is a rod- shaped bacterium that is anaerobic, gram-positive, encapsulated, spore-forming, and non- motile. It is commonly found in both the soil and intestines of all endothermic animals. The population of *C. perfringens* in healthy birds is typically around 102-104 colony-forming units (CFUs) per gram of small intestine digesta. Under disease- challenge conditions, the number increases to 107-109 CFUs per gram of intestinal digesta (Shojadoost et al., 2012). The overgrowth of *C. perfringens*, which triggers the disease, is caused by alterations in the gut's physical qualities and the immunological condition of birds (Moore, 2016).

Implications of NE on Broiler Health and Productivity

Necrotic enteritis usually affects broilers between the ages of 2 to 6 weeks, and it can result in abrupt mortality without any warning symptoms (Cooper et al., 2013). Many factors, such as an imbalanced ration composition, intestinal hypomotility, immunosuppression, stress, excessive stocking density, and simultaneous coccidial infection, predispose birds to this condition. The disease manifests in both subclinical and clinical forms. Clinical necrotic enteritis is characterized by symptoms such as ruffled feathers, diarrhea, weight loss, pseudo-membrane formation, necrotic foci in the intestinal mucosa with "Turkish Towel" appearance, foul-smelling gas accumulation, and high mortality. The sub-clinical form (being 80% prevalent in the worldwide commercial flocks) is; however, associated with less prominent signs, i.e. poor nutrient digestion and absorption, poor feed conversion ratio (FCR), and cholangiohepatitis. Necrotic enteritis causes 10-40% mortality, costing the worldwide poultry sector 2-6 billion US $ annually (Wade and Keyburn, 2015; Wang et al., 2020). *Clostridium perfringens* type A and C are infectious in humans and can cause foodborne disease. Therefore, reducing the occurrence of NE in poultry is of critical importance (Mora et al., 2020).

Use of Antibiotic Growth Promoters in Broilers

Antibiotics have historically been administered at non-therapeutic levels to maximize animal productivity. Antibiotic growth promoters (AGPs) have the following benefits: they decrease subclinical diseases, reduce morbidity and mortality, enhance growth rate, decrease feed cost by 10-15% while achieving the desired growth, optimize the conversion of feed into animal products, and enhance reproductive and meat quality (Rathnayaka et al., 2021).

So far, the functioning of AGPs remains unclear, and understanding their modes of action could help in developing efficient non-antibiotic alternatives. Although the precise mechanisms of action are not well defined, AGPs are believed to enhance performance by modulating the gut microflora (Brown et al., 2017). To account for the enhanced antibiotic-mediated growth in animals, at least four mechanisms of action have been suggested: (1) a reduction of polarized epithelium thickness, which improves the nutrients absorption and utilization; (2) prevention of the subclinical infections; (3) an increase in the nutrient availability by decreasing the competition among microorganisms for nutrients in the intestines; and (4) a reduction in the levels of microbial metabolites in the intestines that hinder the growth (Fig. 1) (Niewold, 2007).

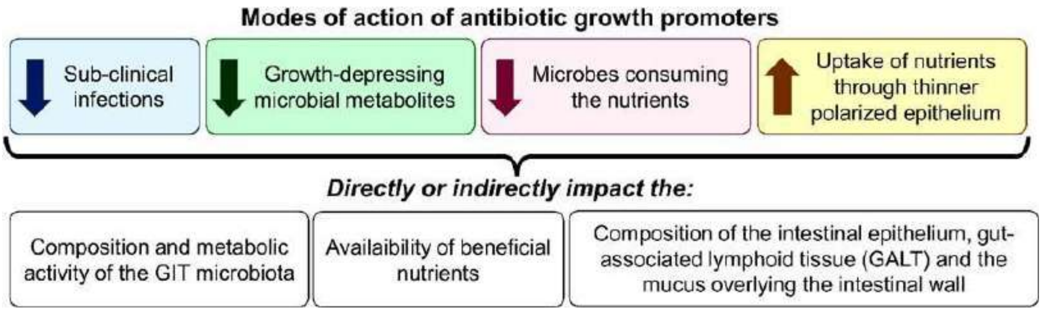

Fig. 1: Proposed mechanisms by which antibiotics function as growth promoters (Broom, 2017).

For several decades, adding AGPs to chicken feed has proven to be an effective and sustainable way for avoiding and treating NE infections. Commonly utilized antibiotics for prophylaxis and treatment of NE include bacitracin, amoxicillin, avoparcin, virginiamycin, lincomycin, and tylosin (Abd El-Ghany et al., 2022).

Concerns Pertaining to the Utilization of AGPs

The use of antibiotics has drawn more attention from consumers, government organizations, and researchers due to an upsurge of the antibiotic resistance. The use of AGPs in poultry and livestock farming presents many notable issues: (1) the development of strains resistant to antibiotics as a result of selection pressure; (2) the horizontal or vertical transfer of antibiotic-resistant genes (3) the dissemination of antibiotic-resistant bacteria into the environment; and (4) the discharge of antibiotic residues and their byproducts into the surroundings (Kumar et al., 2020; Wang et al., 2021).

Rationale for Alternatives to AGPs

The excessive utilization of AGPs in poultry feed has resulted in the emergence of antibiotic-resistant bacteria and the detection of antibiotics residues in the chicken products, thereby compromising the health of both animals and humans. Moreover, consumers now have a demand for animal products that are free from antibiotics. As a result, several countries such as the European Union (EU), Canada, the USA, Hong Kong, and Japan have gradually banned or severely restricted using AGPs in poultry (Salim et al., 2018). Broilers raised without antibiotics are; however, more vulnerable to enteric illnesses, which can have a detrimental effect on their overall welfare and intestinal health. Some of the economic effects of AGPs restrictions on chicken production include lower growth rates and feed efficiency, more mortality and morbidity, and higher veterinary costs due to more therapeutic treatment, which drives up meat prices. Countries that have restricted the use of AGPs in poultry diets have experienced a notable rise in the economically consequential infections such as NE. Hence, it is imperative to find and develop efficient substitutes for AGPs. Prebiotics, probiotics, synbiotics, acidifiers, enzymes, phytochemicals, antimicrobial peptides, and bacteriophages are among the most researched and effective alternatives to replace AGPs (Fig. 2) (Rahman et al., 2022).

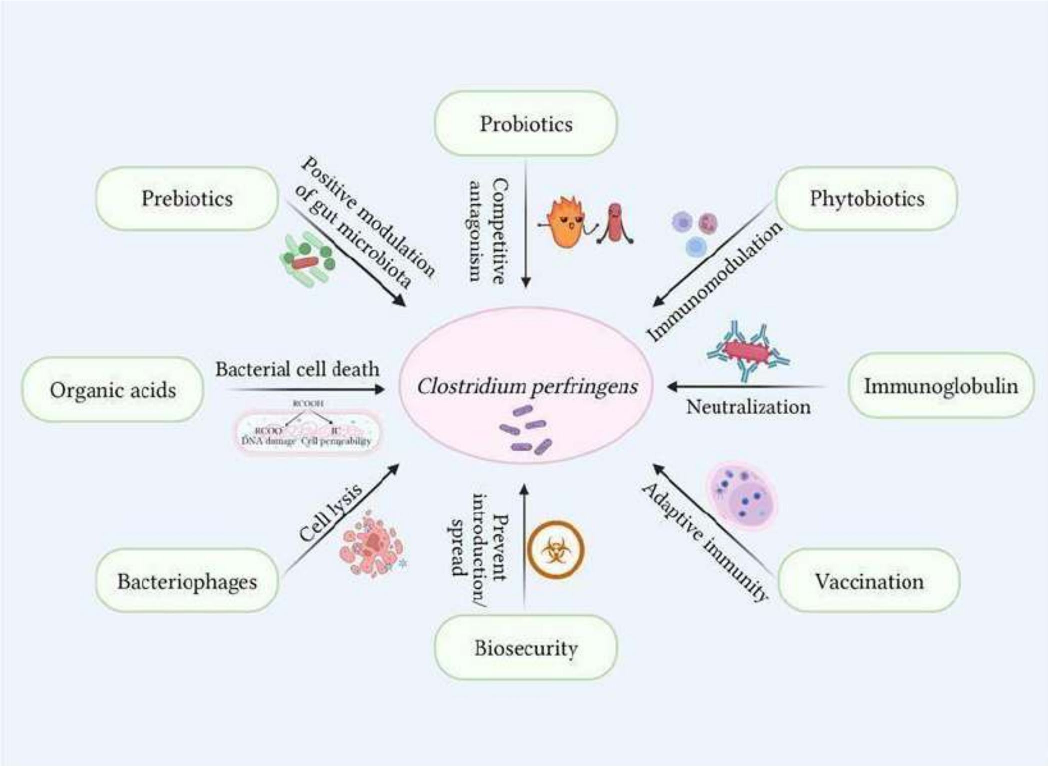

Fig. 2: Some of the proposed alternatives to AGPs for preventing and controlling NE {Source: Fathima et al. (2022)}.

While the majority of these suggested alternatives to antibiotics for poultry production have garnered increasing attention over time, probiotics have been the subject of extensive research with greater international interest. At present, synthetic biological techniques are also being utilized to develop genetically modified probiotics (engineered probiotics) that possess improved therapeutic potentials and greater specificity (Aggarwal et al., 2020).

Probiotics: Tailoring Solutions for Broilers

Lilly and Stillwell coined the term "probiotic" in 1965 to denote growth-promoting substances generated by microorganisms. The World Health Organization (WHO) defines probiotics as “mono or mixed cultures of living microorganisms that provide a health advantage to the host when provided in adequate amounts” (Krysiak and Konkol, 2021).

Characteristics of an Ideal Probiotic

The first important step in selecting a microbial strain for the prospective probiotic usage is determining its taxonomic classification, which can provide information about the strain's origin, domain, and physiological characteristics. The schematic way for the selection of probiotic strains involves consideration of their technological usability (efficient production of large amounts of biomass, viability, stability, desired sensory properties, genetic stability), functionality (resistance to enzymes and bile salts, competitiveness, antagonistic activity towards pathogens, adherence and ability to colonize), and safety (Joint, 2002).

Ideal probiotics have the following characteristics: (1) they are non-toxic, and generally recognized as safe; (2) have a positive effect on the host; (3) can adhere to and colonize the intestinal mucosa; (4) can fight off pathogens; (5) can withstand the acid and bile salts in the gut; (6) can endure the contractions of the intestinal wall and so not be washed out of the gut (7) remain viable during storage and processing operations (Stęczny and Kokoszyński, 2021).

Modes of Action of Probiotics

Broilers' diets supplemented with probiotics have many positive effects, such as: (1) changing the composition of the intestinal microbiota by producing metabolites that inhibit pathogen growth, such as hydrogen-peroxide (H2O2), bacteriocins, and short-chain fatty acids (SCFAs); (2) increasing feed efficiency and, consequently, production performance; (3) boosting the immune system, which increases the levels of immunoglobulins in the serum and mucous membranes, while simultaneously decreasing the intensity of pro-inflammatory processes; (4) competitively excluding pathogens and/or neutralizing their toxins; (5) lower blood cholesterol levels by controlling lipid metabolism; (6) enhance digestion and nutrient absorption; (7) control ammonia production for better litter quality; (8) regulate production of cytokines (9) decrease stress related to the antibiotic administration, temperature fluctuations, vaccination, and transportation; and (10) quickly remove the mycotoxins and other similar substances from the body. The general mechanisms of action of probiotics against pathogens are depicted (Fig. 3).

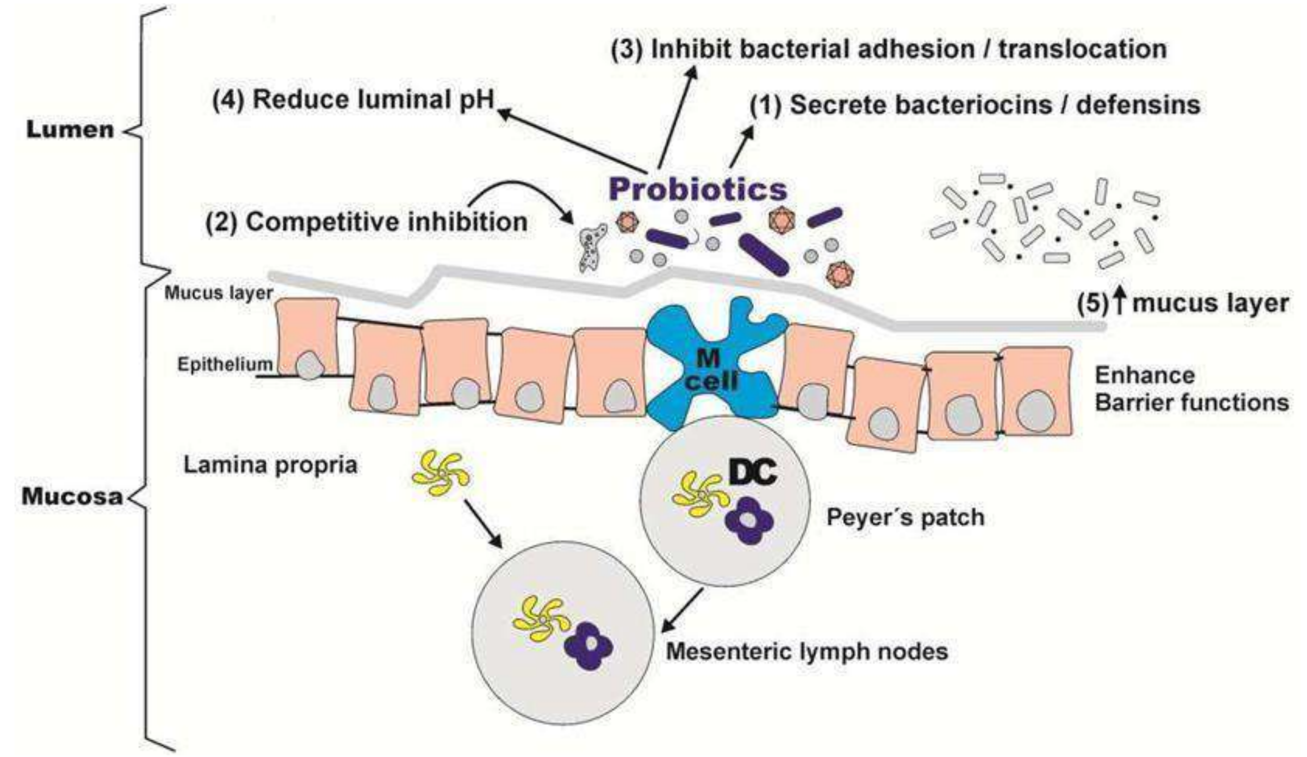

Fig. 3: Diagrammatic depiction of the interaction between gut mucosa and probiotic bacteria (Source: Ng et al. (2009)).

Key Probiotic Strains for the Poultry Industry

Probiotics can be classified as either allochthonous, which refers to microbes that are not naturally found in the intestinal flora of animals, or autochthonous, which refers to microbes that are naturally present in the intestinal flora of animals. Furthermore, probiotics are either bacterial or non-bacterial. In broilers, fallowing probiotic species are commonly employed for improving performance, meat quality, intestinal microbiota modulation, and pathogen inhibition (Table 1) (Bajagai et al., 2016).

Table 1: Probiotic microorganisms commonly used in broiler chickens (Hazards et al., 2017).

Lactobacillus spp.	*Bifidobacterium* spp.	Other lactic acid bacteria	Other microorganisms
L. johnsonii	*B. animalis*	*Enterococcus faecium Leuconstoc*	*Bacillus licheniformis*
L. acidophilus	*B. infantis*	*mesenteroides Lactococcus lactis*	*B. subtilis*
L. paracasei	*B. bifidum*	*Streptococcus thermophilus*	*B. coagulans*
L. reuteri	*B. lactis*	*Pediococcus acidilactici*	*B. cereus*
L. plantarum	*B. longum*	*Enterococcus faecalis*	*Saccharomyces cerevisiae*
L. casei	*B. breve*		*Saccharomyces boulardii*
L. rhamnosus	*B. adolescentis*		*Aspergillus niger*
L. amylovorus			*Aspergillus orizae*

Probiotics against Necrotic Enteritis Specific Mechanisms/Actions against NE

The processes by which probiotics suppress NE rely on a variety of factors such as age and type of the bird, the species and strain of the probiotic agent, the host immunological condition, and particularly the severity of the disease. In general, probiotics work by reestablishing the disturbed microbiota, producing antimicrobial compounds, preventing pathogens from colonizing through competitive exclusion, and modifying the host immune system. However, in the context of NE, specific mechanisms are highlighted (Fig. 4).

Efficacy of different Probiotic Strains against NE in Broilers

The most commonly used probiotics for necrotic enteritis include various strains of lactic acid producing bacteria such as *Lactobacillus acidophilus*, *L. fermentum*, *L. casei*, *L. reuteri, L. johnsonii, L. plantarum, L. salivarius, L. rhamnosus*, and others*; Bacillus* species like *B. subtilis, B. licheniformis, B. coagulans, and B. amyloliquefaciens; Enterococcus faecium, Clostridium butyricum, Butyricicoccus pullicaecorum,* and certain yeasts including *Pichia pastoris* and *Saccharomyces cerevisiae* (Caly et al., 2015). The beneficial effects of various types of probiotics are summarized in Table 2.

Table 2: The ameliorative effects of different types of probiotics on NE in broiler chickens induced by *C. perfringens*.

References	Genera	Strains	Concentrations	Main outcomes
Cao et al. (2019)	*Lactobacillus*	*L. plantarum* 1.2567	1 × 10^9 CFU/Kg feed	Increased average daily gain (ADG), Reduced gross necrotic intestinal lesion scores, Decreased inflammatory responses
Qing et al. (2017); Wang et al. (2018)		*L. johnsonii* BS15 (CCTCCM2013663)	10^5 and 10^6 CFU/g feed	High dose enhanced serum IgA and IgG levels on 21d, Positive effects on peripheral blood T-lymphocyte subpopulations, Improved ADG and FCR, Increased gut-friendly microbes
Li et al. (2022)		*Lactobacillus fermentum*	1 × 10^9 CFU/g in feed	Decreased lesion score in jejunum, Reduced coccidial oocyst counts in ileal digesta
Vieco-Saiz et al. (2022)		*Limosilactobacillus reuteri* ICV416, *Ligilactobacillus salivarius* ICV421, *L. salivarius* ICV430	10^7 CFU/mL orally	Increased body weight, Decreased lesion scores with mixed *Lactobacilli*
Shojadoost et al. (2022)		*L. crispatus* + *Ligilactobacillus salivarius*+ *L. johnsonii* +*Limosilactobacillus reuteri*	1 × 10^7 or 1 × 10^8 CFU orally	Reduced NE lesions in birds treated with 10^8 CFU of the mixed *Lactobacilli*, Improved the ratio of villus height to crypt depth (VH/CD)
Gharib-Naseri et al. (2021)	*Bacillus*	*B.amyloliquefaciens* (CECT 5940)	1.0 × 10^6 CFU/g of diet	Enhanced body weight gain (BWG), Improved FCR, Increased *Ruminococcus* populations and butyrate amount in the ceca, Reduced *C. perfringens* numbers, Enhanced digestibility of amino acids
Zhang et al. (2022)		*B.amyloliquefaciens* (BLCC1-0238)	2×10^5 CFU/g diet	Improved performance, Reduced mortality and intestinal NE lesions
Wu et al. (2018)		*B. coagulans*	4 × 10^9 CFU/Kg of diet	Enhanced BWG (15-28d), Improved FCR, Decreased lesion scores and crypt depths in the small intestine, Reduced Coliform and *C. perfringens* counts in the cecal contents, Increased *Lactobacilli* and *Bifidobacterium* counts
Keerqin et al. (2021)		*B. subtilis* (DSM29784)	10^8 CFU/Kg feed	Increased BWG (4% improvement) than the NE-challenged birds
Sokale et al. (2019)		*B. subtilis* (DSM32315)	1 × 10^6 CFU/g of feed	Improved BWG, Reduced mortality and mean lesion score
Hussein et al. (2020)		*B. subtilis* (DSM 17299)	0.2 g/Kg feed	Increased feed efficiency and livability, Reduced intestinal NE lesions score
Liu et al. (2021)		*B. subtilis* PB6	4 × 10^7 and 6 × 10^7 CFU/Kg feed	Increased BWG and ADFI (Average daily feed intake) with high-dose, Decreased lesion score, Restored ileal microbial composition
Hussein et al. (2020)		CloStat (*B. subtilis*)	0.5 g /Kg feed	Improved the feed efficiency and livability, Decreased intestinal NE lesions score
Koli et al. (2018)		*B. subtilis*	1.2 x10^6 CFU/g feed	Improved BWG and FCR, Reduced counts of *C.perfringens in* the small intestine
Chen et al. (2024)		*B. subtilis* HW2	1 × 10^6 CFU/g, 5 × 10^6 CFU/g, and 1 × 10^7 CFU/g	All doses improved growth, intestinal morphology, gut barrier function, immune response, gut microbial and short chain fatty acids profile
Zhao et al. (2020)		*B. licheniformis* H2	1 × 10^6 CFU /g feed	Ameliorated the negative effects on growth performance at 28 days, Improved VH/CD ratio in ileum

Zhou et al. (2016)		*B. licheniformis*	1.0×10^6 CFU/g feed	Enhanced BWG and Improved FCR (1-14d)
Emami et al. (2020)		*B. licheniformis* spores	3.2×10^9 CFU/g feed	Reduced mortality (0–14d), Decreased lesion scores in the duodenum
Musa et al. (2019)		*B. subtilis* B21 (BS) and *B. licheniformis* B26 (BL)	Both at 2×10^9 CFU/g feed	Improved ADFI (1-21d) in the BL group, Increased ADG in BS group, Improved VH/CD ratio in both groups
Sandvang et al. (2021)		*B. amyloliquefaciens* (DSM 25840) + *B. subtilis* (DSM 32325) + *B. subtilis* (DSM 32324)	1.6×10^6 CFU/g in feed	Improved BWG and FCR (0-42d), Reduced mortality and intestinal lesion score
Ramlucken et al. (2020)		*B. subtilis* (CPB 011, CPB 029, HP 1.6, and D 014) + *B. velezensis* (CBP 020 and CPB 035)	1×10^9 CFU /g feed	Improved FCR (>35d), Increased VH/CD ratio
Wu et al. (2019)	*Enterococcus*	*E. faecium* (NCIMB 11181)	2×10^8 CFU/Kg of diet	Increased BWG compared with NE-challenged birds, Decreased gut lesion score at three days post-infection
Xu et al. (2021)	*Clostridium*	*C. butyricum* (GCMCC0313.1)	2×10^8 CFU/g of diet	Increased ADG and ADFI, Improved FCR and intestinal morphology
Huang et al. (2018)		*C. butyricum* (YH 018)	1×10^9 CFU/g feed	Reduced *C. perfringens* counts
Eeckhaut et al. (2016)	*Butyricicoccus*	*B. pullicaecorum strain* 25-3T (LMG 24109)	10^9 CFU/Kg feed	Improved FCR
Sun et al. (2021)	Compound Probiotics	*L. johnsonii* BS15+ *B. licheniformis* H2	1×10^8 CFU/ml + 10^9 CFU/g in feed	Improved FCR Improved intestinal morphology parameters Reduced intestinal lesions and inflammation

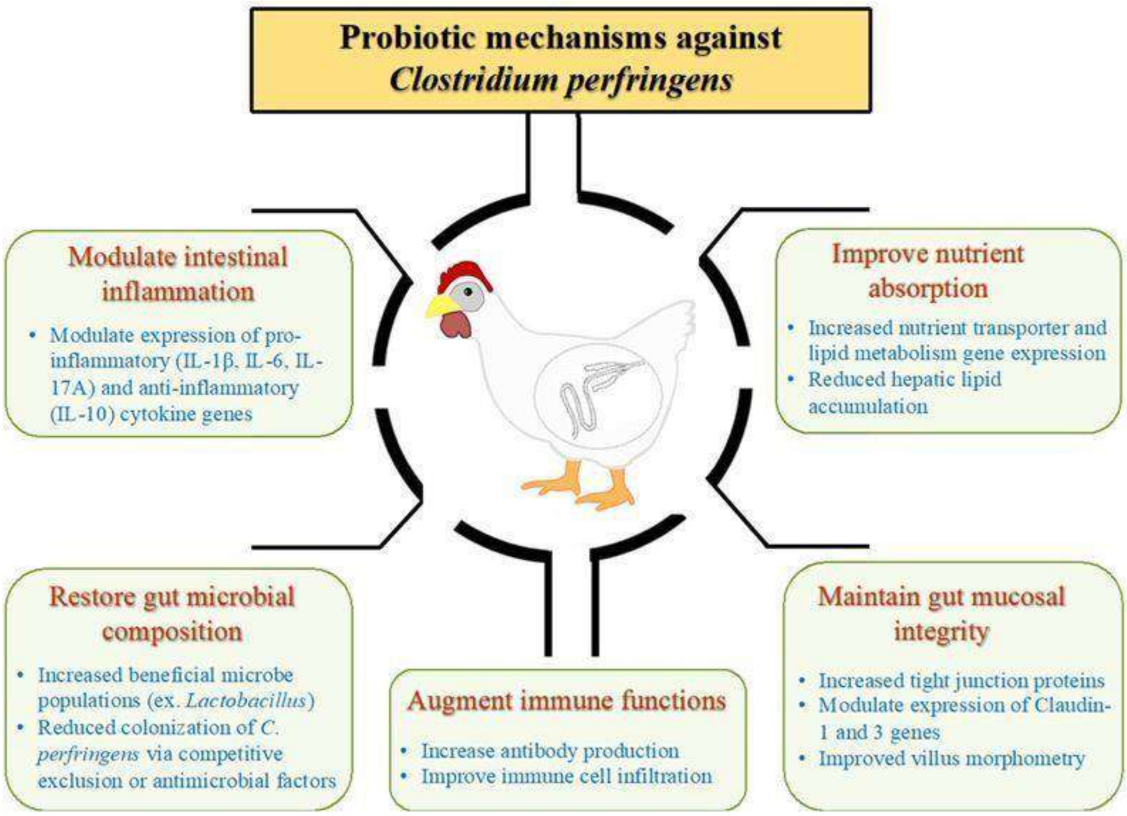

Fig. 4: Anti-*C. perfringens* mechanisms of probiotics (Kulkarni et al., 2022).

Factors Influencing Probiotic Efficacy

The complex and multi-faceted impacts result from the interactions between microbial additives and the microflora of the host's digestive system. Listed below are a few elements that have an impact on this ultimate result (Afshar Mazandaran and Rajab, 2001).

- Quality assurance
- Consumption amount and method
- Age and type of animal
- Microbial flora composition of the host's digestive tract
- Composition and type of product
- Production methods

Challenges and Considerations in Probiotic Application for Broilers Stressors Affecting Performance of Probiotics

The use of probiotics in poultry production has associated risks and constraints. Newly hatched poultry species are exposed to various stress factors in the environment that can weaken their maternal antibody defense system. These stressors hinder the normal colonization of beneficial microorganisms in the birds' gut, making them vulnerable to pathogens during early life (Edens, 2003).

Resistance of Bacteria to Probiotics

The use of various probiotic strains in animal diets has sparked concerns about the possibility that bacteria in the gut microbiota may become resistant to antibiotics. The pathogenic bacteria can acquire antibiotic-resistant genes from probiotic strains by horizontal gene transfer, as these probiotic strains carry genes that confer immunity to some antimicrobials as well as antibiotic resistance. To reduce this risk, it is critical to examine the potential presence of possibly transmissible resistance genes in a prospective probiotic strain. The best outcomes will be achieved by testing a range of different strains of probiotics. Protocols for assessing the safety of probiotics have also beendeveloped to mitigate various risks related to the incorporation of probiotics in animal feed (Choi et al., 2020).

SWOT Analysis of Probiotics

The SWOT analysis for probiotics is described in Table 3. The majority of research

Table 3: The SWOT analysis of probiotics.

Strengths (Angelin and Kavitha, 2020)	**Weaknesses** (Joshi et al., 2018)
● Certain probiotic strains can endure harsh conditions, such as stomach acid and bile acid ● Enhance nutritional value, sensory and chemical properties of meat ● Avoid diarrhea and intestinal disturbances ● Produce more enzymes to improve feed digestion ● Produce organic acids	● The process of preparing, transporting, and storing feed can readily make bacterial strains inactive ● It is not possible to label items that contain probiotics due to the absence of relevant regulations and standards ● Intestinal and bile acid pH levels are too low for the majority of bacteria to survive ● Probiotics may pose a risk to animals that are born with a weakened immune system
Opportunities (Markowiak and Śliżewska, 2018)	**Threats** (Cheng et al., 2014)
● Multistrain probiotic bacteria are utilized for the prevention of neonatal diarrhea ● Probiotics derived from the intestines of animals and people are a safer and more efficacious option for consumption by both humans and animals ● Probiotics can attach to and eliminate various substances such as heavy metals and aflatoxin by excretion in feces	● Interactions between epithelial cells, pathogens, and probiotics ● The gut microbiota has a strong correlation with several neurological diseases ● Antibiotic resistance genes can be passed on by probioticbacteria, which can also promote the development of antibiotic resistance.

Conducted on the utilization of probiotics in animal diets has documented a diverse range of advantageous impacts on animal growth and well-being. In addition to positively affecting gut microbiota and inflammation, probiotics have been found to decrease diarrhea and enhance feed digestion through the production of enzymes or by stimulating the secretion of digestive enzymes in the intestines (Angelin and Kavitha, 2020). However, the use of probiotic-based products may be limited due to various concerns. These concerns include inconsistencies in the quality and dosage of probiotics, low survival rates in the GIT, inactivation during the production, transportation, or storage of the feed, potential allergenic reactions, possible interactions between probiotics, pathogens, and epithelial cells, as well as the potential transmission of antibiotic-resistant genes (Hmidet et al., 2009).

Conclusion and Future Perspectives

Gut health is an important determinant of animal health, and nutritional interventions can improve it. Due to rising limitations on the antibiotics use in chicken production, there is a pressing need for effective alternatives to manage enteric diseases i.e., necrotic enteritis. Among various approaches, probiotics appear to provide a promising option for controlling NE. Several essential elements must be taken into account when selecting a probiotic formulation to manage NE in chickens such as: type of bird, species, breed, and age, probiotic strains of choice, route, and frequency of administration. Although there is a significant amount of literature demonstrating the beneficial effects of probiotics in chicken feed, further extensive research is necessary to completely understand the molecular changes induced by probiotics and the interactions between epithelial cells, pathogens, and probiotics. This will necessitate the integration of metagenomic, nutrigenomic, and metabolomic studies. The elucidation of these unknowns will result in a deeper understanding of probiotics' function in enhancing the broilers' health and growth. Future research should also focus on identifying the precise mechanism of action of probiotics, figuring out the optimal dosage for single or multi-strain probiotics, assessing the impact in birds with intestinal disorders, removing the possibility of antibiotic resistance gene transfer, and establishing selection criteria for novel probiotic species.

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Chapter 34

Probiotics and Liver Diseases: Revamping Therapeutic Strategies in Community

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ABSTRACT

Probiotics have been suggested for the prevention and treatment of a wide range of illnesses, such as liver disorders. Bacterial substances may cause immediate harm to liver cells when they pass into the liver-gut axis under adverse circumstances, these actions also trigger proinflammatory and autoimmune reactions in the liver. Probiotics exert beneficial impacts on various chronic liver disorders by modulating the bacteria in the intestinal tract, prevent adhesion of microorganisms, improve the function of the mucosal barrier, and secrete bioactive compounds and decreasing the microbial toxins production. The types of bacteria found in the human gastrointestinal system serve a variety of purposes, including helping to control the body's immunological response and preserving a microbial barrier against possible infections. The variations in the diversity of gut microbiota are significant in the onset of liver disorders. The use of bacterial strains that promote health may help alleviate the detrimental interactions and liver conditions. The most widely used probiotics are strains of Bifidobacteria or Lactobacillus, which are found in the natural gastrointestinal flora. They may also promote the development of favorable microbes. Probiotics have been found to have a promising effect in the treatment of alcoholic liver disease, non-alcoholic fatty liver, viral hepatitis, hepatic encephalopathy, and liver cirrhosis.

KEYWORDS		
Probiotics, Liver diseases, Alcoholic and Nonalcoholic fatty liver disease, Gut microbiota	Received: 25-Jun-2024 Revised: 20-Jul-2024 Accepted: 08-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Probiotics are live microorganisms that have been found to enhance human health, such as yeast and bacteria. Probiotic" is an appealing term. Probiotics refer to oral microorganisms rather than substances that are used to benefit bacteria, despite the fact that it is evident that antibiotics are substances that are used against them. The World Health Organization defines probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (Reid et al., 2019). Probiotics can be used in living or dead forms and in combinations with prebiotics, immunostimulants like symbiotic and synbiotism, or even in single or multiple strains. The most commonly used probiotics are Lactobacillus, Bifidobacterium, and *Saccharomyces (S.) boulardii*. *Lactobacillus* and *Bifidobacterium* are Gram-positive rods that are obligated facultative anaerobes and *S. boulardii* is a yeast (Islam, 2016).

A significant role for intestinal microbiota exists in both health and illness. Bacterial elements such as lipopolysaccharide and liver receptors (Toll-like receptors) can interact through the gut-liver axis. Liver disorders or the aggravation of liver disorders may arise from the modulation of this interaction by dysbiosis and increased intestinal permeability. The administration of microbial strains that promote health may help reduce these detrimental interactions and liver diseases (Sharma et al., 2013).

Through the systemic circulation, portal vein, and biliary tract, the stomach and liver communicate extensively. We refer to this two-way communication as the gut-liver axis (Chopyk et al., 2020). Through the portal vein, biliary system, and circulatory mediators, the liver communicates with the intestine. In addition to maintaining liver homeostasis, microbes in the intestine can harbor pathogens and chemicals that aggravate fatty liver disorders. We examine alterations in the gut microbiota that may facilitate the onset or advancement of alcohol-related and non-alcoholic fatty liver (NAFLD) disorders, which are the most prevalent chronic liver illnesses in Western nations. (Lang and Schnabl, 2020). The human body has human microbiota in many locations such as the nares, oral cavity, urogenital tract, skin, and gut. The most densely colonized site in humans is, of course, the gastrointestinal system, with almost two thirds of the total microbial load found in the colon. About 100 trillion (1014) microorganisms' total, or one to two kilograms of human weight, reside in our gut. (Sekirov et al., 2010)

Role of Gut Microbiota in Health and Disease

The regular functioning of the host organism is significantly influenced by the gastrointestinal microbiome. The gut microbiota can produce a range of metabolic products that, depending on how they interact with the host, have a favorable or harmful impact on human health. The conversion of dietary components into bioactive food elements is carried out by microbiota.

Indigestible polysaccharides such as cellulose, hemicelluloses, resistant starch, pectin, oligosaccharides, and lignin can be broken down by these bacteria to produce short-chain fatty acids (SCFAs), which are abundant sources of energy for the host, include butyric, propionic, and acetic acids. These fatty acids enter the colon after evading absorption in the upper gastrointestinal tract (Lin and Zhang, 2017).

The host organism can also benefit from the microbiota's beneficial effects because it plays a crucial part in the synthesis of several vitamins, including B and K, cobalamin, riboflavin, nicotine, and pantothenic acids (LeBlanc et al., 2013). As an independent organ with a broad metabolic capacity and significant functional plasticity, in addition to shedding epithelial cells, the gut microbiota obtains nutrition from host dietary components (Sonnenburg et al., 2005). It is well established that dietary fibers and a balanced gut flora improve health, using synbiotics to maintain gut health. The prebiotics in them nourish both the probiotic bacteria in the dietary supplement and the naturally occurring bacteria that live in our gut on their own (Hemarajata and Versalovic, 2013). The human gut's barrier function involves chemical, physical, and immunological elements. Defensins, mucins, and angiogenin 4 are examples of antimicrobial peptides. Secretory immunoglobulin A is involved in immunologic and luminal chemical processes that support the integrity of the gut's barrier (Yu et al., 2012).

Table 1: Several instances of both potentially detrimental and advantageous gut microbiota bacterial species (Scotti et al., 2017; Singh et al., 2017)

Bacterial Strain	Basic Features	Related physiological changes	Diseases related states
Bacteroides spp.	Gram-negative obligate anaerobe	Activate CD4 (+) T cells	Raised in obesity and with an animal-based diet.
Bifidobacterium spp.	Gram-positive obligate anaerobe	Short chain fatty acids production, enhance the intestinal mucosal barrier and reduce intestinal LPS levels	Reduced prevalence in obesity and in smokers Rise in Rett syndrome Used as a probiotic
E. coli	Gram-negative facultative anaerobe	Activate Toll-like receptors	Potential advantages for ulcerative colitis Elevated in inflammatory bowel disease and in type 2 diabetes
Clostridium spp.	Gram-positive obligate anaerobe	Encourage the production of TH17 cells	Elevated upon exposure to smoke, autism and in Rett syndrome Decreased in inflammatory bowel disease. Positive relationship between weight gain and plasma insulin.
Lactobacillus spp.	Gram-positive facultative anaerobe	Short chain fatty acids production, anti-inflammatory activity	Decreased obesity (L. lantarum). Increased obesity (L. reuteri) L. casei boosts defenses against illness. L.reuteri used as a probiotic and it prevents tooth decay. L. rhamnosus reduce stress and depression
Neisseria spp.	Gram-negative obligate aerobe	Sugar fermentation	Pathogenic species are: Neisseria meningitides causes (Meningococcal disease) and Neisseria gonorrhoeae. Decreased after tobacco use and smoke, especially in the mouths of smokers
Staphylococcus spp.	Gram-positive facultative anaerobe		Pathogenic S. aureus caues (pneumonia, bone and joint infections). Raised in obesity.
Streptococcus spp.	Gram-positive facultative anaerobe		Some species are pathogenic. Streptococcus mutans cause dental caries following a high-carb diet. Streptococcus salivarius used as a probiotic.

Various bacterial species induce physiological alterations in the body and lead to illnesses.

Liver Diseases

Liver disease is the cause of two million fatalities each year and 4% of all deaths globally (1 out of every 25 deaths). Nearly two thirds of liver-related deaths are in men. Acute hepatitis caused a smaller proportion of deaths, with complications from cirrhosis and hepatocellular cancer being the main causes of death **(**Devarbhavi et al., 2023). It is estimated that alcohol usage accounts for 5.3% of all fatalities globally. Moreover, liver damage caused by alcohol accounts for 5.1% of all illnesses and injuries worldwide. In low-, middle-, and high-income nations, alcohol use disorder (AUD) causes a considerable loss of years of life due to disability, affecting males more often than women worldwide (Aslam and Kwo, 2023). Metabolic syndrome, alcoholism, and obesity are linked to fatty liver disorders. This disease's appearance is influenced by the gut flora, lifestyle, and nutrition. Triglycerides make up the majority of the fat in the hepatocytes of patients with NAFLD. Hepatocellular carcinoma and liver cirrhosis are possible outcomes. Although the basic pathophysiology of NAFLD is unknown, changes in the gut microbiota are thought to have a significant impact in the development of the disease (Betrapally et al., 2016). The proliferation of Gram-negative bacteria brought on by increasing alcohol consumption increases gut permeability, which increases the access of bacterial metabolites to the liver as well as pro-inflammatory chemicals like lipopolysaccharides and bacterial toxins (Betrapally et al., 2016). The liver is a key organ of the immune system that is especially rich in innate immune cells and is in continual contact with endotoxins produced by the gut microbiota as well as circulating nutrients. Understanding the pathogenesis of different liver illnesses has been made easier by research on the relationship between liver and intestinal microbiota (Wang et al., 2021). Clinical characteristics of liver diseases in human patients is illustrated in figure 1.

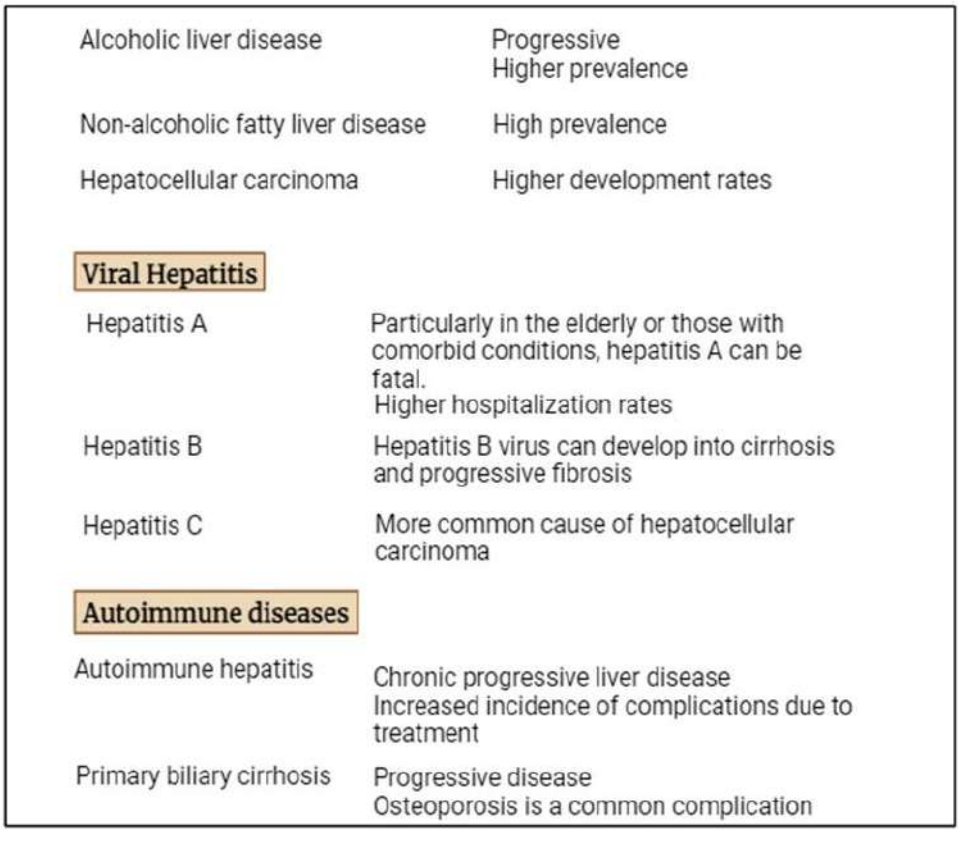

Fig. 1: Clinical characteristics of liver diseases in patients (Tajiri and Shimizu, 2013)

Microbiota and Liver Diseases

The term "gut-liver axis" has gained popularity because of the relationship between the gastrointestinal system and the liver, as well as the fact that nutrients are absorbed by the gut before being absorbed by the liver **(**Sharma et al., 2013). Intestinal bacteria have been the main focus of microbiota research in liver disorders. Decreased diversity of gut bacteria and modifications to the microbiome's makeup, including a transition toward gram-negative bacteria and a decrease in beneficial bacteria, are linked to alcohol consumption (Duan et al., 2019; Smirnova et al., 2020).

The nature of the gut microbiome is affected by various environmental factors, including alcohol intake. People who drink alcohol see an increase in gut microorganisms. In addition to maintaining liver homeostasis, microbes in the intestine can harbor pathogens and chemicals that aggravate fatty liver disorders. We examine how alterations in the gut microbiota may contribute to the onset or advancement of alcohol-related and non-alcoholic fatty liver disorders, which are the most prevalent chronic liver illnesses in Western countries (Lang and Schnabl**,** 2020)**.** Long-term alcohol use does change the microbiota in relation to alcohol-associated liver injury, according to findings from animal study **(**Mutlu et al., 2009).

Alcohol-related liver disease, hepatocellular cancer, and NAFLD all are connected to alterations in the microbiota. Hepatic encephalopathy has also been linked to changes in microbiota (Bajaj et al., 2012). The severity of these changes increases with the progression of the disease and suggests that modifications to the microbiome may impact brain function; additionally, the gut–brain axis may be a potential target to lower the risk of relapse in alcohol consumption. Even in the case of cirrhosis and alcoholic hepatitis, microbial function—specifically, that which is connected to bile acid metabolism—can govern alcohol-related harm (Bajaj, 2019). Another study suggests that although microbiota may not be directly responsible for the onset of hepatocellular carcinoma, it may have contributed to its growth and development (Dapito et al., 2012).

The foundation of the probiotics' therapeutic effects, involving four processes, was discovered through molecular and genetic investigations (Fig 2).

1. Antagonistic interactions through the synthesis of antimicrobial agents
2. Competition with pathogens for adhesion to the epithelium and for nutrients
3. Immunomodulation of the host
4. Inhibition of bacterial toxin production

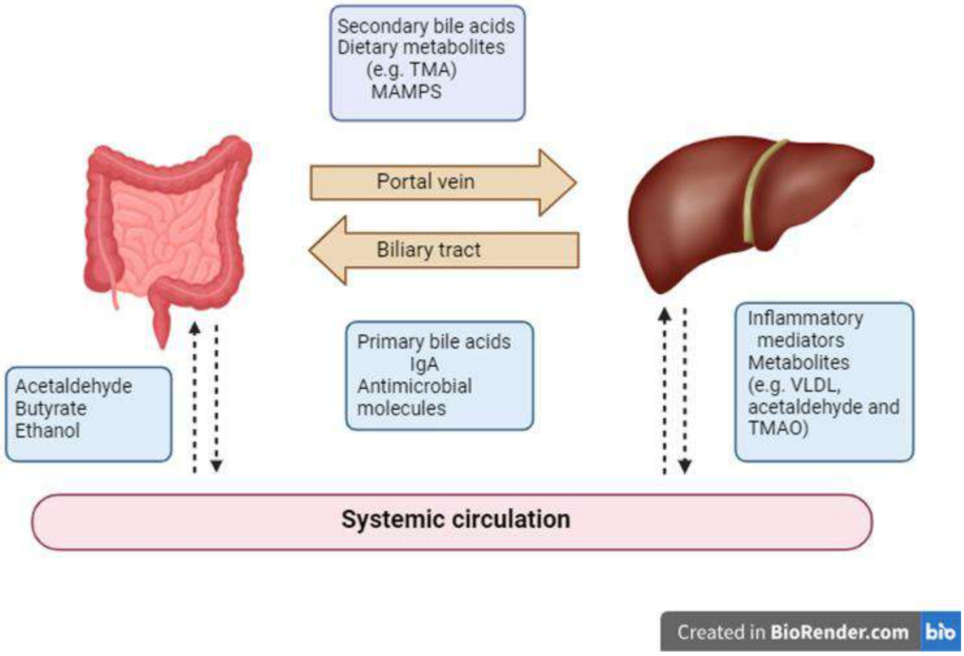

Fig. 2: The gut-liver axis and the interaction with the microbiome

The Liver and intestine communicate extensively through the biliary tract, portal vein and systemic mediators. Liver products primarily influence the gut microbiota composition and gut barrier integrity, whereas intestinal factors regulate bile acid synthesis, glucose and lipid metabolism in the liver.

Probiotics in Liver Diseases:
Alcoholic Liver Disease

The majority of probiotics contain the saccharolytic bacteria Lactobacillus and Bifidobacterium, which can ferment carbohydrates to produce lactic acid. It is well known that lactic acid effectively inhibits the growth of pathogenic bacteria. There is strong evidence from preclinical and clinical research that the gut microbiota is an important factor in ALD.

In addition to bacterial dysbiosis, patients with alcohol-use disorder and ALD also have alterations in their gut mycobiome. The fecal mycobiota of these individuals exhibits a decrease in fungal diversity and an increase in Candida spp **(**Lang et al., 2020).

Intestinal permeability is higher in people with moderate liver illness and alcohol use disorders. It will be necessary to conduct more research to find out if people with elevated intestinal permeability also have progressive liver disease (Leclercq et al., 2014).

In the intestine, butyrate, propionate, and acetate are the most prevalent SCFAs produced by bacteria through the fermentation of nondigestible carbohydrates. Patients who drink alcohol on a regular basis had lower fecal levels of SCFAs. Similarly, compared to samples from heavy drinkers, patients with alcohol-related hepatitis exhibited reduced levels of SCFAs and fewer bacteria that produce SCFAs in their feces (Smirnova et al., 2020).

Together, changes in the gastrointestinal microbiota cause ALD through a variety of pathways, including as microbial chemical metabolism, toxin production, and disruption of the intestinal barrier. New therapeutic strategies have been put out to modify the intestinal microbiota, and numerous recent research has shown the effectiveness of these strategies, which include probiotics, prebiotics, synbiotics, fecal microbiota transplantation (FMT), bile acid regulation, and others (Hong et al., 2019). Some strains of Bifidobacterium and Lactobacillus that are frequently used as probiotics and found in yogurt and probiotic pills are given in Table 2.

Probiotics Treatment in Patients with ALD

Probiotic use resulted in a reduction in the level of steatosis, inflammation in the liver, lipogenesis, oxidative stress, a decrease in the level of biomarkers of systemic inflammation, gastrointestinal dysbiosis, creating an environment that is anti-inflammatory so that intestinal permeability can be decreased, and bacterial components (LPS) can be transferred to the systemic circulation **(**Tsai et al., 2020). By stimulating the production of antimicrobial peptides (AMPs), certain probiotics control the host defensive peptide response. In actuality, human beta-defensin-2 was highly expressed in epithelial cells by the probiotic E. coli strain Nissle (EcN) and a few species of Lactobacilli. Other probiotics, such as Lactobacillus reuteri, can also enhance the release of interleukin-22 (IL-22), which stimulates AMPs to facilitate intestinal mucosa repair and defense (Patnaude et al., 2021). Probiotics activate the epidermal growth factor receptor, which repairs the intestinal epithelium barrier that was harmed by alcohol. Probiotics' ability to prevent alcoholic liver damage also depends on this receptor's ability to function. Probiotics prevent hepatocyte apoptosis brought on by alcohol. Treatment with a prebiotic and a synbiotic combination of several bacterial strains in ten patients, all of whom were chronic alcohol consumers with a median daily consumption of 150 g of pure ethanol, as opposed to baseline values, dramatically reduced liver damage and function (Li et al., 2016). Probiotics' ability to prevent the growth of pathogenic microorganisms is one of its many health advantages.

Table 2: Frequently used bacteria in probiotics

Lactobacillus strains	Bifidobacterium strains	Other lactic acid bacteria	Other microorganisms
• L. acidophilus • L. rhamnosus GG • L. bulgaricus • L. plantarum • L. reuteri • L. salivarius • L. casei	• B. bifidum • B. lactis • B. longum • B. breve • B. infantis,	• Enterococcus faecium • Lactococcus lactis • Streptococcus thermophilus	• Escherichia coli Nissle 1917 • Saccharomyces cerevisiae (boulardi)

These bacterial strains are frequently used as a probiotics and found in yogurt and probiotic pills and they restore the gut microbiota and delay liver damage and fibrosis.

According to pre-clinical research, LPS endotoxin caused by alcohol and fat infusion can be reduced and alcoholic liver damage can be avoided by pre-treating the gut flora with antibiotics to clear it out or with probiotics (lactobacilli) to replenish it (Vassallo et al., 2015). Probiotic supplementation has been shown in several human studies to enhance the immune response against enteric infections, reduce oxidative stress and damage, and lower endotoxin levels.

Additionally, the researchers performed numerous therapeutic experiments on probiotic-using ALD patients. The findings demonstrated that probiotics significantly improved the condition of ALD patients as well, and the treatment method used was essentially the same as it was for animal models. In a clinical trial involving patients with alcoholic cirrhosis, Lactobacillus casei Shirota (6.5 x 109 CFU) administered 3 times a day for four weeks to restore phagocytic ability, reduce Toll like receptor 4, soluble TNF receptor (sTNFR1), sTNFR2, and enhance IL-10 levels (Liu et al., 2023). A study stated that probiotics (Bifidum, B. lactics, B. longum, L. acidophilus, L. rhamnosus, S. thermophiles) significantly reduced small intestine bacterial overgrowth (SIBO) in individuals with chronic liver disease. Probiotics affect each of these organs directly as well as indirectly through the interaction of the axis's components, operating at various levels of the liver, brain, gut, and microbiota axis.

Probiotics can alter a number of pathophysiological pathways that contribute to the development of liver damage, some of which are as follow: **(**Fuenzalida et al., 2021)

1. Probiotics have a protective effect on the mucous layer and crypts while also enhancing tight junction expression and digestion at the gut level.
2. This alteration increases the benefits of probiotics in the microbiota, repairing it and lowering alcohol-induced dysbiosis. This will reduce the amount of pathogenic bacteria and enhance the amount of beneficial bacteria, lowering the gut's high permeability and the liver PAMP translocation.
3. A probiotic-based therapy can reduce systemic and neuroinflammatory inflammation because of the effect that probiotics have on the brain, which also lowers levels of proinflammatory cytokines. One way to manage alcohol intake and psychological symptoms like depression and anxiety is through the regulation of inflammation. Controlling elevated permeability and substance translocation also helps to manage blood-brain barrier disruption and neuroinflammation.
4. Probiotics have shown several advantages for the liver, ranging from the reduction of steatosis to encephalopathy and cirrhosis. The reduction in pathognic associated molecular patterns in the systemic circulation, particularly LPS, which causes the inflammatory processes linked to the TLR4 pathway to normalize, explains these effects on the liver. As a result, alcohol's harmful effects on the liver are lessened, including less Küpffer cell activation, reduced liver enzymes, proinflammatory cytokines, and less fibrosis.

Non-alcoholic Fatty Liver Disease

The buildup of fat in the liver in individuals who do not drink excessive alcohol is known as nonalcoholic fatty liver disease (NAFLD), which is an indication of metabolic syndrome in the liver. About 25% of people worldwide suffer from NAFLD, which is a worldwide public health issue. Patients with obesity, type 2 diabetes (T2DM), and metabolic syndrome are more likely to have NAFLD (Cao et al., 2023). NAFLD is primarily defined by fat accumulation in hepatocytes above 5% of liver weight when excessive alcohol is not consumed.

It is possible that the gut microbiota has a role in the formation of fatty liver disease. Prebiotics, probiotics, and synbiotic supplements can help treat NAFLD by altering the gut microbiota, according to the data obtained from animal studies. The majority of people with NAFLD do not exhibit any symptoms, and it is linked to obesity and metabolic syndrome characteristics such as central adiposity, dyslipidemia, high blood pressure, and insulin resistance (IR) or diabetes (Hassan K et al., 2014). NAFLD can develop into cirrhosis, fibrosis, non-alcoholic steatohepatitis (NASH), and possibly hepatocellular carcinoma (HCC) (Liu et al., 2022). Fig 3 shows the spectrum and progress of NAFLD in patients.

In histologically confirmed MAFLD, a combined probiotic (1 g twice daily for three months) reduced the serum levels of ALT, AST, GGT, total cholesterol, triglycerides, as well as the steatohepatitis activity (NAS) and the percentage of those suffering from dysbiosis. However, it had no discernible effect on the serum levels of total bilirubin and high density lipoprotein cholesterol (Cai et al., 2020). In patients with nonalcoholic steatohepatitis, they reported that a complex combination of probiotics, prebiotics, vitamins, and minerals helped lower aminotransferase levels. The serum levels of triglycerides, ALT, AST, GGT, and ALP decreased when a probiotic (Lactobacillus, Rhamnosus, Acidophilus, Bifidobacterium longum, and Breve) was used for MASH (Behrouz et al., 2020). One of the most successful approaches to control gut microbiota is the use of probiotic supplements. Probiotics have been shown to benefit NAFLD in a number of animal studies by lowering inflammation, hepatic triglycerides, total body weight, visceral fat tissue weight, as well as insulin resistance (Kobyliak et al., 2017).

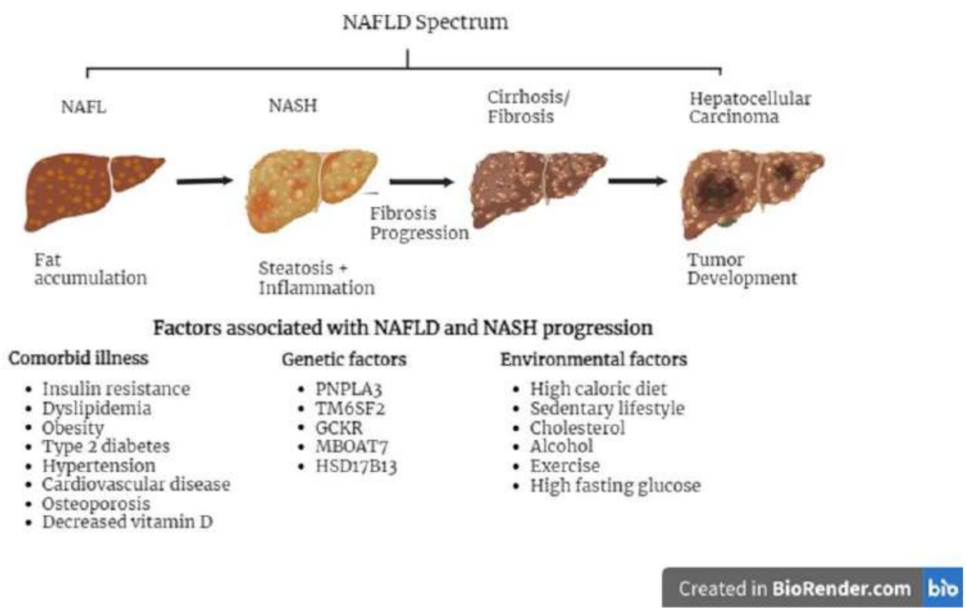

Fig. 3: The spectrum of NAFLD divided into four stages and the different factors contribute the the development of hepatocellular carcinoma.

The Gut Microbiota's Function in the Pathophysiology of NAFLD

Many metabolic, genetic, and microbiome-related variables influence the pathophysiological mechanisms underlying non-alcoholic fatty liver illness. Patients with NAFLD have altered gut microbiota composition, and some research indicates a faecal microbiome profile linked to progressive fibrosis.

In the gut microbiota, the phyla Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria include the majority of commensal organisms. The gastrointestinal microbiome structure and composition can be impacted by a number of variables, including age, nutrition, health, and illness. The microbiota can exacerbate NAFLD by modifying the ability of the diet to provide energy, impacting the process of de novo lipogenesis and the synthesis of SCFAs, modifying the metabolic signaling pathways of choline and bile acid, causing a rise in intestinal permeability and inflammation, and generating endogenous ethanol within the gut (Yao et al., 2021). Usually initiated in adipose tissue and the liver, de novo lipogenesis is a complex and tightly controlled lipid metabolic mechanism. Under normal circumstances, surplus carbohydrates are converted by de novo lipogenesis into free fatty acids, which turn into storage triglycerides after being esterified that can be used for β-oxidation to provide energy (Sanders and Griffin, 2016).

Probiotics Treatment in Patients with NAFLD

The therapeutic impact of probiotics on NAFLD has been assessed through a number of randomized clinical trials (RCTs). Probiotic strain composition and other supplements have a significant impact on their effectiveness. The activity of the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) considerably reduced after taking Lactobacillus acidophilus orally for a month, and some patients experienced relief from dyspepsia, according to the results of an RCT involving thirty patients (Yao et al., 2021). Probiotics have been demonstrated in numerous studies to have important therapeutic benefits in models of fatty liver in mice. A variety of strains, including Lactobacillus acidophilus, L.

plantarum, L. casei, L. bulgaricus, Bifidobacterium breve, B. longum, and B. infantis, are mixed together to form VSL#3. Giving VSL#3 to obese mice on a high-fat diet for four weeks improves the histological analysis of liver fat deposition, lowers the amount of total fatty acids, and lowers the amounts of amino-transferase plasma via inhibiting the c-Jun N-terminal kinase and NF-kB synthesis pathways. In addition, VSL#3 can reduce hepatic steatosis and insulin resistance in the same mouse model (Abenavoli et al., 2013).

Hepatic Encephalopathy

Hepatic encephalopathy (HE) is a brain dysfunction caused by liver insufficiency. It manifests as a wide spectrum of neurological and psychiatric abnormalities ranging from sub clinical alterations to coma. It is most commonly observed in patients of cirrhosis. HE encompasses a wide range of non-specific clinical indicators that have an impact on patients' and their families' quality of life. HE poses a significant threat to the healthcare system because of its high rate of hospitalization and contacts.

Minimal HE

As the most common type of HE, up to 80% of people with liver cirrhosis may have minimal hepatic encephalopathy (MHE). MHE is defined by impairment in cognitive function in the domains of attention, alertness, and integrative function; however, there are no overt clinical symptoms. It has been demonstrated that MHE has an impact on driving, quality of life, everyday functioning, and total mortality.

Overt HE

Overt hepatic encephalopathy is a generally reversible neurologic complication of cirrhosis. It is significant to note that overt hepatic encephalopathy has been linked to poor hospitalization and mortality consequences, since hospitalizations due to hepatic encephalopathy have been rising over time **(**Rahimi et al., 2021). Ammonia influences and other mechanism leading to development of overt hepatic encephalopathy such as impaired blood-brain barrier, changes in neurotransmission, proinflammatory cytokines, oxidative stress.

Pathogenesis of Hepatic Encephalopathy

In health, there is a physical space (the gut) and a common metabolism between the host and microbiota (Asnicar et al., 2021). The common metabolism between the microbiota and host is changed in cirrhosis and HE. The fermentation of non-digestible polysaccharides from the host's diet by bacteria results in the production of short chain fatty acids. By raising the output of tight junction proteins and mucin by intestinal epithelial cells, which both support barrier function, SCFAs in turn provide a vital energy source for the host colonic epithelium (Woodhouse et al., 2018). The function and permeability of the intestinal barrier are affected by these modifications to the host-microbiota connection, which facilitate the transfer of neurotoxic substances. Studies conducted at the interface between microbiology and neurology have revealed multiple mechanisms connecting microbiome to neuropsychiatric disorders **(**Skolnick and Greig, 2019). Ammonia was first identified as one such chemical with neurotoxic effects in HE. Serum ammonia levels are elevated in cirrhosis due to portosystemic shunting and poor hepatic ammonia metabolism, with further assistance from muscular and renal sources (Levitt and Levitt, 2019).

Ammonia can penetrate the blood-brain barrier and reach astrocytes, where it is transformed into glutamine, an osmole that causes swelling in the astrocytes, oxidative stress, cellular malfunction, and finally, abnormalities in brain activity (Jaffe et al., 2020). The pathogenesis of hepatic encephalopathy (HE) involves changes in the levels of inflammation, endotoxemia, and intestinal microbiota. Probiotics and symbiotics are shown as a treatment for HE since they may have positive effects on gut microbiota.

Probiotics Treatment in Hepatic Encephalopathy

Patients with HE are thought to benefit most from nonabsorbable disaccharides as their initial therapy. Lactulose decreases the generation and absorption of ammonia from the intestines by altering the gut microbiota. It also acts as an osmotic laxative, prebiotic, and gut acidifying agent (Kornerup et al., 2018). Through bacterial fermentation and the osmotic therapeutic mechanism, lactulose lowers the pH in the colon and the intestinal lumen's level of aminogenic components (Fu et al., 2022). The US Food and Drug Administration authorized rifaximin, an oral antibacterial drug, in 2010 for the prevention and treatment of HE (Jesudian et al., 2020). For individuals with HE, lactulose and rifaximin should be taken together as a therapeutic approach.

Conclusion

The advantages of probiotics for those suffering from ALD, NAFLD and other liver diseases. Strong evidence has been found linking the consumption of probiotics to improvements in liver function as measured by serum levels of ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), GGT (Gamma glutamyl transpeptidase). To stop intestinal leakiness brought on by alcohol and the onset of ALD, treatments that modify the gut microbiota are required. In individuals with non-alcoholic fatty liver disease, the use of probiotics and synbiotics lowers liver fibrosis and increases levels of the proinflammatory marker high-sensitivity C-reactive protein. Growing interest has been shown in researching probiotics as a potential alternative treatment approach for patients with NAFLD and/or NASH due to their capacity to reverse gut dysbiosis.

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Chapter 35

Probiotics and Prebiotics: Boosting Fish Gut Health, Immunity and Disease Resistance

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ABSTRACT

Probiotics are defined as live microorganisms that help in the prevention and management of particular pathological conditions. Probiotics also help to maintain a healthy balance of gut microbiota, supporting digestive and immune system functions. Prebiotics are indigestible fiber that serves as food for probiotics and other healthy microorganisms that already exist in the gut. The local or native anaerobic fauna of the alimentary tract shows resistance against pathogens. Probiotics and prebiotics have been considered for enhancing health and resilience in fish within the aquaculture system. In more important ways, these supplements modulate the structure of the microbial community for the creation of a beneficial bacteria-conducive environment and at the same time, can repress the spreading of the pathogenic strain. Such microbial modulation allows the fish better nutrient uptake, digestion and utilization for better growth performance and feed efficiency. Besides, probiotics and prebiotics are also known to have an immunomodulatory effect and have been upregulated in both innate and adaptive immune responses in fish for the decrease in incidences of infectious diseases. The use of probiotics and prebiotics in aquafeeds is an area that holds a substantial framework for enhancing the sustainability and productivity of aquaculture enterprises through improvements in gut health, immunity and disease resistance. Further, the sensible use of these supplements meets the command to reduce dependence on antibiotics and chemical interventions to maintain environmental compatibility in aquaculture practices.

KEYWORDS		
Probiotics, Prebiotics, Fish health, Immunity, Disease resistance	Received: 24-Jun-2024 Revised: 09-Jul-2024 Accepted: 10-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Prebiotics and probiotics are two main components that promote gut health and the overall well-being of a variety of organisms including animals and humans. Probiotics are defined as live organisms that, when added in adequate quantities, give health benefits to the host by regulating the balance of intestinal microbiota and enhancing immune function. These healthy bacteria can be obtained from supplements or naturally occurring in fermented foods (Venema and Do Carmo 2015). Prebiotics, on the other hand, are undigestible fiber that serves as food for probiotics and other healthy microorganisms that already exist in the gut. Prebiotics aid in developing a positive gut setting by increasing the development and activity of helpful quantities of bacteria. Probiotics and prebiotics have been gaining popularity in recent years, with an increase in research on these supplements. These may give positive outcomes to an organism's health because they work together to develop healthy gut microorganisms (Holzapfel, 2006).

Brief Overview of the Importance of Fish Gut Health

Gut health, immunity and disease resistance are very important to the health and survival of fish. The gut microbiome is of extreme importance in the maintenance of these aspects of fish health. The gut microbiome is a complex community of microorganisms symbiotically living in the gut of fish and interacting with the host. Some of their functions are digestion, nutrient absorption and regulation of the immune system. A healthy gut microbiome can help prevent pathogenic bacteria colonization and improve fish disease resistance. On the other hand, an altered gut microbiome could cause dysbiosis (an imbalance in bacterial composition) which may lead to an increase in diseases among fishes. (Gómez and Balcázar 2008).

Explanation of the Role of Probiotics and Prebiotics in Fish Gut

Probiotics and prebiotics contribute to gut health, immunity and disease resistance. Probiotics regulate the gut bacteria, help the immune system to boost itself and assist in treating several disorders related to digestion. Prebiotics help bind and remove extraneous pathogenic bacteria from the gut and preserve the health of the fish through improved gut integrity, digestion, nutrient absorption and immune function. Prebiotics promote healthy, balanced, diverse populations of gut bacteria, or healthy microbiomes (Merrifield and Ringo 2014).

Non-digestible carbohydrate fractions like inulin, oligosaccharides (galactose, fructose or mannose), β-glucans, organic acids, fructo-oligosaccharides (FOS) and mixtures of these components are the main sources of prebiotics (Van Doan et al., 2020). Further development in sustainable aquaculture develops because these prebiotics promote the overall health and growth of fish, reducing the application of antibiotics and vaccinations. Many fish species such as rainbow trout, brook trout, sturgeon, common carp, koi, African catfish, European sea bass and sea bream have been regarded to derive the advantages out of the application of prebiotics on gut morphology, pathogen-binding capability, immunostimulant property and nutrient digestibility (Hasan et al., 2023). Both probiotics and prebiotics work in conjunction to regulate the gastrointestinal microbiota (Kalita et al., 2023).

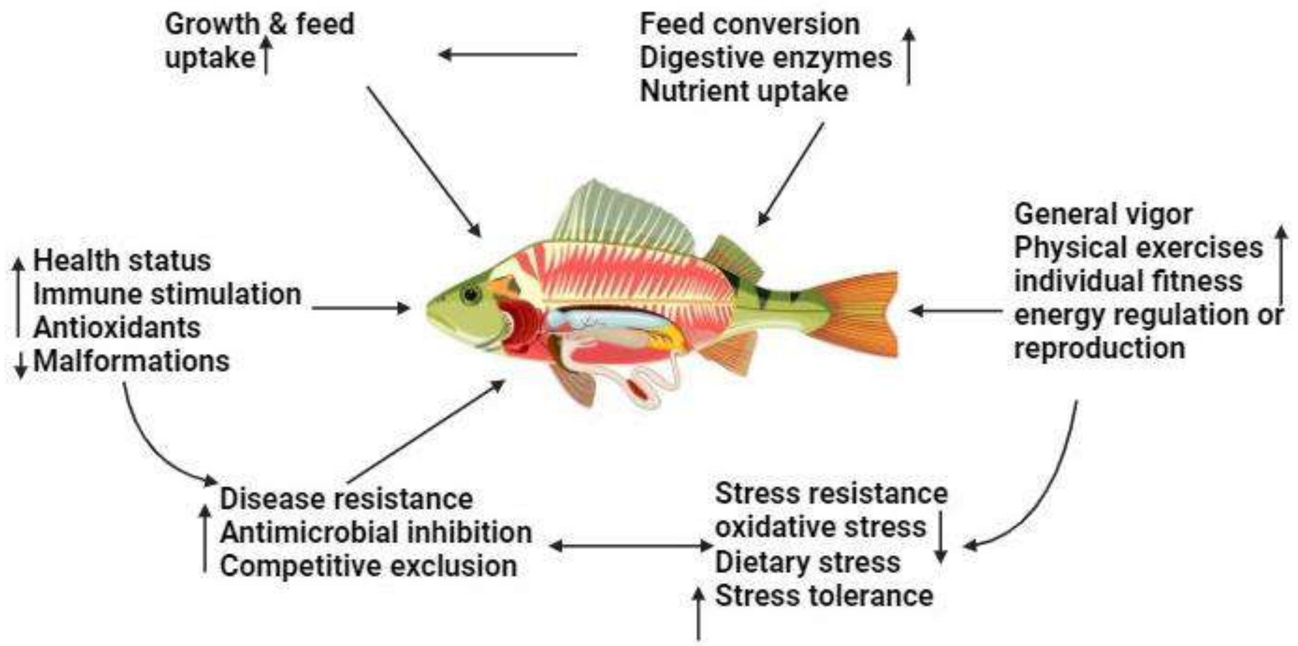

Fig 1: Diagrammatic view of the role of Probiotics and Prebiotics in fish health (small upward arrows show an increase in growth, FCR, General vigor, stress tolerance, health status, disease resistance and small downward arrows show a decrease in dietary and oxidative stress and malformations).

Mechanism of Action of Probiotics in Fish Health

Probiotics support fish gut health through many ways. Among them is the alteration of the gut microbiome. Probiotics can colonize the gut, thereby reducing the number of harmful bacteria through nutrient and binding site competition. They may also produce inhibitory substances that completely inhibit the growth of harmful pathogens (Plaza-Diaz et al., 2019). The other way is through enhancement of the gut immune system. Probiotics can also enhance fish resistance to infection by elevating immune cell production and cytokines. It boosts mucus production and tight junction proteins, which are substances that block dangerous bacteria from getting into the blood and toxins from entering the gut. These probiotics shall also increase the uptake and utilization of nutrients in the fish, thus resulting in improved growth performance of the fish (Loh, 2017).

Beneficial Examples of Probiotics in Fish Health

A large number of probiotics useful for fish have been studied and developed for aquaculture industries. For instance, probiotics benefit the utilization of feed and resist diseases, as well as improve growth in fish cultured.

1. Lactobacillus spp: *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus casei* (Wuertz et al., 2021).
2. Bifidobacterium spp: Both *Bifidobacterium longum* subsp. *Longum*
3. Enterococcus species: *Enterococcus faecium* and *Enterococcus faecalis* (Sayes et al., 2018).
4. Streptococcus species: *Streptococcus thermophiles*.
5. Bacillus species are the *Bacillus subtilis* (Martínez et al., 2012).

These probiotics can be given to fish via feed, water or direct application to their skin or gills.

Beneficial Examples of Prebiotics in Fish Health

Some of the prebiotics studied in fish are fructooligosaccharides (FOS), β-glucan (GLU), chitosan (CTS), mannan-oligosaccharides (MOS), and xylooligosaccharide (XOS) (Wee et al., 2022). All these reports showed that the prebiotics enhanced disease resistance and antioxidant potential in hybrid grouper, grass carp and Nile tilapia (Li et al., 2021). However, the effectiveness of prebiotic supplementation depends on the prebiotic structure, dosage, supplementation period, fish species and age/stage/weight. (Amillano-Cisneros et al., 2023).

Effects of Probiotics on Fish Gut Microbiota Composition

Some probiotics introduced into new fish species result in the alteration of gut microbiota and lead to changes in both growth and immunity. For example, the use of probiotic supplementation (Bacillus velezensis and Lactobacillus sakei) to rainbow trout increased beneficial microorganisms while reducing harmful bacteria, hence the regulations in growth and immunity (De Marco et al., 2023). In addition, probiotics were able to modulate the metabolome (total number of metabolites present in an organism) and therefore affect a wide range of metabolic processes in fish. All these findings emphasize the importance of probiotics for the fish gut microbiota and fish health, thus making them quite important tool in aquaculture for improving the growth and disease resistance of the host (Rohani et al., 2022). Probiotics help in the absorption and effective digestion of nutrients in fish (Wuertz et al., 2021).

Probiotics can increase the length of the intestine villi, increase the growth of beneficial microbes and decrease the load of pathogenic bacteria in the intestine with improved nutrient absorption and digestion in the fish (Assan et al., 2022). The activities of digestive enzymes in all species of fish are further boosted by probiotics, thus improving digestion and nutrient absorption (Ghori et al., 2022). Moreover, probiotics have also been shown to enhance growth and survival, as well as the intestinal morphology of fish, thereby increasing nutrient digestibility and nutrient metabolism of the organism (Gaffar et al., 2023).

Probiotics and Prebiotics in Enhancing Fish Immunity

Probiotics and prebiotics maintain healthy gut microbiota, promote beneficial bacteria, such as Lactobacillus and Bifidobacterium and suppress the growth of pathogens. Alteration of the gut microbiota supports digestion and an increase in nutritional absorption, thus resulting in better health and an increased immunity in fish. This enhances the gut barrier by stimulating mucin production and tight junction protein that serves to exclude pathogens and toxins from reaching the systemic circulation. A healthy gut barrier is very important in the maintenance of fish free from infection and diseases (Akhter et al., 2015).

Table 1: The Effects of Probiotics (gram-positive and gram-negative bacteria) against pathogens in fish.

Sr.#	Probiotics	Fish Species	Pathogens	Beneficial Effects	References
Gram Negative Bacteria					
1.	"Pseudomonas spp.	"Rainbow trout	"F. psychrophilum	"Low mortality rate.	"(Korkea-aho et al., 2011).
2.	*P. aeruginosa*	Rohu	*Aeromonas hydrophila*	High survival rate.	(Giri et al.,2012).
		Zebrafish	*Vibrio parahaemolyticus*	Improves defence mechanism	(Vinoj et al., 2015).
3.	*P. chlororaphis*	Perch	*A. sobria*	Control "*A. sobria*" infection	(Chi et al.,2014).
4.	*P. fluorescens*	Rainbow trout	*V. anguillarum*	Reduced mortality rate.	(Capkin and Altinok, 2009).
5.	Aeromonas hydrophila	Rainbow trout	A. salmonicida	Low rate of infections.	(Kim et al.,2010).
		Goldfish	*A. salmonicida*	High level of infections control.	(Wu et al.,2015).
6	*A. sobria*	Rainbow trout	*Lactococcus garvieae* and *Streptococcus iniae*	Increased disease resistance.	(Giri et al.,2012).
		Rainbow trout	*A. Bestiarum*	Protection against several pathogens.	(Vinoj et al.,2015).
7.	*A. veronii*	Common carp	*A. hydrophila*	High rate of disease resistance. .	(Chi et al.,2014).
8.	*Shewanella putrefaciens*	Gilthead seabream	*Vibrio anguillarum*	Reduced mortality rate.	(Chabrillón etal., 2006).
		Senegalese sole	*Photobacterium damselae* sub sp. Piscicida	Improved growth and disease resistance	(Diaz-Rosales et al., 2009).
9.	*S. xiamenensis*	Grass carp	A. hydrophila	Increase immunity.	Wu et al., 2015
10.	*Enterobacter cloacae*	Rainbow trout	*Yersinia ruckeri*	High survival rate.	(Capkin and Altinok 2009).

11.	*Enterococcus faecalis*	Rainbow trout	*A.salmonicida*	Low mortality rate.	(Rodríguez-Estrada etal., 2013).
12.	*Enterobacter amnigenus*	Rainbow trout	*Flavobacterium psychrophilum*	Improvement in infections control.	(Burbank et al., 2011).
13.	*Roseobacter sp*.	Turbot	*V. anguillarum*	Infection control.	(Planas et al.,2006).
14.	*Vibrio alginolyticus*	Atlantic salmon	*A. salmonicida*	Reduction in cumulative mortality.	(Hjelm et al.,2004).
15.	*Flavobacterium sasangense*	Common carp	*A. hydrophila*	Immunity increase.	(Chi et al.,2014).
16.	*Zooshikella* sp.	Olive flounder	Streptococcus inane	Increased immunity.	(Kim et al.,2010).
17.	*Phaeobacter gallaeciensis*	Cod larvae	*V. anguillarum*	Reduction in death rate.	(D'Alvise et al., 2012).
Gram-Positive Bacteria					
18.	*Carnobacterium divergens*	Atlantic cod	*V. anguillarum*	Reduction in Vibriosis.	(Al-Dohail et al., 2011).
19.	*Carnobacteria. inhibens*	Atlantic salmon, rainbow trout	*A. salmonicida, Vibio ordalii, Yersinia ruckeri*	Reduced mortalities.	(De la Banda et al., 2012).
20.	*Lactobacillus rhamnosus*	Rainbow trout	*A. salmonicida*	Decreased mortality.	(Nikoskelainen et al., 2001).
21.	*L. sakei*	Rock bream	*Edwardsiella tarda*	Reduction in cumulative mortality.	(Harikrishnan et al., 2011).
22.	*L. acidophilus*	Nile tilapia	*Pseudomonas fluorescens, Streptococcus iniae*	Strong immunity.	(Aly et al.,2008a).
23.	*L. lactis*	Olive flounder	*Streptococcus iniae*	Activated "innate immune system" and protection against pathogens.	(Kim et al., 2013).
24.	*L. plantarum*	Rainbow trout	*Lactococcus* (*Lc.*) *garvieae*	Reduction in death rate. "	(Vendrell et al., 2008).
25.	*L. pentosus*	Japanese eel	*Edwardsiella tarda*	Improve immunity.	(Lee et al., 2013).
26.	*L. brevis*	Tilapia	*A. hydrophila*	Reduction in death rate.	(Liu et al., 2013).
27.	*Leuconostoc mesenteroides*	Rainbow trout	furunculosis	High Disease resistance.	(Balcázar et al., 2007).
		Brown trout	*Aeromonas salmonicida*	Increased immunity and disease resistance.	(Balcázar et al., 2009).
28.	P*ediococcus acidilactici*	vertebral column compression syndrome (VCCS)	*Pediococcus acidilactici*	Increase survival.	(Aubin et al., 2005).
29.	*P. pentosaceus*	Grouper	*V. anguillarum*	Reduction in cumulative mortality.	(Huang et al., 2014).
30.	*Enterococcus faecium*	European eel	*Edwardsiella tarda*	Reduced edwardsiellosis.	(Aubin et al., 2005).
31.	E. *casseliflavus*	Rainbow trout	*Streptococcus iniae*	Improve growth rate.	(Safari et al., 2016).
32.	*E. gallinarum*	Sea bass	*Vibrio anguillarum*	Protection against pathogens.	(Sorroza et al., 2013).
33.	*Bacillus pumilus*	Tilapia	*A. hydrophila*	Increased immunity of fish species.	(Aly et al., 2008b).
34.	*B. circulans*	Catla catle	*A. hydrophila*	Increased immunity of fish species.	(Bandyopadhy and Das 2009).
35.	*Vagococcus fluvialis*	Sea bass	*Vibrio anguillarum*	Increased survival.	(Sorroza et al., 2012).
36.	*Bacillus subtilis* and *Bacillus licheniformis*	Trout	*Y. ruckeri*	Increased survival.	(Safari et al., 2016).
37.	*B. subtilis*	Indian major carp	*A. hydrophila*	Control of Infection rate.	(Kumar et al., 2006).
		Rainbow trout	*Aeromonas*	Increase survival of fish species.	(Newaj-Fyzul et al., 2007).
		Channel catfish, striped catfish	*Edwardsiella ictaluri*	Reduced mortality rate.	(Ran et al., 2012).
		Red hybrid tilapia	*Streptococcus agalactiae*	Reduced mortality rate.	(Ng et al., 2014).
		Grouper	*Streptococcus* sp.	Increased survival rate.	(Liu et al., 2012).
38.	*Kocuria* sp.	Rainbow trout	*V. anguillarum* and *V. ordalii*	Reduced mortality rate.	(Sharifuzzaman and Austin 2010).

39.	*Brochothrix thermosphacta*	Rainbow trout	*A. bestiarum*	Protection from skin infections.	(Ng et al., 2014).
40.	*Rhodococcus* sp.	Rainbow trout	*V. anguillarum*	Batter protection against pathogens.	(Sharifuzzaman et al., 2011).
41.	*Micrococcus luteus*	Nile tilapia	*A. hydrophila*.	Reduced mortality rate.	(Abd El-Rhman et al., 2009).
		Rainbow trout	*A. salmonicida*	Better survival.	(Sharifuzzaman et al., 2011)
42.	*Clostridium butyricum*	Rainbow trout	Vibriosis	increase disease resistance.	(Pan et al., 2008).
		Chinese drum	Vibriosis	Increased phagocytic activity, resistance to Vibriosis .	(Pan et al., 2008).
43.	*Kocuria* sp.	Rainbow trout	*V. anguillarum* and *V. ordalii*	Reduced mortality rate.	(Sharifuzzaman and Austin 2010).
44.	*Brochothrix thermosphacta*	Rainbow trout	*A. bestiarum*	Protection from skin infections.	(Ng et al., 2014).
45.	*Rhodococcus* sp.	Rainbow trout	*V. anguillarum*	Batter protection against pathogens.	(Sharifuzzaman et al., 2011).
46.	*B. subtilis B. licheniformis*	Olive flounder	*S. iniae*	Higher survival rate.	(Cha et al., 2013).
47.	*B. licheniformis*	Tilapia	*S. iniae*	Increase disease resistance.	(Han et al., 2015).
48.	*B. amyloliquefaciens*	Nile tilapia	*Yersinia ruckeri*, *Clostridium perfringens type D*	Increased survival rate.	(Selim and Reda 2015).
Yeast					
49.	*Debaryomyces hansenii*	Leopard grouper	*A. hydrophila*	Enhance disease resistance.	(Reyes-Becerril et al., 2011).
50.	*Saccharomyces cerevisiae*	Tilapia	*A. hydrophila*	decreased death rate.	(Abdel-Tawwab et al., 2008).
51.	*Saccharomyces cerevisiae* var. *boulardii*”	Rainbow trout”	*A. hydrophila”*	Enhance disease resistance.”	(Quentel et al., 2005).”

Probiotics and prebiotics enhance the immune system of fish by increasing the cell production such as macrophages, neutrophils and lymphocytes. They also enhance the secretion of immunoglobulins and cytokines, which are important in immune defense and regulation. This means that the use of probiotics helps fish, to protect themselves from pathogens through the modulation of fish immune response. Some of probiotic bacteria produce antimicrobial compounds, such as bacteriocins, organic acids and H2O2 that prevent the growth of pathogenic bacteria in the gut (Hoseinifar et al., 2015).
Improved health status was given to histo-morphological changes in the fish gut by the prebiotic and probiotic treatment; for instance, Nile tilapia (Oreochromis niloticus) shows growth and resistance to pathogens and an improvement in physiological conditions by dietary supplementation with probiotics and prebiotics (Sîrbu et al., 2022).

Overview of Fish Immune System

Innate immune as well as adaptive immune components, classify the fish immune system, which together performs vital role in the defense against pathogens and securing fish from death in various aquatic environments. Fish immune components consist of physical barriers, cellular and humoral factors, hence making part of the innate immune system and serves as the first line of defense. (You et al., 2022). Macrophages, neutrophils and natural killer cells recognize and phagocytize pathogens, while other structures include complement proteins and antimicrobial peptides that neutralize and eliminate the invaders. More structures that are added are the mucosal-associated lymphoid tissues (MALT) in the gut and gills which have an important role in immune surveillance and response. (Bermudez-Brito et al., 2012).

In contrast, the adaptive immune system is much more specific, made up of lymphocytes B and T-cells and antibodies. B cells are responsible for producing antibodies against particular antigens, while T cells take a controlling function in immunity reactions and the killing of infected cells (Smith et al., 2019). For example, in jawless fish, the adaptive immune system is characterized by variable lymphocyte receptors (VLRs), while in jawed fish, it consists of major histocompatibility complex (MHC) molecules, enabling recognition and response to a wide array of pathogens (Plaza-Diaz et al., 2019). Also, the immune system of fish can be influenced by environmental factors, stressors and management practices. Thus, there is a need for the optimum husbandry practices to maintain immune function and health in aquaculture operations. (Mokhtar et al., 2023).

Probiotics and Prebiotics in Preventing and Treating Fish Diseases

Probiotics when applied in adequate quantities increase the phagocytic, lysozyme, complement, respiratory burst activity and cytokine expression in fish while stimulating the gut immune system with significant increases in the number of Ig (+) cells and acidophilic cells (Nayak, 2010). Prebiotics, on the other hand, have been shown to enhance growth, non-specific immunity, disease and stress resistance and antioxidant activities in fish species (Zhu et al., 2023). Probiotics and prebiotics in fish and shellfish act on the innate immune system, thereby increasing disease resistance and the overall health of the organisms (Akhter et al., 2015).

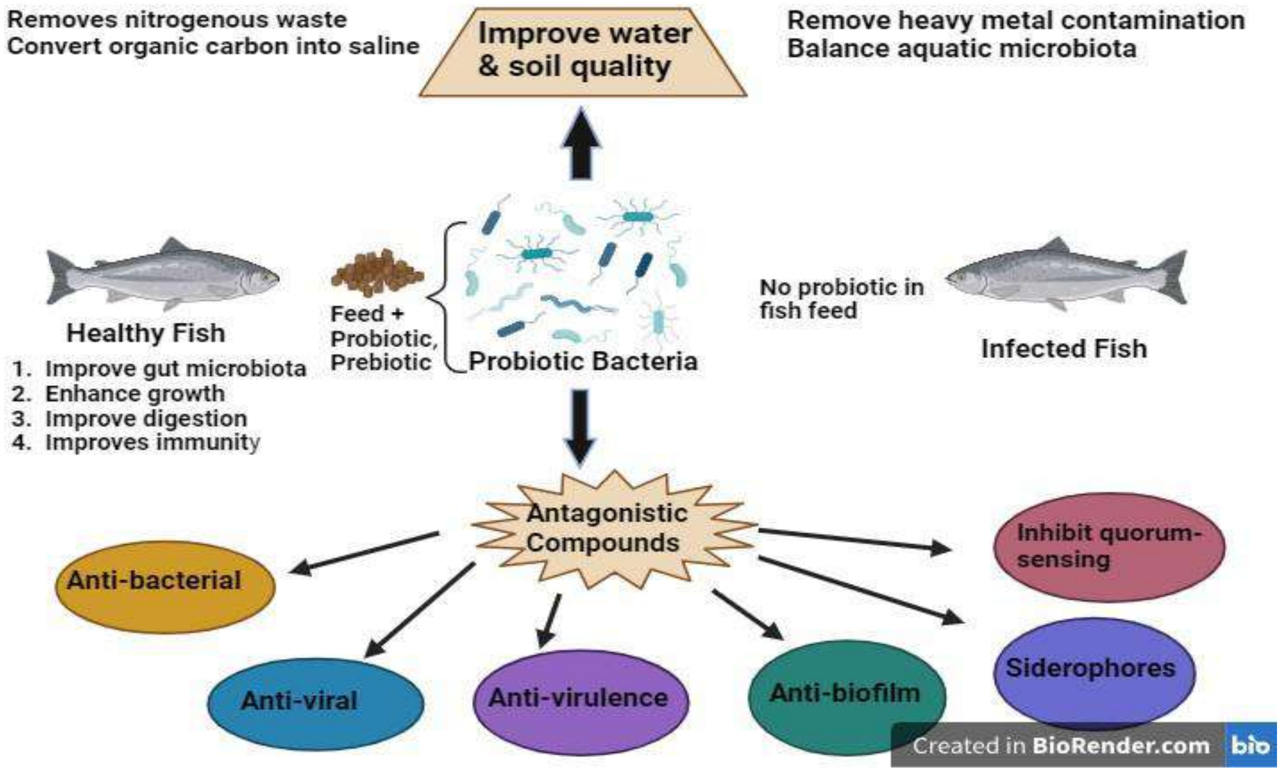

Fig 2: Diagrammatic representation of the role of probiotics and prebiotics in enhancing fish immunity

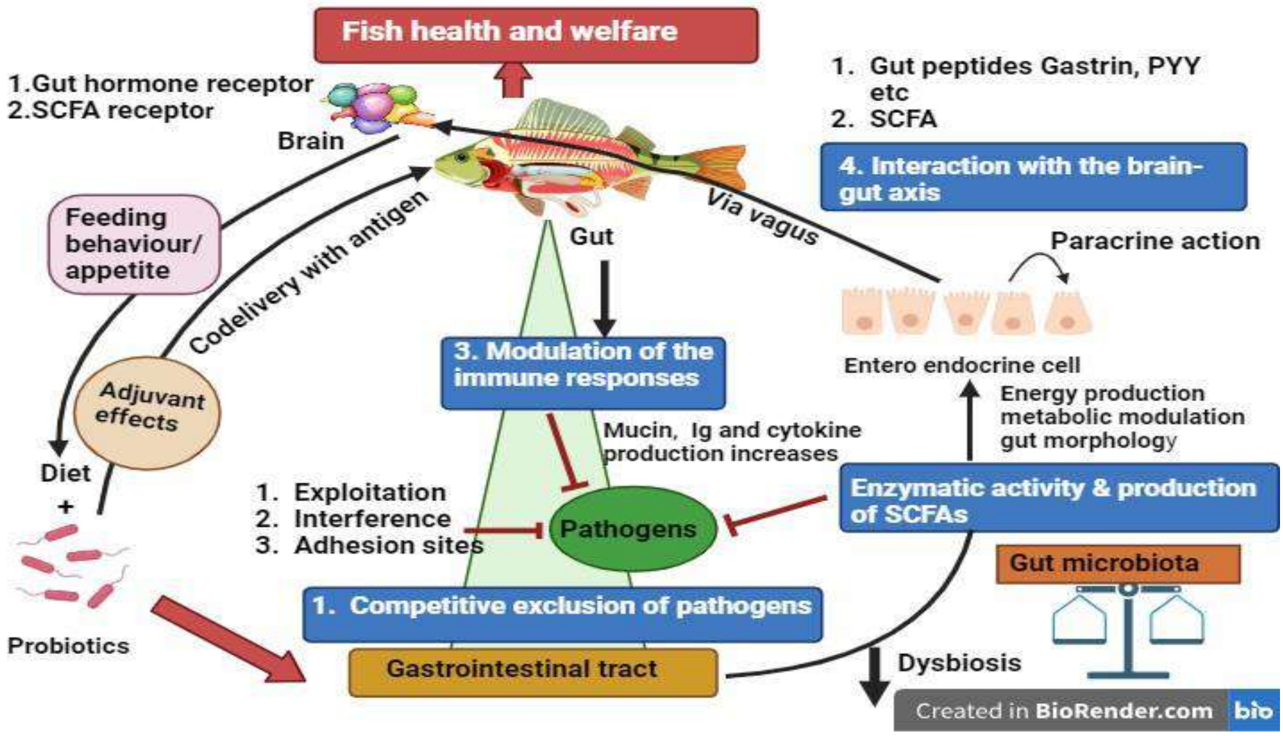

Fig 3: Probiotics and Prebiotics in Preventing and Treating Fish Diseases

Other methods being developed for the prevention and treatment of diseases in fish include the use of probiotics, prebiotics and synbiotics. Prebiotics stimulate gut microbiome, which enhances host immunity and the production of antibacterial substances to regulate bacterial, viral and parasitic diseases in the various species being used in aquaculture. Probiotics have been documented to reduce mortality in fish species such as the Atlantic salmon and rainbow trout (Hoseinifar et al., 2018). It improves growth performance, immune response and disease resistance in aquaculture species. (Wei et al., 2022).

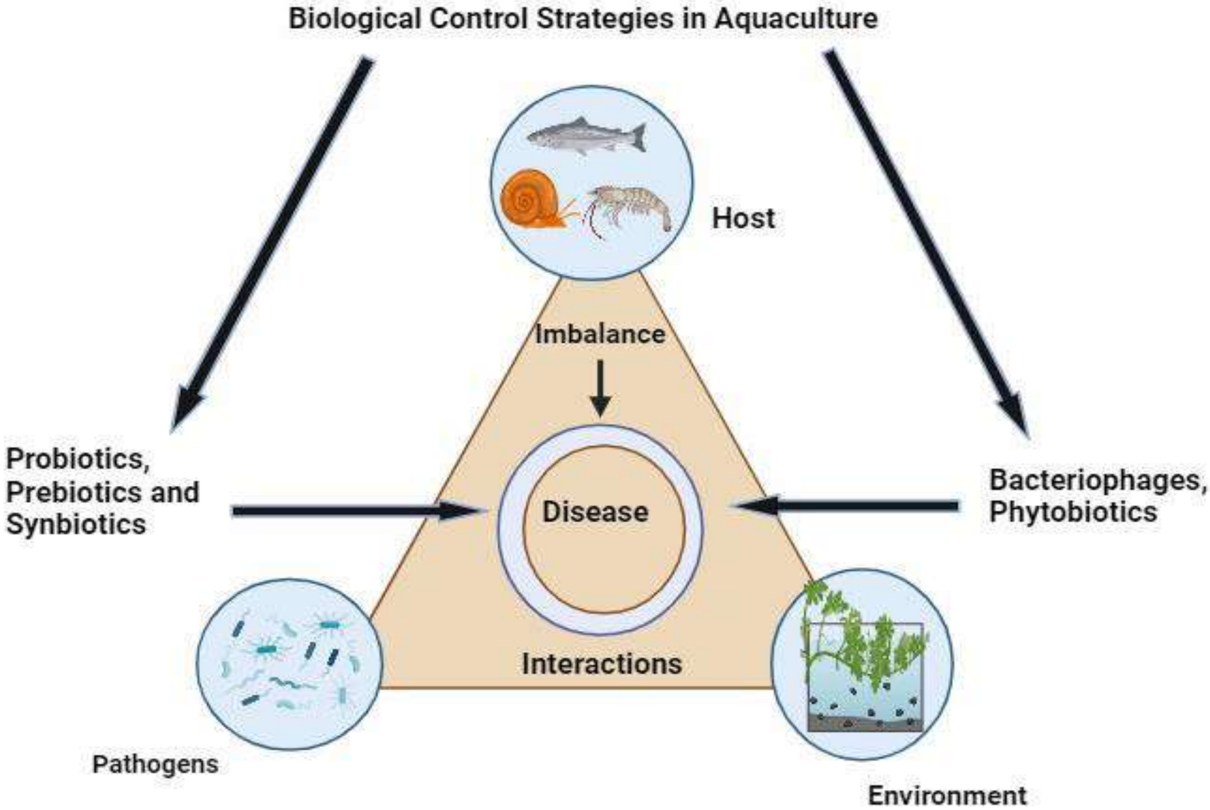

Fig. 4: Control strategies to control diseases through the use of Probiotics, Prebiotics and Synbiotics

Case Studies and Examples

Survival was enhanced in Rainbow trout by the use of the probiotic Micrococcus luteus. The application of probiotics has been shown to bring about lower mortalities in a variety of fish species (Wuertz et al., 2021). Prebiotic administration enhances the activity of probiotics, make them more resistant to reactive oxygen species (ROS) and enables them to pass through the gastrointestinal tract. In addition, growth and physiological conditions were increased in Nile tilapia exposed to a bacterial pathogen in the presence of probiotics and prebiotics. The potential of probiotics and prebiotics in disease control within aquaculture is increasingly recognized; as a result, numerous studies are currently being published with the use of these agents as antibiotic alternatives. (Hoseinifar et al., 2018).

Challenges and limitations of Probiotics and Prebiotics in Fish Health

Limitations of the use of probiotics and prebiotics in fish health are species-specific because of the differences in the gut microbiome of each species. Other challenges due to the use of probiotics in aquaculture include the persistence of probiotic strains in the digestive tract, resistance to acid and bile salts and interaction with host gut defenses (Wuertz et al., 2021). Other potential risks from the use of probiotics that should be taken into consideration are such as antibiotic resistance and risks of transferring genetic elements to other microorganisms in the gut of the fish. The general health status, along with further factors like the quality of the water, diet, stressors or other adverse situations, would condition the efficacy of the probiotics and prebiotics in prophylaxis and treatment of diseases in fish (Sîrbu et al., 2022). These highlight the limitations and need for more research on the interactions of probiotics and prebiotics with fish health and the possible risks and benefits in aquaculture practice. (Cruz et al., 2012).

Stability and Shelf-life of Probiotics and Prebiotics

Probiotic stability is affected by many factors, including packing, moisture and temperature. Most of the shelf-stable probiotics are meant to be used within one to two years (Butt and Volkoff 2019). Additionally, through un-opened blister packs, there is a protection from heat and humidity, which increases the shelf life of the probiotics. For example, Bacillus is one of those probiotic strains that are more heat- and environment-friendly and therefore more stable. The usual storage condition recommended by manufacturers is a cool, dry place away from direct sunlight to extend the shelf life of the probiotics. (Alvanou et al., 2023).

Freeze-dried probiotic products should be able to achieve low water activity levels for a long shelf life at room temperature. The probiotic supplements that require refrigeration are likely to have this indicated on their labels (Ringo et al., 2022). The composition and formulation of the probiotics are critical in the determination of their stability. By their prebiotic content, they are usually combined with other ingredients like fibers, sugars, or sugar alcohols which might influence their stability and shelf life. Manufacturers are in the process of developing formulations that will reduce the potential for contamination while retaining prebiotic activity upon storage (Alvanou et al., 2023).

Potential Risks and Side Effects of Probiotics and Prebiotics

Probiotics and prebiotics for fish have relatively few risks and side effects, but some considerations should be made:

Probiotics

Thus, the use of inappropriate probiotic strains for specific fish species causes negative effects (Hoseinifar et al., 2018). Probiotics could theoretically lead to systemic infection in some susceptible hosts, although the actual risk is generally low (Martínez et al., 2012).

Prebiotics

High levels of prebiotics may lead to some negative effects on aquatic animals, and some types of prebiotics enhance growth without elevating immunity. Probiotics and prebiotics in fish are safe and beneficial, but careful selection with proper dosage and monitoring for any potential side effects is necessary (Wee et al., 2022).

Conclusion

In conclusion, this chapter emphasized the importance of these supplements to improve fish gut health, immunity and disease resistance in aquaculture. From the data and research findings, it is obvious that probiotics and prebiotics have multiple effects on the fish's gut microbiota. This has a beneficial balancing effect, which improves the absorption of nutrients, digestion and general metabolic efficiency. These supplements, in addition, possess immunomodulatory properties that further improve the innate defense mechanisms of the fish, decrease susceptibility to infectious agents and increase resilience to diseases. All these combined approaches enhance production indices such as growth performance and feed utilization while supporting the goal of sustainable aquaculture through reduced reliance on antibiotics and chemical interventions. It is this order of thinking that leads us to modern aquaculture complexities of integrating probiotics and prebiotics as a promising avenue toward optimized fish health and welfare, assuring long-term viability and resiliency of aquaculture operations in the face of evolving challenges

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Chapter 36

Mitigating Zoonotic Transmission: Probiotics and Prebiotics Strategies in Control of Brucellosis

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ABSTRACT

Brucellosis, a zoonotic disease caused by different *Brucella species*, poses various threats to public health globally. The chapter provides an overview of brucellosis and the challenges associated with its control. The primary focus is on the use of probiotics and prebiotics to reduce zoonotic transmission. It initiates with the etiology, pathogenesis, transmission routes, and epidemiology of brucellosis and discusses the mechanisms behind prebiotics and probiotics. Probiotics are live microorganisms that intend to have proven health advantages, improve gut health, modulate immune response, and mitigate brucella colonization. On the other hand, prebiotics are high-fiber, non-digestible components in food that foster the activity of beneficial bacteria. They offer complementary therapeutic approaches by promoting a microbiome-friendly environment for host defense mechanisms. After thoroughly analyzing assorted studies, this chapter explains the synergistic effects of probiotics and prebiotics in mitigating brucellosis incidence. Utilizing prebiotics and probiotics to meliorate the gut health of the host and their role as adjuvant strategies in brucellosis transmission advocate the initiative to reduce zoonotic diseases and improve public health.

KEYWORDS
Animal health, Cross-species transmission, Gut microbiota, Immunomodulation, Malta fever, One Health

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INTRODUCTION

Brucellosis is a zoonosis that affects humans, wildlife, and animals, including cows, goats, sheep, and swine. This disease is caused by different *Brucella species*, with an estimated two million new human cases per year. The infection has had sufficient impacts on public health yet is ignored, causing economic downturns, especially in low-income countries. The zoonotic ailment is transmitted from animals to humans by direct contact with infected animals or using products obtained by those animals (Qureshi et al., 2023; Ahmad et al., 2024). In humans, brucellosis can cause respiratory illnesses, Malta fever, and osteoarthritis in case of chronic infections (López-Santiago et al., 2019). Brucellosis, also named Malta fever, is historically prevalent in the Malta region (Koul, 2015). A significant portion of growing infectious diseases that affect humans is of animal origin, with a gauge showing that 60% of all human pathogens and 75% of growing human pathogens are zoonotic (Bueno-Marí et al., 2015). Therefore, the importance of zoonotic transmission is highlighted (Dubey et al., 2021). Zoonotic diseases can also be transmitted indirectly via vectors such as vertebrates and arthropods (Razgūnaitė et al., 2019). The proximity of humans to animals, such as in animal exhibits, is strongly associated with zoonotic outbreaks, highlighting the necessity for enhanced surveillance to mitigate these diseases (Bender and Shulman, 2004).

Probiotics and prebiotics are essential to regulate the immune system's activity and maintain gut health. Probiotics are active microbes that offer health benefits to the host when administered in sufficient quantity. They are typically yeast and bacteria with lactic acid bacteria, and bifidobacteria are more common. These microorganisms can potentially eradicate some hidden diseases (Ogueke et al., 2010). Prebiotic compounds are the food we cannot digest but tend to have a healthy effect on the body. Gas production is helpful because it enhances probiotic bacteria functioning by releasing growth-stimulating substances in the gut (Antoniadou and Varzakas, 2021). Also, the status of digestive health associated with using probiotics and prebiotics depends on the strain and structure due to many influencing factors, including age, gender, and health condition (Bender and Shulman, 2004).

The disease spreads contagion at the public health scale and has a far-reaching economic influence, mainly affecting rural living near animals (Mitiku and Desa, 2020). Incorporating probiotics and prebiotics as microbe managing agents of brucellosis is based on the fact that they can improve immune functions and host health, which may contribute to reducing zoonotic diseases. Thus, they are less than receptive to both the problem of antibiotic resistance and abusing the necessity of it (Ogueke et al., 2010; Pattanaik et al., 2022).

Brucellosis: A Zoonotic Threat

Etiology and Pathogenesis of Brucellosis

Brucella pathology is significantly affected by components of etiology in terms of pathogenesis. (Al-Tubaikh, 2010; Goldman and Schafer, 2020). Different Brucell*a species cause brucellosis*. *Brucella abortus, B. melitensis, and B. suis* are primary etiological agents in cattle shows the pathogenesis of Brucella. The pathogenesis of brucellosis is complex, as bacteria enter the host cell and escape the immune defenses, causing prolonged infection. This deft pathogen survives and replicates within the host cells, avoiding the immune system. Brucella decreases bactericidal activity, stops phagocytosis, abates endotoxic reactions, and hinders antigen presentation (Byndloss and Tsolis, 2016; Elrashedy et al., 2022). The pathogenesis of Brucella further expands its capability to persist and multiply in both phagocytic and non-phagocytic cells and its potential to exploit host cellular processes, prevent host cell apoptosis, and disrupt phagocytosis. Brucella persistence in water, dairy products, and meat further enhances its transmission (Głowacka et al., 2018). This disease significantly impacts the economy and public health, necessitating ongoing efforts in prevention, treatment, and control (ur Rahman et al., 2019).

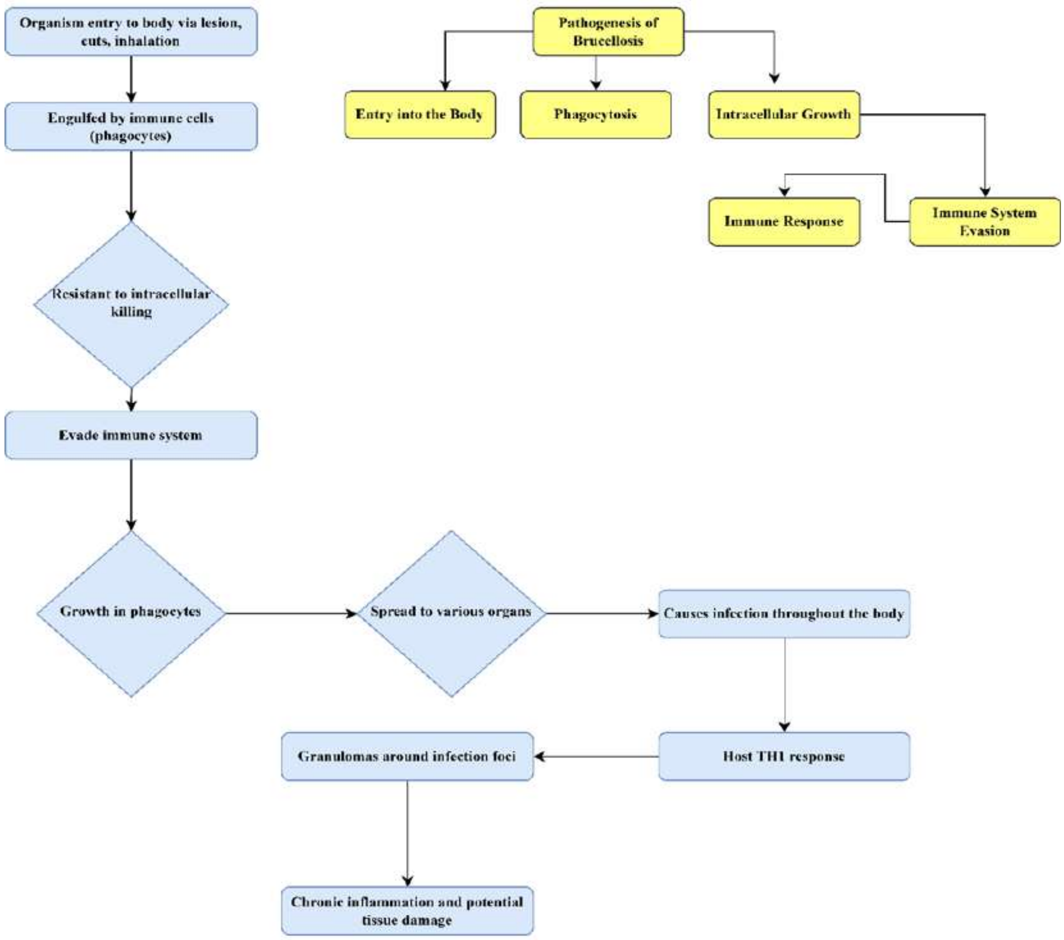

Fig. 1: Illustrates the pathogenesis of brucellosis, depicting bacterial invasion of host cells, immune evasion mechanisms, and the establishment of chronic infections.

Transmission Routes of Brucellosis to Humans

Brucellosis spread to humans via direct contact with infected animals, their secretions, and by consuming unpasteurized products obtained from infected animals (Mostafavi et al., 2011; Babaei et al., 2020). The disease, also transmitted by inhalation of aerosols or mucous membranes representing a threat to people close to animals such as herdsmen and butchers, demonstrated an elevated infection rate among butchers in Nigeria (Cadmus et al., 2006). Apart from this, seroprevalence studies point out that people who live close to livestock have a higher risk of transmission (Nakeel et al., 2016; Babaei et al., 2020). Although brucellosis is a zoonotic disease that transmits from animals to humans, there is evidence of cross-species infection among animals, which complicates the epidemiology of the disease (Shoukat et al., 2017). For instance, cattle can be hosts of *B. melitensis and B. abortus*, and it is the host that resolves their genetic links in either sense. The spread of harmful infections (cross-species transmissions) brings the infectious person and the human population into one health circle. The issue of Brucellosis has been unwavering since the 19th century. This disease is Indigenous in various parts of the world. i.e., the Mediterranean region (Carlson et al., 2018) depicts the transmission routes of the disease adapted from (Bugeza et al., 2023). In small ruminants, a particular variation in the prevalence of brucellosis has been stated in different regions of the world (ranging from 1% to 32%) (Jamil et al., 2020; Madan et al., 2022).

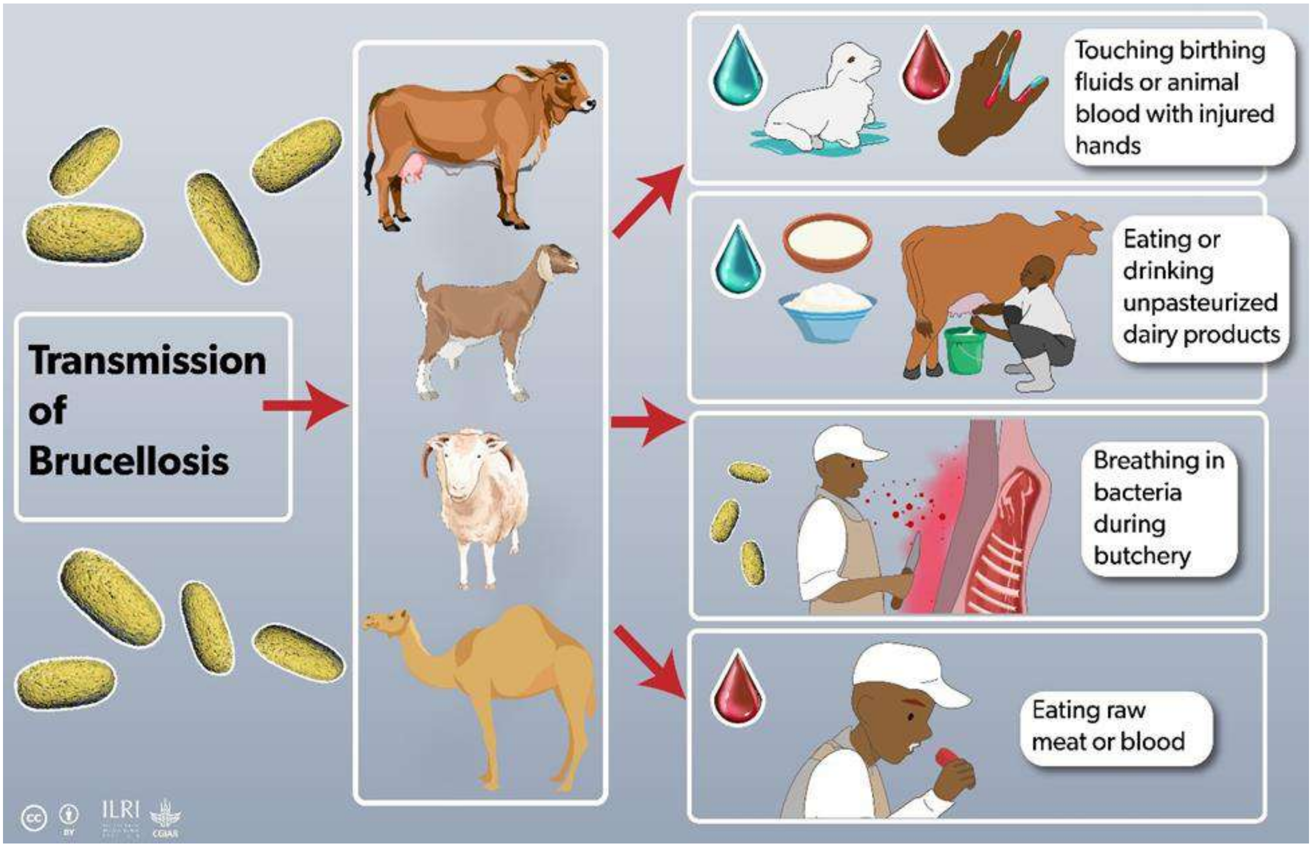

Fig. 2: Highlights the intricate pathways through which brucellosis spreads, providing a comprehensive visual representation of its transmission routes.

Global Impact and Epidemiology of Brucellosis

The epidemiology of this disease is complex, with variations in occurrence observed across many regions. For example, data from a recent case study showed a decline in the number of human cases over time in Iran, attributed to the success of implementing guidelines for the management and control of the outbreak. The decrease in vaccination rates and the spread of brucellosis among livestock might increase brucellosis incidence among livestock (Mostafavi et al., 2011). On the other hand, the significant prevalence of pyretic patients who consumed unpasteurized milk denotes a continuous spread from animals to humans (Badri and Mohamed, 2018). The continuous existence of this disease is due to several reasons, including cultural practices, rural customs, and insufficient vaccination schedules, leading to rapid transmission (Koul, 2015). Epidemiological surveillance is a technique used to collect, analyze, interpret, and publicize health data to prevent and control diseases. In the human population, there is a need to guess the magnitude of the problem (illness rates, death rates, the proportion of cases resulting in death, disability rates, and patterns of infection over time), risk factors (direct contact with animals, unpasteurized milk, fresh cheese) and identifying people are at high risk, improving detection and treatment levels. Outbreak detection and brucella species change in a specific population of animals are crucial for successful activity (Garin-Bastuji, 2012; Ismail et al., 2016).

Challenges in Brucellosis Control

The main challenge in controlling brucellosis in humans is to control animal diseases. By preventing animal diseases, human cases can be reduced due to zoonotic transmission. In developed countries, brucellosis is eradicated or controlled through long-term and costly animal vaccination programs and culling of infected animals. Live vaccines such as *B. abortus* S19 and *B. melitensis* Rev.1 have been proven fruitful for controlling brucellosis in bovines and small ruminants worldwide. Controlling brucellosis requires effective vaccination (Aragón-Aranda et al., 2020).

One Health is a collaborative approach to controlling brucellosis. This approach involves coordination between human health sectors, animals, and livestock holders and initiating educational programs to raise awareness. Proper food hygiene is crucial to prevent human diseases. Contaminated dairy products are the source of transmission of diseases to humans, so pasteurization of milk is super important (Chen et al., 2023). Other challenges include more national infrastructure, public health education, and limited public awareness. It is essential to address these issues for effective control (Seimenis et al., 2019).

Role of Probiotics in Brucellosis Control

Mechanisms of Action of Probiotics

Probiotics produce their effects via a variety of mechanisms of action. They oppose microbes directly by exerting such substances that inhibit infectious agents and by competing for nutrients and attachment sites on the host's epithelial cells, in this way preventing colonization for pathogens (Lukic et al., 2017; Savitri et al., 2021). They also improve the integrity of the gut epithelial barrier, which serves as a primary defense against microbes (Lukic et al., 2017; Zhou et al., 2024).

Appealingly, probiotics possess immunomodulatory effects, interacting with immune cells like macrophages, intraepithelial lymphocytes, and natural killer cells. They influence the formation of cytokines, including signal molecules that regulate inflammatory and immunity reactions. Probiotics exhibit the ability to change both the activities and composition of the gut microbiota such that the beneficial bacteria occupy a more significant portion of the gut, hence leading to the makeup of a good gut environment (Azcarate-Peril, 2019; Zhou et al., 2024). These qualities allow them to practice stopping and treating disorders, which are mainly immune-related. More studies in this area would help characterize the mechanisms and optimal strategies in the clinical environment (Keerthi et al., 2023). Furthermore, the emerging findings of the studies have shown that probiotics are closely related to an extensive and significant improvement of inflammation and oxidative stress biomarkers in humans (Tabrizi et al., 2019; Zamani et al., 2020). Brucella commonly occurs through mucosal surfaces showing diverse mechanisms of probiotic action in promoting health adapted from (Latif et al., 2023). That is why developing mucosal-administered vaccines could favor managing brucellosis at the microbe entry sites. Mucosal vaccines can simultaneously enhance humoral and cell-mediated immune response at a systemic level. In lactic acid bacteria, the probiotic. The probiotic strain of *lactobacillus casei* is a suitable candidate for antigen delivery in inactivated lactic acid bacteria (Mohammadi and Golchin, 2020).

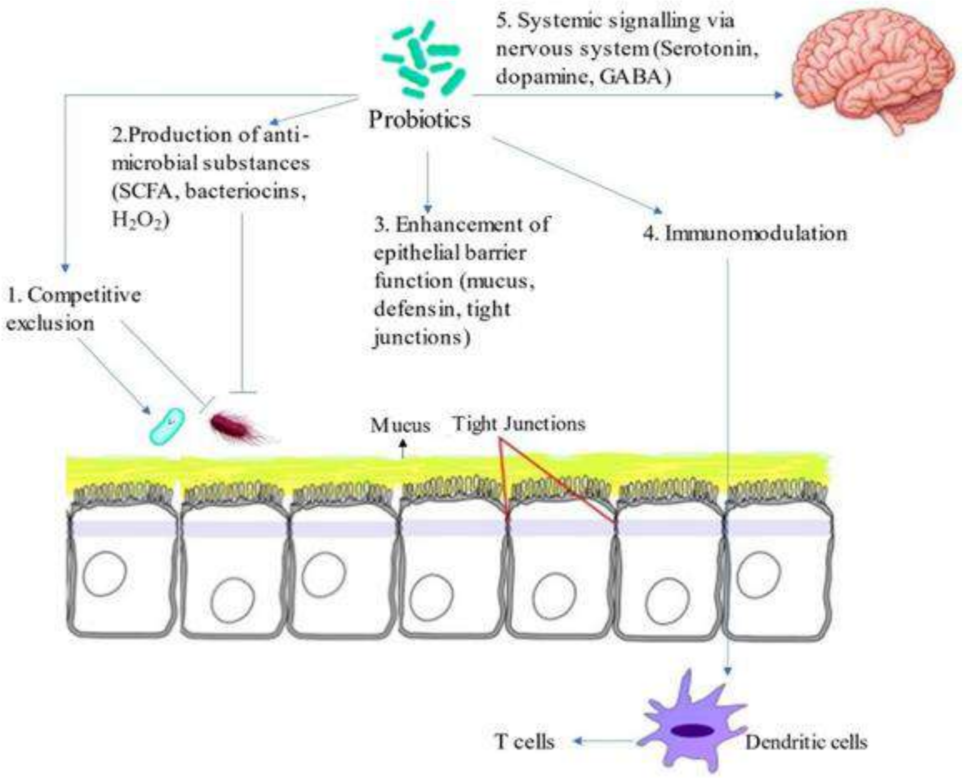

Fig. 3: This figure illustrates the multifaceted roles of probiotics in maintaining and enhancing gut health.

Evidence of Probiotics in Animal Models of Brucellosis

Numerous studies have extensively reported the use of probiotics in animal experiments and the ability to regulate inflammation associated with lesions of the intestines, including brucellosis (Devi et al., 2021). The human gut reaches 70% of its overall composition from bacterial cells widely dispersed in the colon. The gut microbiome mediates a crucial function while guarding the gut's health. Normal microbiota is a critical element of mucosal immunity of gut epithelium and animal cellular models. Lactobacillus and Bifidobacterium emerge as pivotal bacterial strains renowned for managing gut inflammation, exhibiting robust proliferation in mouse models (Devi et al. et al., 2021). The findings from the research on the animal models likely reveal that probiotics can lower the infection rate and help reduce bacterial load. An example of antibiotic-like effects of probiotic use in the experimental study is the prophylactical and therapeutical treatment of white mice exposed to the highly harmful *B. melitensis* 16M strain (Gavrilova et al., 2020). The gut-brain linkage is highly complicated and involves the enteric nervous system and hypothalamus-pituitary-adrenal axis, which results in inflammatory bowel disorders. Probiotics care about the defensiveness via dendritic cells and guide the system to strengthen and hinder the microbe transition (Gavrilova et al., 2020).

Potential Benefits and Limitations of Probiotics

Probiotics support the clinical immune response, sustain biota in the gut, and benefit diseases such as type 2 diabetes mellitus (T2DM), gestational diabetes mellitus, and rheumatoid arthritis (RA), which broaden their application (Khalesi et al., 2019; Zheng et al., 2021b). Several studies have been conducted, and they have proved that probiotic levels can be the most effective in reducing visceral fat and triglyceride levels when used as a powder (C. Wang et al., 2020). There has been documented evidence that probiotics reduce CRP (C-reactive protein) and inflammation cytokines significantly in people who suffer from RA; on the other hand, the probiotics increase the level of anti-inflammatory IL-10 (interleukin) in these patients (Pan et al., 2017). In addition, they have been found to positively affect the fastening rate of blood sugar and rejuvenate the diversity in the gut microbiota of pregnant ladies who suffer from gestational diabetes mellitus (Zheng et al., 2021a). However, there are some limitations regarding probiotic use. Their efficiency is uncertain because they have poor tolerance to bile salts and acids, which may sabotage their benefits and risk of antibiotic resistance (Y. Wang et al., 2020).

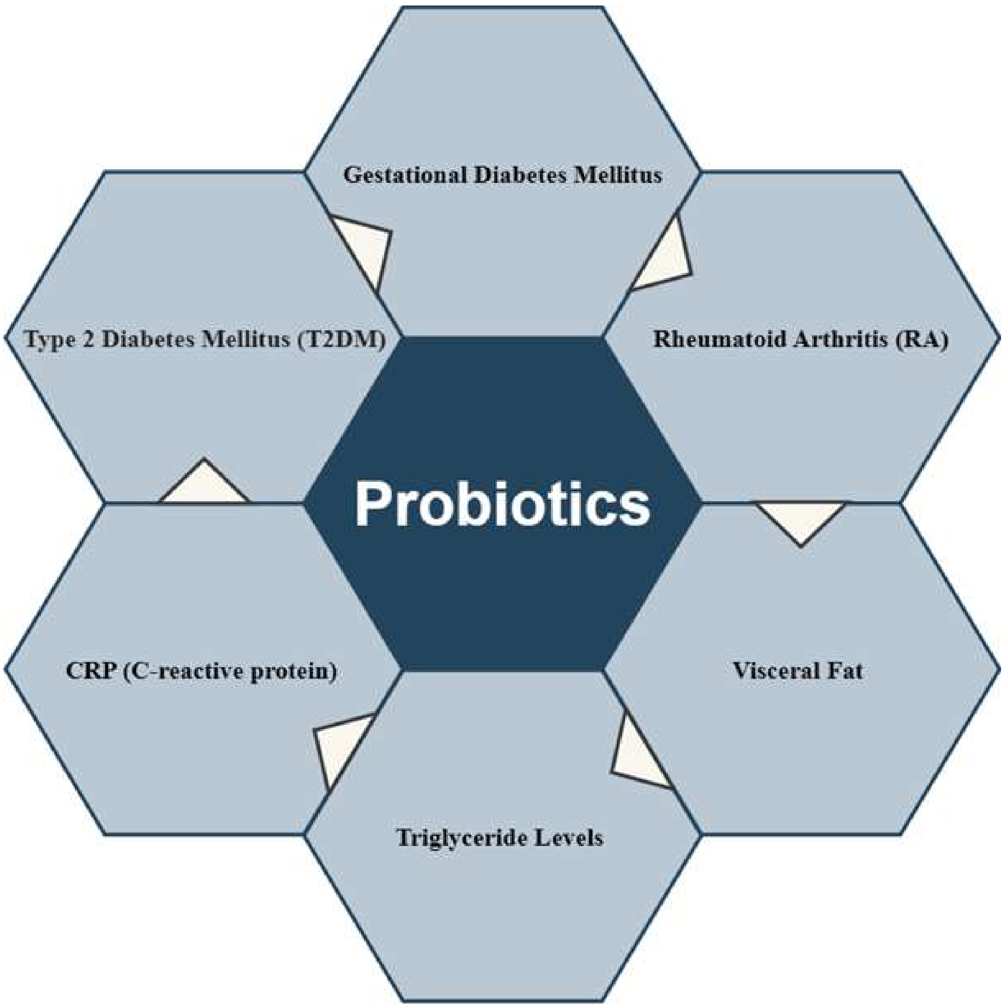

Fig. 4: This figure highlights the diverse therapeutic benefits of probiotics in managing various diseases and conditions.

Prebiotics as Adjunctive Therapy in Brucellosis Management

Overview of Prebiotics as Adjuvant Therapy

Prebiotics are a type of dietary fiber that aids in the growth of normal microflora. It has many types, including inulin, oligofructose, fructooligosaccharides, and galactooligosaccharides (Korcz et al., 2018). However, the extent of prebiotic effects and the range of their compounds containing prebiotic activities are subjects of continuous research, and new findings have the potential to expand the benefits and definition of prebiotics (Valcheva and Dieleman, 2016; Carlson et al., 2018).

A process known as "cross-feeding" occurs when various microbes work together to use complex carbohydrates effectively. To use partially broken-down products from dietary carbohydrates or to ingest fermentation end-products like lactate and acetate, for example, bifidobacteria cross-feed with butyrate-producing bacteria (Belenguer et al., 2006). These findings provide insight into a more comprehensive understanding of prebiotics, which monitors favorable changes in the gut microbiota as a population rather than concentrating on certain target bacteria. Within this context, prebiotics are considered suitable candidates for dietary carbohydrates that are fermented by the gut microbiota and improve the synthesis of beneficial metabolites in the gut (Bindels et al., 2015).

Experimental and Clinical Evidence of Prebiotics in Brucellosis

Although data on the role of prebiotics in brucellosis is minor, it is essential to know prebiotics' effects on gut health. Let us examine the information: According to the World Health Organization (WHO), which measures the frequency and seriousness of human brucellosis's clinical signs and symptoms. Medical professionals were indeed in a position to drive this historic change as they had seen patients suffering varying systemic infections and neurological disorders. They also observed patients fully recover after ST. Among these patients, about half were revealed to have similar conditions, such as muscular pain, joint pain, and backache (Dean et al., 2012). The disability weight of acute brucellosis would be 0.190; however, chronic, and localized brucellosis will have 0.150 disability weights based on the 2004 disease weight. Often, this results in calves being born ignorant of brucellosis disease, which undermines the efforts in production in such areas. On the other hand, the main advantage of probiotics is their effect on the two types of healthy bacteria named Lactobacillus and Bifidobacterium (Martinez et al., 2015). Presently, probiotics help promote the growth of the right kind of overwhelming bacilli and immunologic function by nurturing the formation of suitable species. Although a known promise of prebiotics is the stimulation of the gut microbiome, which is crucial to let them be effective against brucellosis, the scientific evidence of their effect on brucellosis is still few due to the nature of having to alter the gut flora (Hedin et al., 2007). Therefore, ample research must be pursued to unequivocally ascertain the exact role of prebiotics in gut health upkeep with brucellosis (Dean et al., 2012; Jadhav et al., 2023).

Indeed, both probiotics and prebiotics work together beneficently when combined into a product line, in which they bind to the intestinal lining, thus helping prevent disease, promote healthy digestion, and sustain proper immune function (Akhter et al., 2015; Carlson et al., 2016). Moreover, it becomes clear that certain probiotics and prebiotics are intricate and unique; hence, the need for a customized approach emerges (Wu et al., 2016; Wang et al., 2022).

Challenges and Opportunities

The legal aspect pertains to safety, while science can validate these treatments. Through scientific proof, one can be assured that prebiotics and probiotics are verified precisely (Sanders et al., 2005). It is a big issue due to diverse levels of consumer understanding. Official interpretations and standards contribute a significant role in examining health claims (Brink et al., 2005). Consistent and accurate labeling is essential to guide consumer decisions and choices (Brooks and Kalmokoff, 2012). Although prebiotics and probiotics contribute to essential health advantages and are recognized as crucial for preserving a balanced microbiome, various economic, regulatory, and consumer perception challenges suppress their utilization in low-resource communities (Figueroa-González et al., 2011). Resolving these challenges is very important for adopting and utilizing such functional foods in low-resource communities (Quigley, 2019; McFarlane et al., 2023). Brucellosis management by studying the role of probiotics is an emerging interest. Current studies target the interpretation of nutrient metabolism, immune modulation, microbe protection, and the mechanism of action of probiotics. As the industry progresses, probiotics may have applications for impacting various sectors beyond gut health. In conclusion, probiotics have potential for brucellosis control, and future studies will determine their further medicinal potential (Kumar et al., 2022; Sajankila et al., 2023). Probiotics and prebiotics offer considerable potential for tackling global health concerns. Here are some opportunities and research needs: Researchers can genetically characterize and manipulate probiotic microorganisms to improve their effectiveness. Understanding strain-specific features enables focused therapy. Identifying microbial structure and interdependence at diverse body sites will improve our understanding of the probiotic and prebiotic effects (Abraham and Quigley, 2016). Metabolomics can assist in identifying necessary chemicals that mediate host advantages. Bridging the gap between research findings and practical applications is critical. Probiotic strains and prebiotic items can majorly impact human health if effectively translated. Dual isotope/radio labeling of novel prebiotics can create databases for annotating metabolic networks. New techniques enable real-time experiments in humans, allowing researchers to observe how bacteria integrate into the existing microbiome. Quantifying health levels can provide significant insights. Interconnected teams or thorough experiments are required (Spacova et al., 2020; Alam et al., 2022)

Conclusion

In conclusion, incorporating prebiotics and probiotics to control Brucella has enough potential to boost therapeutic and preventive strategies. By modulation of gut microbial composition and efficiency, prebiotics and probiotics can minimize microbe colonization, intensify immune responses, and improve overall health. Innovative interventions for enhancing resilience against Brucella can utilize the symbiotic interaction between the gut microbiota and the host immune system. Moreover, safety profiles, strain specificity, and long-term effects need thorough examinations to successfully integrate this biomedicine into brucellosis control strategies. Nevertheless, with ongoing multidisciplinary collaboration and advances in microbial ecology, probiotics and prebiotics have the potential to become indispensable adjuncts in the comprehensive plan for combatting brucellosis and protecting public health.

Data Availability

Not applicable.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Chapter 37

Unmasking the Relationship of Prebiotics and Iron Bio Availability in the Management of Anemia

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ABSTRACT

Anemia is a low iron level in the body and the most common cause of disability in women globally. Blood loss, recurrent infections, inflammatory diseases and problems with absorption are among the complications brought on by anemia. Anemia can be treated with prebiotics and iron supplements. The amount of iron that the body can more effectively use in a particular food is referred to as iron bioavailability. Two forms of dietary iron are absorbable: heme and non-heme. Heme iron is found in meat, fish and poultry and is obtained from the hemoglobin and myoglobin components of these foods. Heme iron appears to have a bioavailability of 15-35% higher than non-heme iron. Prebiotics help improve the health of gut and improve the absorption of several minerals, most notably iron. Non-digestible foods called prebiotics nourish probiotics to keep the gut healthy. Short-chain fatty acids (SCFAs) such as propionate, butyrate and acetate are produced in the large intestine by the gut microbiome's fermentation of prebiotics. Prebiotics can be found in foods including milk, honey, soybeans, bamboo shoots, fruits, vegetables and wheat bran. Low vitamin D levels may cause pernicious anemia because vitamin D is directly linked to iron absorption through its influence on hepcidin. Dairy products are the main source of vitamin D and the most popular way to treat anemia is to take iron supplements.

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INTRODUCTION

Anemia results from a prolonged, non-progressive loss of iron stores, known as iron deficiency, a more severe condition where the body does not have enough iron. Signs and symptoms of anemia include weakness or exhaustion, breathlessness, pale skin and sclera, abnormal heartbeat, pain in the chest, acrylic nails, symptoms of pica (*Pica* is a mental health condition where people compulsively swallow non-food items) and most prevalent cases of physiological iron deficiency and chronic fatigue (Cook, 2005).

Anemia roughly affects one third of the population and half of the cases are affected by iron deficiency. Iron deficiency occurs in two forms absolute and functional. Absolute anemia occurs when the body's whole supply of iron is depleted resulting in iron shortage. In contrast, functional iron deficiency is a condition when the bone marrow does not receive enough iron, despite overall iron reserves being elevated or normal (Lopez et al., 2015).

Iron-limited erythropoiesis, regardless of sufficient stocks, predicts reduced iron transport to erythroid precursors. Anaemia is prevalent and can strike individuals with chronic kidney illness (Gibson et al., 2017). According to the Global Burden of Disease Study 2016, iron deficiency anemia (IDA) ranks 1st among women and is one of the major causes of disability globally. Particularly at risk are young children, adolescents, pregnant women, women of reproductive age and infants under the age of 5 Low iron intake, poor absorption, blood loss, recurrent infections and inflammatory diseases are common risk factors (Gibson et al., 2017).

Common methods for improving a population's iron status include oral iron supplementation, food fortification, dietary variety, the prevention and treatment of chronic illnesses like tuberculosis, hookworm and malaria. Prebiotics added to iron fortificants help enhance the absorption of iron among anemic models. In many circumstances, oral iron supplementation is used to avoid iron deficiency and iron deficiency anemia. In most cases, it is also the first line of treatment for both conditions.

Oral iron therapy does have certain disadvantages (WHO, 2008).

A non-digestible food element called prebiotics is selectively metabolized by host bacteria to provide a plethora of health benefits. These bioactive substances have a variety of beneficial effects on the health of humans and animals, especially in GI tract (immune system modulation, pathogen inhibition), mental health (vigor and cognition), bones (better absorption of minerals) and cardio-metabolism (lower cholesterol). Additionally, prebiotics offer favorable temperature, acidic stability and organoleptic qualities, making them particularly intriguing food ingredients. They serve as food for probiotics, which are microscopic microorganisms like bacteria and yeast. To put it briefly, prebiotics are employed to balance the microbiota of the gut (Cardoso et al., 2021).

Prebiotics enhance digestion, lower inflammation, promote the growth of beneficial bacteria and inhibit the growth of pathogenic bacteria. Prebiotics lessen the likelihood of infection and inflammation, which supports a strong immune system. Prebiotics may lessen the signs and symptoms of sadness and anxiety by enhancing brain-gut connection. Prebiotics may help prevent cancer. They encourage the absorption of calcium and vitamins, which lowers the risk of developing other types of cancer (Liu, 2023).

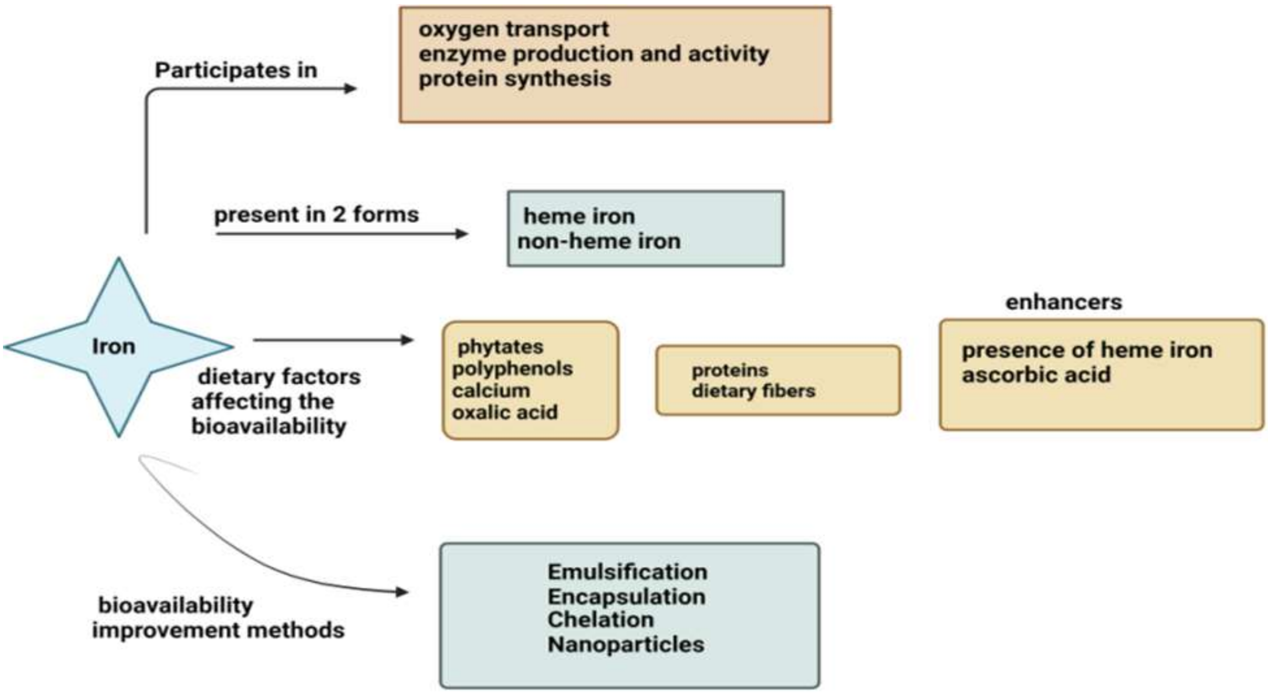

Fig. 1: Iron is essential for delivering oxygen to cells and facilitating the synthesis of energy.

Importance of Hepcidin in Anemia Management

Since iron is necessary for numerous cellular processes, maintaining iron homeostasis requires maintaining the proper balance between iron absorption, transport, storage and utilization. Iron balance is primarily regulated by absorption because the human body lacks a defined mechanism for excreting iron (DeDomenico et al., 2007). The liver secretes hepcidin, a peptide circulatory hormone that is crucial for the central nervous system and iron homeostasis maintenance. It is the primary control mechanism for maintaining systemic iron homeostasis, balancing the supply and use of iron. This material is mostly made by hepatocytes and functions as a negative regulator of iron absorption into the blood. To work, hepcidin binds to the Ferro protein, an iron transporter present in macrophages, enteral duodenal cells and placental cells. Ferro protein internalization and breakdown are triggered by hepcidin binding. The blood cannot absorb iron when the Ferro protein on the cell surface is lost. The absence of saturation changes and decreased iron delivery to growing erythroblasts are the outcomes of decreased iron entry into plasma (DeDomenico et al., 2007).

Sources and Challenges in Iron Absorption

Vitamin D enhances iron absorption and many minerals function best when combined with vitamins. Pro-inflammatory cytokine synthesis is inhibited and anti-inflammatory cytokine production is increased when vitamin D levels are adequate. Pernicious anemia may result from low vitamin D levels because vitamin D is directly linked to iron absorption through its action on hepcidin. Iron supplementation is the most often utilized strategy to treat anemia, while vitamin D is primarily found in dairy products. Beef products including ground beef, beef liver and bottom round steak cuts are excellent providers of iron (Shoemaker et al., 2023). Animal sources of Iron are meat, poultry, fish and cereals while plant sources of Iron are beans, lentils, seeds, nuts, vegetarian and vegan diets and dark green vegetables.

Iron absorption and cell surface Ferro protein were elevated in the presence of reduced hepcidin expression. Various factors, including anemia, hypoxia, cytokines, and plasma iron, influence the levels of plasma hepcidin.

Table 1: Iron contents in different food groups

Feed	Iron content
Fish	0.2/100mg
Poultry	0.4-1.5/100mg
Meat	1.0-3.3/100mg
Wholegrain bread and wheat	0.7-3.7/100mg
Pulses and legumes	1.7-3.2mg/100mg

(Domellöf and Sjöberg, 2024).

Iron intolerance is brought on by hepcidin expression deregulation. Chronic disease-related anaemia is caused by overexpression of hepcidin, whereas hemochromatosis (HFE) is caused by hepcidin shortage and results in iron buildup in key organs. It has been demonstrated that mutations in one of four genes—transferrin receptor 2 (TFR2), hemochromatosis type 2 (HFE2), and hepcidin antimicrobial peptide (HAMP) cause hepcidin deficiency. Iron overload disease is brought on by mutations in the HAMP gene, which codes for hepcidin. Hepcidin insufficiency results in deregulated iron absorption. It is unknown how TFR2, HFE, and HFE2 function in controlling the generation of hepcidin (DeDomenico et al., 2007).

Classification of Anemia

Nutrition Therapy and Pathophysiology, 2nd edition lists the following nutritional anemia. Anaemia comes in three primary forms.

Microcytic Anemia

Reduced hemoglobin levels, decreased red blood cell volume per deciliter of blood, or decreased red blood cell density per cubic millimeter of blood are all indicators of iron deficiency anemia (Moestrup, 2006).

Megaloblastic Anemia

Megaloblastic anemia is characterized by massive, irregular, and immature red blood cells with impaired oxygen delivery. Bone marrow and circulation contain these cells. Deficiency in folate or vitamin B12 can essentially hinder DNA synthesis (Hoffbrand, 2015).

Hemochromatosis

The main symptoms of hemochromatosis, also known as iron overload, are pro-oxidative iron damage to cells and iron accumulation due to the body's inability to operate. Hemochromatosis type I is inherited, whereas type II is brought on by other illnesses such liver disease (Beutler, 2004).

Non-Nutritional Anaemia Types

Sickle cell Anemia

Patients with sickle cell anemia, the most prevalent kind of heme illness, experience cell inflammation due to a homozygous defect in heme polymerization. The crescent-shaped brain becomes obvious after staining and magnification (Kennedy et al., 2005).

Hemolytic Anemia

According to Phillips et al. (2018), it is the premature and potentially fatal loss of red blood cells. Autoimmune hemolytic anemia, which is characterized by self-produced RBC antibodies against oneself, must be taken into consideration when diagnosing hemolytic anemia (Gehrs and Fridberg, 2002).

Anemia of Premature

It usually manifests as pre-birth of a newborn child, occurring prior to the completion of erythropoiesis and iron storage. Very low birth weight newborns classified as preterm are those weighing less than 1kg and birth before 29 weeks of gestation (Strauss., 2010).

Thalassemia

A mutation in either beta or alpha globin causes thalassemia. Reduced globin levels cause hemoglobin production to decline (Origa et al., 2007).

Polycythemia

A myeloproliferative tumor, polycythemia is defined by abnormal myeloid lineage hematopoiesis with an excessive synthesis of red blood cells and pro-inflammatory cytokines (Benevolo et al., 2023).

Conventional Approaches for the Treatment of Anemia

Oral Treatment

An increase in hemoglobin of 1 g/dl after 1 month of treatment indicates an adequate response to treatment and

Clotting and bleeding disorders

Hemophellia	Thrombosis
Hemophilia is a hereditary disorder characterized by insufficient thrombin generation and poor coagulation. Blood plasma essential protein fibrinogen, or plasma protein VIII insufficiency, is defined as hemophilia A; deficient in plasma protein factor IX is classed as hemophilia B.	Thrombosis is a multifactorial etiology linked to numerous diseases and environmental variables; platelet aggregation, oxidative impacts, inflammatory response, and blood viscosity all contribute to improved coagulation.

confirms the diagnosis. In adults, treatment should be continued for another 3 months after diabetes treatment to provide additional iron support. In children, the dose is 3 mg/kg/day. When treating gastrointestinal diseases such constipation, diarrhea, nausea, and epigestric discomfort, following oral therapeutic instructions can have unfavorable consequences. When taking iron with meals, these effects will lessen, although absorption will be 40% lower (WHO, 2001).

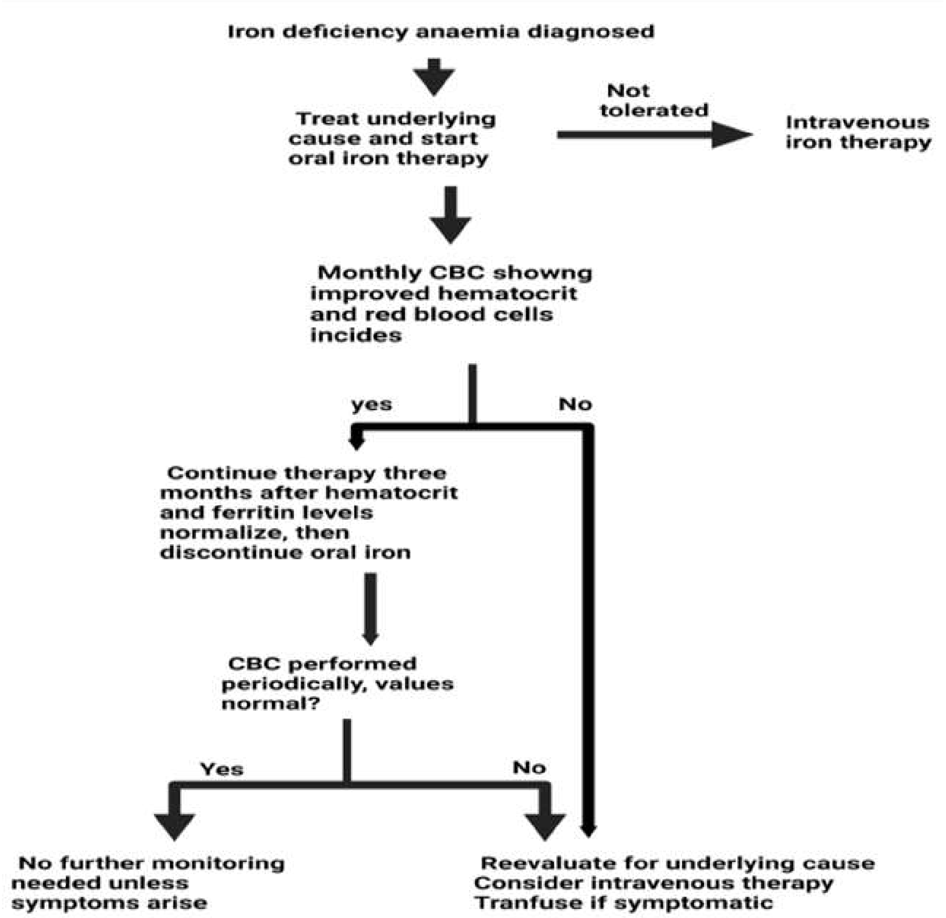

Fig. 2: Treatment of iron deficiency using oral and injectable medications

Blood Transfusion

Packeted red blood cell transfusions for individuals with iron insufficiency are not widely recognized. Transfusion is indicated by most standards based on a certain heme value; however, other significant considerations to consider include the patient's clinical condition and symptoms. If a pregnant woman's heme level is less than 6 g/dl, blood transfusion is advised since it may cause harm to the fetus. Low amniotic fluid volume, fetal cerebral vasodilation, and fetal death are all brought on by oxygenation. In order to determine the course of treatment, two units of red blood cells should be obtained and reevaluated clinically (WHO, 2001).

Sources and Types of Prebiotics

Primarily found in vegetables, prebiotics are generally significant substances that can be produced synthetically through the enzymatic conversion of sugars. Presumptive foods like soybeans, uncooked oats, onions and maize flour are rich sources of oligosaccharide, lignin and cellulose. A prebiotic food is also thought to be honey. Finans, which are typically found in wheat, bananas, onions and garlic, include inulin and fructo-oligosaccharides, are the best prebiotics (Ballini et al., 2023).

Fructans

Lactic acid bacteria are stimulated by fructans, although some research indicates that the fructan chain length establishes a criterion for identifying the fermenting bacteria.

Galacto-oligosaccharides

Probiotics formed from lactulose are oligosaccharides that mostly originate from this sugar.

Non-carbohydrate Oligosaccharide

They are categorized as prebiotics rather than carbohydrate oligosaccharides. Such prebiotics include flavonols derived from cocoa, which promote the growth of lactic acid bacteria.

Table 2: Sources, types and industrial production of prebiotics

Types of Prebiotics	Sources of Prebiotics	Industrial production of prebiotics
Fructo-oligosaccharides	Wheat, honey, banana, sugarcane, onion	Produced by the controlled enzymatic action of the polysaccharides, extracted from chicory root.
Isomaltulose	Honey and sugarcane juice	Naturally occurring disaccharides.
Galacto-oligosaccharides	Human's milk, cow's milk	Commercially produced by the action of B-galactosidases from lactose.
Lactulose	lactose(milk)	Also manufactured from lactose.
Lectosucrose	Lactose	Is producing using lactose and sucrose as raw material.

(Al- Sheraji et al., 2023).

Role of Prebiotics in Gut Health

For a period of six weeks, a daily dosage of roughly 10 mg of inulin may assist to improve the glycemic control of prediabetics and Type 2 diabetes. According to reports, middle-aged persons' gastrointestinal problems and inflammatory indicators can be alleviated with a 30-day regimen of daily inulin administration. Inulin supplementation can be used to control lipid metabolism, particularly in the case of dyslipidemia, which is linked to obesity and other cardio-metabolic diseases (Ferarri et al., 2022). Generally, 50-90% of the development of colorectal cancer (CRC) can be attributed to nutrition. The development of colorectal cancer is more common in those who consume large amounts of processed and red meat, refined starches and sugars, low vitamin intake, dietary fiber and whole wheat (Fareez et al., 2023).

Relationship between Gut Microbiota and Nutrient Absorption

The number of microbial species in the gut microbiota has been found to grow with age, ranging from 400 to 500. The main cause of these shifts in the abundance of microbial communities is, in essence, nutrition. Not all fibers are prebiotic, but they do include prebiotics. Bifidobacterium and lactobacilli can proliferate when exposed to Galacto-oligosaccharides (GOS). The gastrointestinal tract, blood cholesterol, immunological system, brain and bone health and iron absorption have all been positively impacted by prebiotics. (Gibson et al., 2017). It will be crucial to look at the mechanism by which prebiotics enhance iron absorption before figuring out the optimal prebiotic combinations and dosages. Prebiotics are typically utilized in the manufacturing of meat, beverages and baked goods due to their technological advantages and health benefits (Ferreira et al., 2023).

Iron Bio-availability

The term "iron bioavailability" describes the quantity of iron that the body can absorb or use from food. A fraction of an ingested nutrient that is available for utilization in normal physiologic functions and for storage is called bio-availability. The dietary supply, vitamin C, phytates, and oxalates are among the variables that affect bioavailability.

Factors Influencing Iron Absorption

Anaemia has become the most common condition worldwide. Worldwide, reports of anemia have indicated that 42% of children aged 6-59 months and 33% of women within the reproductive age range are affected. Heme iron, however, absorbs more readily than non-heme iron because to its heterogeneous origin and dependence on boosting and inhibiting variables. According to reports, fermented sauces, vegetables, meat and fish are also essential ways to increase the bioavailability of iron, as dose ascorbic acid. Some iron-inhibiting foods and beverages include phenol, which is mostly found in tea, coffee, some vegetables, dairy products, cheeses, and milk because they are high in calcium, as well as bran-rich foods like bran bread, breakfast cereals, oats, rice, and unpolished rice (Singh and parasad, 2023).

Thus, several techniques that enhance iron absorption are:

Chelation

By preventing iron absorption inhibition, this method helps to increase the bioavailability of minerals by two to three times. Several metabolic diseases, such as those affecting the liver, heart, and hormones, can be prevented using chelation therapy (Pogialli et al., 2011).

Encapsulation

A technique that helps to entrap vital factors within a transporter medium. The main purpose is to create a strong hemodynamically and physically fluid barrier with environmental conditions like water vapor, enzymes, pH and oxygen (Piskin et al., 2022).

Supplementation

Addition of synthetically produced vitamins, minerals, amino acids, iron, herbs and enzymes to the diet. It is an affordable method of improving health and has advantages for the economy and society (Olson et al., 2021).

Fortification

It is a process of adding micro-minerals like vitamins and minerals to improve the nutritional quality of foods. Fortification is done at various stages including food processing, packing and labeling (Shubham et al. 2020).

Heme vs Non-Heme iron

In the crust of the earth, iron is the fourth most prevalent element. Iron from diet enters the human body as both heme and non-heme iron. The absorption of heme iron is unaffected by inflammation and has a high bioavailability rate (Bah et al., 2024).

Non-organic iron is non-heme and is found in foods derived from plants and animals, whereas heme iron is an organic iron found in animal meat. Heme makes up 10–15% of the total iron in food, but non-heme absorbs 10% of the iron, which is 2.5 times less than heme. But animal flesh contains both heme and non-heme iron, while plant-based diets are exclusively non-heme, and dairy and eggs are classified as heme (Charlebois and Pantopoulos 2023).

Mechanism of Body Iron Absorption

Humans have an average daily reference intake of 10-15 mg, of which only 1-2 mg are absorbed through the digestive tract. About 70% of the iron in a human body attaches only to hemoglobin; the remaining iron binds to proteins such as ferritin, myoglobin, and transferrin. Enterocyte cells initiate absorption in the small intestine's upper jejunum and duodenum (Piskin et al., 2022).

In the digestive system, different proteins i.e. lactoferrin, ferritin, hemoglobin and bacterioferritin control the iron transport system. Mucin binds with iron in the acidic condition of the stomach to maintain it is solution state for later uptake in the alkaline conditions of the duodenum, then this mucin-bound iron passes across the mucosal cell membrane subsequently. After entering the cells, iron is transferred to the basolateral side to export to the blood plasma through cytoplasmic iron-binding proteins. Iron can be absorbed both in non-heme or in heme form. In the duodenal enterocyte, an earlier heme carrier protein (HCP-1) is responsible for iron absorption in duodenal enterocyte but later it was assumed that folate carrier is accountable for this system. Non-heme that is present in the Ferric (Fe^{+3}) or Ferrous form (Fe^{+2}), only the ferrous form is absorbed by the enterocyte with the help of reductase or cytochrome b enzyme present in the apical membrane of the duodenal enterocyte, then Divalent Metal Iron Transporter (DMT-1) transport it into duodenal cytoplasm. Acidic conditions are friendlier for iron absorption. A glycoprotein, hepcidin, is involved in the circulation and metabolism of iron metabolism, in case of excess iron absorption DMT-1 production retarded at the enterocyte by hepcidin (Shubham et al., 2020).

Prebiotics and Iron Absorption

Mechanism of Interaction

Prebiotics improve the absorption of several minerals, most notably iron, and also support gastrointestinal health (Ahmad et al., 2021). Prebiotics appear to improve iron absorption through a variety of methods, while the precise mechanism is still unknown. Prebiotics are consumed and then fermented, producing osmotically active sugars. By raising passive absorption, these carbohydrates improve the absorption of metals like iron. Furthermore, weak organic acids produced by fermentation have properties that facilitate the absorption of minerals (Nishito and Kambe 2018). These weak organic acids naturally lower the pH of the luminal solution, which helps iron change from its ferric form ($Fe3+$) to its more easily bioavailable ferrous form ($Fe2+$) (Moustarah and Mohiuddin 2019).

Short-chain fatty acids (SCFAs) such as propionate, butyrate, and acetate are produced in the large intestine by the gut microbiome's fermentation of prebiotics. The synthesis of these short-chain fatty acid molecules can lead to increased proliferation of epithelial cells, increasing the surface area that is available for absorption. Increased iron absorption follows as a result (Alexander et al., 2019). Moreover, the bifidogenic effect of both short- and long-chain fructo-oligosaccharides (FOS) lowers the pH of the colon, producing lactate and short-chain fatty acids (SCFA), which increases the bioavailability of essential minerals for a healthy diet, including calcium, iron, manganese, magnesium, and zinc (Costa et al., 2021).

Furthermore, the HAMP gene, which controls iron absorption, is thought to express itself more when prebiotics and iron are fed together. The colon's anti-inflammatory properties, which reduce the amount of Hepcidin in the blood, could be another way (Parikh and Bos 2018).

Specific Prebiotics and their Effects on Iron Absorption

Numerous research using human and animal models have demonstrated the ability of prebiotics to improve iron absorption. (Wang 2017).

Fructo-oligosaccharides (FOS)

Normally, inulinase hydrolyzes inulin to yield FOS. (Santos and Maugeri, 2007). A study was carried out on weaned rats to find out how well soya supplements with FOS absorbed iron. The results of the study showed that, in addition to having higher haemoglobin levels, the rats fed with FOS-supplemented soy drink showed an increase in DMT 1 protein expression (P-value < 0.05). As a result, it was suggested that giving weaned rats fructose (FOS) improved their iron absorption (Silva et al., 2018).

The addition of fructo- and glucto-oligosaccharides to young child formulas (YCF) enhanced the bioavailability of iron, according to studies. The young child formula (YCF) with the highest amount of GOS and FOS added also had the best iron bioavailability, per the study's findings. The study found a direct correlation between the presence of prebiotics and the bioavailability of iron in formulae intended for young children (Christides et al., 2018).

Galacto-oligosaccharides (GOS)

GOS is a naturally occurring oligosaccharide with a terminal glucose unit that contains galactose in small amounts in breast milk. Commercially produced GOS is produced from lactose by employing β-galactosidases to transglycosylate it (Zhang et al., 2021).

To treat infants with iron deficient anaemia under the age of five, a novel Micro Nutrient Powder (MNP) formula containing GOS prebiotic was evaluated in a study. A four-month randomized controlled experiment involved the purposeful shaping of three groups of newborns from Kenya. A control group received MNP without iron, while the first treatment group received MNP plus 2.5 mg of ethylene diamine tetra-acetic acid (NaFeEDTA) and ferrous fumarate, a dicarboxylic acid ($C4H2FeO4$). 7.5g of GOS and MNP were given to the second treatment group. Anaemia was shown to have significantly decreased in both therapy groups (P-value < 0.001) in the study. Moreover, the negative effects of high iron content on gut health were found to be mitigated by GOS incorporation. The study concluded that 7.5g of GOS and the relatively lower iron content of MNP helped to significantly minimize anemia and lessen the effects of iron deficiency on gut health (Paganini et al., 2017).

Inulin

Inulin is a naturally occurring substance in hundreds of different plant-based foods (Zhang et al., 2021). Inulin is useful for absorbing iron in human models, especially in cases of iron insufficiency (Shoaib et al., 2016). According to the study's findings, inulin may increase the caecum's concentrations of DMT-1, a protein that transports ferrous iron ($Fe2+$) from humans and other animals.

Inulin's effect on iron absorption and bifidobacteria's effect on short-chain fatty acids in anemic women were investigated by Petry et al. in 2012. Still, the results of the study showed that the women who took inulin supplements did not experience a substantial change in their iron status. While inulin was found to reduce fecal pH, the effect was not statistically significant (Petry et al., 2012).

Conclusion

Prebiotics such as FOS, GOS, and inulin have been investigated for their ability to enhance iron absorption and mitigate iron deficient anemia. FOS is the result of inulinase's hydrolysis of inulin. Breast milk naturally contains a small amount of GOS, an oligosaccharide with a terminal glucose unit that contains galactose. The trans glycosylation of lactose utilizing β-galactosidases yields GOS, which is commercially synthesized. Inulin is found naturally in hundreds of different plant-based diets. Inulin has been demonstrated in human models to be beneficial for iron absorption, especially in cases of iron shortage. However, several methods, such as chelation, encapsulation, supplementation and fortification, can improve iron absorption. Iron-rich foods such as meat and meat products, beans, lentils, and so on can be added to treat anemia. Lean meat, ground beef, and bottom-round steak cuts are all excellent providers of iron. Vitamin D enhances iron absorption, for example, and many minerals function best when combined with vitamins. On Earth's crust, iron ranks as the fourth most prevalent element. Both heme and non-heme iron are absorbed by the body from meals. The absorption of heme iron is unaffected by inflammation and has a high bioavailability rate. Chicken, fish, meat, and cereals are examples of animal-based sources of iron. Beans, lentils, seeds, nuts, vegetarian and vegan diets, and dark green vegetables are examples of plant sources of iron.

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Chapter 38

Encapsulated Probiotics Exploring the Role in Functional Food Industry

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ABSTRACT

Generally speaking, it is a mechanical or physicochemical process whereby bacterial cells are ensnared in encapsulating materials with varying characteristics that can lessen or prevent the encapsulated microorganisms' harm or cellular losses, particularly, in contrast to the anticipated positive effect on the host. Probiotics have gained popularity for their numerous health benefits, but their sensitivity to environmental factors and poor survival rates during processing and storage hinder their effectiveness. Encapsulation technology offers a solution by protecting probiotics from adverse conditions and enhancing their delivery to the target site. This chapter reviews the benefits and methods of encapsulating probiotics, including spray drying, emulsification, micro fluidization, and coacervation. The advantages of encapsulation, such as improved stability, controlled release, and enhanced functionality, are discussed. The chapter also highlights the importance of selecting suitable encapsulation materials and techniques to ensure the survival and viability of probiotics. By encapsulating probiotics, their therapeutic potential can be unlocked, leading to the development of innovative functional foods and supplements that promote gut health and overall well-being.

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INTRODUCTION

The health benefits of probiotics, which are live microbial supplements, are well-known. Among other health benefits, the usage of probiotics has been connected to enhanced gut flora composition and greater disease resistance. In recent years, probiotic-based food products have grown in popularity. Bifidobacterium has been associated mostly with the human digestive system and has been used as probiotics over the years (Sharifi Rad et al., 2020).Foods containing probiotics were first discovered in cheeses and milks made by lactic acid bacterial and fungal fermentation, as well as in leavened bread fermented by yeast (Suvarna and Boby, 2005). Furthermore, it is commonly recognized that fermented foods are healthy. Probiotic-rich milk was suggested as a potential treatment for gastrointestinal disorders by Hippocrates and other ancient physicians. Additionally, fermented milk products have been proposed as a treatment for gastroenteritis by the Roman historian Plinius. Viable nonpathogenic microorganisms were frequently employed in the treatment of intestinal illnesses in order to modify or replace the intestinal microbiota. Nissle 1917 is one of the rare Escherichia coli strains that is not laboratory produced. It wasn't until the 1960s that the term "probiotic" was used to describe substances produced by bacteria that promote the growth of others (Beswick and Mullins, 1964). Although little has been discovered about how probiotics act in the gut, more research should be done to determine the role they play in human health as well as the safety of using them. Because there is no scientific understanding or categorization study of the probiotics found in fermented food, people are unaware of their full nutritious potential. Our above concerns can be resolved with the aid of a probiotic database derived from fermented foods. By adding vitamins, proteins, essential fatty acids, and necessary amino acids to dietary substrates, fermentation can enhance the food's nutritional value and digestibility. More specifically, fermentation may connect process energetics and product quality to the variety of the fermenting microbial community and their characteristics. Probiotic fermented foods have gained popularity in recent years, which has sparked creativity and accelerated the creation of new products globally. Foods containing probiotic bacteria are being added more frequently in an effort to maintain the microbial balance in the gastrointestinal tract and enhance gut health. Some significant bacteria are Bacillus, which is linked to the fermentation of legumes, and Acetobacter, which produces acetic acid and is involved in the fermentation of fruits and vegetables. Furthermore, we offer some details regarding the bacteriocins that are generated by bacteria in foods that have undergone fermentation. Because yeast produces the enzymes that lead to desired biochemical reactions, it is essential to the food business. This is demonstrated by how ethanol is produced in beer and wine as well as how bread rises. As such, it is advantageous for the industrial development of probiotics. We have gained a better understanding of probiotics and their active ingredients by studying the biological data of probiotics in various fermented meals. Furthermore, PBDB can be used to understand the traits and roles of distinct microorganisms in a variety of fermented foods. Even though no study has completely examined the probiotics in fermented foods using an integrated database, this effort is crucial to the advancement of the medical field (Zhao et al., 2019).

History of Probiotics

The term "probiotic" was first used in 1953 to describe "active substances that are essential for a healthy development of life" by German scientist Werner Kollath. It is derived from the Latin pro and the Greek βιοσ, which means "for life." This word was first used in a different context in 1965 by Lilly and Stillwell to describe "substances secreted by one organism which stimulate the growth of another." More accurately, Fuller (1992) defined probiotics as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance"(Gasbarrini et al., 2016). Elie Metchnikoff discovered around 1900 that eating living microbes (Lactobacillus bulgaricus) in fermented milk or yogurt improved certain GIT properties. Today, the Food and Agricultural Organization of the United Nations and the World Health Organization define probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." The International Association for Scientific Prebiotics and Probiotics has reviewed and approved this definition.

Commonly used Probiotic Microorganisms

Numerous bacteria belonging to various genera and species may possess probiotic qualities. However, over time, probiotics have been made from the genera Lactobacillus and Bifidobacterium (Fijan, 2014). Mainly because these are the most common bacteria in the human digestive tract and are regarded as GRAS (generally recognized as safe) (Brodmann et al., 2017). Specifically, the dairy industry often uses the probiotic strain Lactobacillus spp. Lactobacilli are rod-shaped, gram-positive, non-spore-forming microorganisms that often inhabit an anaerobic environment. Despite this, they are acid- and aero-tolerant, fermentative, and selective.

Health Benefits of Probiotics

Functional foods have a role in improving human health that goes beyond nutrient content. Functional foods are used to promote health and balance out minor physiological problems that healthy hosts may encounter. They fall between nutrients, which provide basic physiological functions, and medications, which treat diseases. Apart from the well-known functional ingredients like vitamins, minerals, and micronutrients, probiotics are a part of the new wave of active ingredients that also includes phytonutrients, lipids, and prebiotics.

Probiotic bacteria enhance the equilibrium of the gut microbiota and strengthen the body's defenses against infections, which is helpful to human health. Probiotics are also said to have anti-bacterial, anti-carcinogenic, blood cholesterol-lowering, immune system-stimulating, and vitamin-synthesizing properties. Probiotics have several main advantages, including the reduction or elimination of conditions like constipation, diarrhea, and colon irritation (Rehaiem et al., 2014). Several studies have demonstrated their beneficial effects on gastrointestinal infections, antimicrobial activity, lactose metabolism enhancement, serum cholesterol reduction, immune system stimulation, antimutagenic, anti-carcinogenic, anti-diarrheal, and reduction of Helicobacter pylori infection through the addition of specific strains to food products (Pereira et al., 2011).

Encapsulation

Encapsulation is a useful method for improving the way that living cells and bioactive compounds are transported into food products. It requires covering active ingredients in a carrier medium. It is also possible to encapsulate proteins and lipids. The most commonly utilized encapsulation technique in the food sector is spray drying since it's a continuous, adaptable, and most importantly, economical procedure. Most encapsulates are produced by spray-drying; the remainder are made via freeze-drying, melt extrusion, melt injection, and spray-chilling (Nedovic et al., 2011). During the manufacturing process and storage of food, non-encapsulated probiotic microbes may be subjected to high temperatures, low pH, high osmotic pressure, and high oxygen levels (Kailasapathy,2006). Research into surrounding probiotics in a physical barrier has been conducted by numerous investigators. Encapsulation is a process that involves the incorporation of protective elements into miniature capsules that can be released under specified conditions at a regulated rate.

Encapsulation Components

Typically, encapsulated particles are made up of two parts: the covering material/shell and the core. The active components to be coated, known as core materials, can be in any of three physical states: liquid, solid, or gas. Using the solid-phase separation approach, solids are disseminated in a polymeric solution and then polymer precipitation occurs (Duduković et al., 2002). Furthermore, depending on their core solubility, solids can dissolve into suitable liquids. If the core dissolves in an organic solvent, the coating materials and the core are also dissolved in the solvent. The organic solvent is then emulsified and evaporated, using the single-emulsion solvent evaporation procedure, to produce Nano-precipitation (Yu et al., 2018). The solid is dissolved in water and then emulsified in the case of a water-soluble core. While gas cores can be adsorbed on an inert material and then enclosed as a solid core, liquid cores can be emulsified. The inert polymeric material that coats the core materials to the appropriate thickness is known as the coating material or shell. These materials should offer desired qualities including stability, strength, flexibility, impermeability, and non-hygroscopicity and be compatible and non-reactive with the core material. Naturally occurring polymers like polysaccharides are among the frequently utilized coating polymers. Matrix and vesicular encapsulated particles fall into two primary categories according to the dispersion of the core substance. Systems with a physically and evenly distributed active component, or core, are called matrix systems. On the other hand, vesicular systems, also known as capsules, have the core material contained within a hollow surrounded by a polymer membrane (Duduković et al., 2002). Different terminology can be used to refer to vesicular and matrix systems based on their composition shapes, coating materials, and production procedures.

Benefits of Encapsulation

Enhancing stability in finished products and during processing is one of the main justifications for encapsulating active substances. Less evaporation and degradation of volatile actives, including scent, is another advantage of encapsulation. Encapsulation is also employed to cover up unpleasant food-related sensations, such the astringency and bitter taste of polyphenols. Preventing reactions with other ingredients, such oxygen or water, in food products is another reason to use encapsulation. Apart from the aforementioned uses, encapsulation can also be employed in food processing applications, such the fermentation and metabolite synthesis processes, to immobilize cells or enzymes. The need to identify practical solutions that offer great production while also ensuring that the finished food items are of a satisfactory quality is growing (Livney, 2010).

Methods used in Microencapsulation

This study highlights the key principles behind probiotics' ability to withstand stress and describes novel techniques to probiotic microencapsulation. Additionally, a study of current in vivo and in vitro models is done in order to evaluate the efficacy of probiotic administration techniques. Probiotics must be encapsulated in order to maintain their viability both in storage and in the human gut, which increases the likelihood of colonization.

These solutions work by protecting the probiotics from harmful environmental elements and enhancing their mucoadhesive properties. Usually, the probiotics are coated or embedded with food-grade materials such as lipids or biopolymers. To improve their chances of life, other components like nutrition or defense compounds are occasionally encapsulated. The importance of having suitable in vitro and in vivo models to evaluate the efficacy of probiotic administration techniques is also emphasized. Encapsulation is a commonly used method for creating customized products in the food industries and specialized food production, food processing sectors (Yao et al., 2020).It involves covering a functionally linked central substance in an inert material matrix for protection. The material that will be enclosed is referred to as "core" or "active material". It also goes by the title's payload, internal phase, and fills. On the other hand, the material that surrounds the active ingredient is called the shell, carrier material, matrix, coating material, wall, capsule, and membrane. Microencapsulation technology has attracted more attention in industrial applications due to its ability to protect unstable bioactive components, provide designed food products additional functional qualities, and distribute active chemicals at controlled rates to specified areas. Encapsulation strategies have therefore been researched extensively for a very long time. The optimum process depends on a number of factors, including the type of the active substance, the characteristics of the shell material, and the attributes that the finished product must have based on its intended use. Several encapsulation techniques may be used in delivery system design.

There are Four Factors that Guide the Design

The physical and chemical characteristics of the substance that need to be encapsulated; the mode of distribution, which influences the choice of wall material; the capsule's dimensions; and, finally, the encapsulation process. Encapsulation helps to screen the contents from the external environment, prolong and facilitate the process of storage or transit, and protect the inside of the item. The application of encapsulation in commerce was initially recorded in 1957 (Kłosowska et al., 2023). Encapsulation has now become more common in several industries, such as food, lipids, essential oils, agriculture (pesticides), cosmetics and fragrances, and nutritional supplements (fish oil and vitamins). In these fields, knowledge has also continued to advance. There are many different ways to encapsulate information. The ideal approach relies on a number of variables, such as the intended use, the size of the encapsulates, the chemical makeup, cost, and availability of the coating, as well as the contained core substance. Blends of organic fragrance compounds, such as artificially produced naturally occurring substances like essential oils or resins with a natural equivalent, make up scent compositions and tastes. Encapsulation changes the material's structure to make it solid from a liquid or gas, immobilizes the active material by encasing it, shields the core from the damaging effects of the environment, releases the core material gradually to increase its exposure to the active material, and facilitates functionality, among other things (Kliszcz et al., 2021).

Advantages to Taste and Fragrance Encapsulation

Taste and aroma encapsulation has following benefits:

1. Extended toughness
2. Greater stability in the final product, which undergoes a structural change from liquid to solid and has improved dispersibility, fluidity, and dosage precision.
3. The time of exposure to taste or odor is prolonged by the controlled and progressive release of fragrance components.
4. Masking taste and aroma
5. Protection from external factors, severing highly volatile and chemically unstable components from their surroundings, safeguarding against ultraviolet radiation, deterioration processes, heat, oxidation, and dryness.
6. Safety increases when volatile substances become less flammable.

Proper Process of Encapsulation

a) Physico-mechanical Methods

Spray Drying

The basic idea behind spray drying is to emulsify and dissolve the core material in an aqueous solution of the carrier substance. The combination is then atomized in a heated chamber, where the active particle is coated, and smaller water molecules evaporate. This method finds application in the culinary, pharmaceutical, and cosmetic sectors, as well as in the manufacturing of milk powder. Because strongly flammable scent ingredients evaporate more quickly than water, it's important to use the right carrier to prevent losing volatile fragrance compounds while letting water evaporate. When selecting a suitable carrier, the following aspects should be considered:

- flavor and odor release beneath the appropriate conditions
- low cost and accessibility
- taste impartiality
- Stability
- good solubility in water
- and good emulsifying characteristics

Emulsification

One popular way for co-encapsulating probiotics is this procedure. This procedure is divided into two stages: the dispersed phase, which contains the suspension of the cell polymer, and the continuous phase, which is composed of oil (mineral or vegetable oil) or organic solution. The mixture is homogenized with the help of surfactants to create the emulsion. When a cross-linking agent is applied or the water-soluble polymer is cooled, the particles are created inside the oil phase. After that, the microbeads are either filtered or centrifuged. The agitation speed, surfactant concentration, rate of cross-linking agent addition, and water to oil ratio—which can vary from 25µm to 2mm—all affect the size of the beads. Emulsifiers reduce surface tension, which makes microspheres smaller and increases the stability of emulsions (Chandramouli et al., 2004). This approach is simpler to scale up, produces microcapsules with a lower diameter, and increases probiotic life. The main drawbacks are that it creates microcapsules with varying sizes and shapes and necessitates the use of a second polymer solution for the extra coating on the cel. Milk protein is used in emulsification with gelification to encapsulate probiotics since it has gelation capabilities and serves as a natural probiotic carrier (Misra et al., 2021).

Micro Fluidization Method

Using the previously described technique, microcapsules are produced using the Microfluidic device, which has micro channels that permit laminar fluid flow and the creation of double emulsions. This apparatus can produce consistent microcapsule sizes and ensure process repeatability.

Pan Coating

This is a technique to microencapsulate solid particles that are greater in diameter than 600µm. This apparatus has perforations all around it and a revolving disk that spins in the opposite direction of the drum's motion (Kłosowska et al., 2023). The cores are provided into the disc's center, where they are transformed into a coating material layer. Holes are then filled with an encapsulating material solution. When the combined mass of the shell and core reaches a certain threshold, centrifugal force exceeds the forces that hold the hole together membrane, causing the microcapsules to be discharged external to the person who receives. Heat-treated air is used to remove the solvent after the shells have undergone chemical or physical curing. This method has high production efficiency and the advantages of being rapid, efficient, and manageably simple to utilize.

Physicochemical Methods

Coacervation Method

Microencapsulation seems to originate from a physicochemical process called coacervation. Two varieties of coacervation are available, based on how the technique is conducted out: basic and sophisticated (Rutz et al., 2017). Simple coacervation comes in one type only of polymer, and there is also an extremely hydrophilic substance present in the colloidal solution. In sophisticated coacervation, two or more polymers can be used. Anionic and cationic polymers need to be able to communicate with each other underwater in order to become complex coacervate, a liquid phase rich in polymers. In this case, the most typically used cationic polymer is gelatin. Gelatin with several synthetic and natural water-soluble polymers combines to form complex coacervates suitable for encapsulation. These coacervates take place in balance with a transparent liquid at the surface. Phases in a colloidal or polymeric solution separate during the coacervation process, and more than two liquid stages are created Consequently of the solution environment being carefully changed. This includes modifying the solubility, temperature, ionic strength, pH, and adding salt or a polymer with a countercharge. The coacervation process yields an equilibrium phase and a colloid-rich coacervate phase.

Chemical Methods

Polymerization

Among the chemical methods for making encapsulates is the polymerization procedure, which encompasses both in situ interfacial polymerization. The previous technique polymerization produces encapsulates by polymerizing monomers at the interface between the dispersing (ethyl alcohol, glycerol, chloroform, water) and scatter (vegetable oils, animals fats and synthetic oils) stages. The dispersion stages are combined with the encapsulating monomer and the suspended or dissolved active component until an o/w emulsion is formed. The most widely utilized monomers have multifunctional organic acid chlorides and multifunctional isocyanates. Furthermore, to the components mentioned above, vinyl acetate, methyl methacrylate and a mixture of vinylbenzene styrene or diamines are also used. As monomers permeate between the phases, the process generates an oil-insoluble polymeric membrane (polyurea, poly-nylon, or polyurethane). On the other hand, unlike interfacial polymerization, in situ polymerization doesn't call for the inclusion of additional reactive substances. When the resulting polymer first forms, it has a low molecular weight, but it gradually becomes larger. After that, the core material is covered with this polymer to form a solid capsule shell.

Encapsulation Efficiency

Capture efficiency (EE) is the most important parameter for assessing the performance of the encapsulation process. EE determines the proportion of bioactive material trapped inside the inert core during the encapsulation process. This can be calculated by dividing the mass of the core material used in the formulation by the mass of the core material that is entirely contained within the wall material. Simply said, the bioactive is more stable throughout the encapsulation process when almost all of it is completely entrapped inside the shell matrix, which is when the best encapsulation efficiency happens. Put differently, the technique provides high-level protection for the bioactive core. It is better to encapsulate PUFA-rich oil and trap almost all of the oil within the shell matrix, leaving very little oil on the surface of the shell matrix. Any oil that remains on the surface of the shell remains outside of the shell matrix. We call this surface fat or free fat. The surface oil rapidly undergoes oxidative degradation as soon as the capsules come into contact with ambient air. Elevated surface oil so frequently correlates with microcapsule off-flavor and worsens consumer approval of the finished product. Many emulsion formulations have far more surface oil than the recommended 0.1% (w/w) concentration for microcapsule preservation (Kaushik et al., 2015).

Conclusion

Encapsulation of probiotics is a crucial technology to enhance their stability, viability, and functionality in food products. Various encapsulation methods, including spray drying, emulsification, micro fluidization, pan coating, and coacervation, have been developed to protect probiotics from environmental stresses and ensure their delivery to the target site. The choice of encapsulation method depends on the type of probiotic, desired release profile, and food application. Encapsulation materials, such as polysaccharides, proteins, and lipids, play a vital role in determining the properties of the encapsulated probiotics. The benefits of encapsulated probiotics include improved gut health, enhanced immune system function, and increased tolerance to stress conditions. Future research should focus on developing more efficient and cost-effective encapsulation methods, exploring new encapsulation materials, and investigating the impact of encapsulated probiotics on human health. By advancing encapsulation technology, we can unlock the full potential of probiotics and create innovative food products that promote human well-being.

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Chapter 39

Role of Probiotics in the Immunity of Humans and Animals

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ABSTRACT

One of the most diverse and active microbial ecosystems is the gastrointestinal tract (GIT), and intestinal microbes are critical to the growth and development of gut-associated immunity. Probiotics work by regulating immune cells such as effector lymphocytes, dendritic cells, epithelial cells, B cells, T regulatory and natural killer cells. Intestinal microbes are critical to the modulation and development of mucosal immunity, and disturbances can result in illnesses related to the microbiota. The immunomodulatory actions of probiotics are primarily driven by two mechanisms gene expression regulation and signaling pathways in hosts. The host's gut mucosal immune system starts immune responses on exposure to foreign antigens to maintain homeostasis, which involves both adaptive immune responses and inflammation induction. Advances in metagenomics have greatly enhanced our understanding of the mechanisms by which gut microorganisms influence bodily functions. A substantial amount of promising data from studies on animal models and humans suggests that probiotic supplementation is a potential approach for preventing and treating intestinal and immune diseases. The benefits of probiotics are extended from the GIT, with research showing the relation between the central nervous system and GIT, that highlights the activity of neurochemical signaling in mental health and intestinal homeostasis. Probiotics have been used for the treatment of diseases in humans as well as in other animals. *Bifidobacterium* and *Lactobacillus* are commonly used.

KEYWORDS		
Alternative medicine, Probiotics, Immunity, Humans, Animals	Received: 09-Jun-2024 Revised: 16-Jul-2024 Accepted: 15-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Probiotics consist of live microbes that are beneficial to health when administered or consumed in the right amounts by the body. Primarily, these are composed of beneficial bacteria and yeasts, that work to combat harmful bacteria, enhance immunity, and rebalance the microbial environment in the gut. Through various molecular mechanisms, probiotics can eradicate detrimental pathogens and regulate the host animal's immune response, promoting overall animal well-being. Probiotics can remove harmful microorganisms from the intestinal tract and can change the microbial density population inside the gastrointestinal tract (GIT). This causes the shifting of balance between beneficial and harmful microorganisms to favorable conditions (Gadde et al., 2017). Probiotics, once established in the gastrointestinal tract (GIT), trigger immunological responses, causing gut cells to release various immunoregulatory components in response to the presence of bacteria. Furthermore, probiotic-produced metabolites can affect several cellular metabolic pathways. For example, some targets within various metabolic pathways are interacting with metabolites like as bacteriocins, hydrogen peroxide, and amines, controlling activities including cell inflammation, differentiation, proliferation, and apoptosis (Plaza-Diaz et al., 2019).

The formation and maturity of the corresponding GIT immunity are significantly influenced by the varied bacteria found in the gastrointestinal tract (GIT), which is a dynamic ecosystem. Through indirect interactions with gut epithelial and immune cells in the lamina propria influence the immune system. The gut microbiota elicits a particular immune response by communicating with underlying immune cells through the intestinal epithelium through the generation of metabolites. Concerns about drug residues in meat, milk, and eggs as well as the emergence of drug-resistant bacteria in veterinary and medical settings make the use of antimicrobials in animal feed extremely concerning. As a result, probiotics have become increasingly popular in place of antimicrobials, and this trend has shown promise. Probiotic use improves gastrointestinal health and nutrition absorption in livestock while reducing disease prevalence and transmission (Begum et al., 2018).

However, the most intriguing and significant aspect of incorporating probiotics into animal and human diets lies in their ability to influence the mucosal immune system of the gastrointestinal tract at both systemic and local levels. This highly complex immunomodulation process results from the association between immune cells associated with the GIT and probiotics. Probiotics exert their effects by regulating immune cells, such as B cells, T natural killer and regulatory cells, dendritic cells, effector lymphocytes, and epithelial cells (Hooper et al., 2012).

The majority of well-established probiotics are categorized under the genera *Bifidobacterium* and *Lactobacillus*. Although, several bacteria from genera such as *Streptococcus Leuconostoc*, and *Enterococcus*, as well as yeast from *Saccharomyces* genus have also been utilized as probiotics in both human and animal nutrition. Recent studies have shown that probiotics stimulate the functions of beneficial intestinal bacteria, especially their immunomodulatory activities. In recent decades, research has highlighted the significant potential of using the microorganisms within the human body to treat and prevent various diseases (Georgieva et al., 2015). Probiotics have been employed for over a century to combat inflammation and infections. Species from the *Bifidobacterium* and *Lactobacillus* genera are the most commonly utilized. These have proven effective in the prevention and treatment of gastrointestinal infections, dental caries, urogenital infections and periodontal diseases. The gastrointestinal tract is a home to a vast diversity of microbial species, plays a crucial role in directly or indirectly influencing immune responses and host metabolism. Targeting the intestinal microbiota through probiotic therapies can impact the biology of immune cells, offering potential treatment for inflammatory disorders or immune-mediated diseases. Consequently, probiotics have shown promising results in modulating human inflammatory disorders such as multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and others (Sales-Campos et al., 2019).

Mode of Action of Probiotics

Probiotics demonstrate their effectiveness by a variety of mechanisms, which include inhibiting and managing enteric pathogens while simultaneously enhancing the functioning and productivity of animals. These mechanisms encompass several fundamental actions such as preventing pathogens from adhesion, generating antimicrobial compounds such as defensins and bacteriocins, excluding pathogenic microorganisms competitively, strengthening barrier functions, reducing the luminal pH and regulating the immunity. By inhibiting harmful bacteria, probiotics contribute to promoting better conditions. For example, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* can prevent adhesion of *Escherichia coli* in the GIT. Bacteria typically engage with cells of hosts through the release of chemical signals which impact bacterial behavior (Waters and Bassler, 2005).

Probiotics generate antibacterial substances that hinder bacterial adhesion and movement. Various bacteria such as *Bifidobacteria, Lactococcus, Lactobacillus*, *Streptococcus*, *Enterococcus*, *Pediococcus*, and *Leuconostoc* can generate bacteriocins or proteins, which inhibit the growth of closely related bacterial species. These probiotics work to diminish the population of harmful microorganisms within the GIT. Bacteriocins which are bioactive antimicrobial peptides produced by bacterial ribosomes, bind to the cells of pathogenic microbes, penetrating their phospholipid membranes. The main process of the bacteriocin-mediated pathogen response is the penetration of the cytoplasmic membrane by pathogenic bacteria, which inhibits the production of DNA and RNA and eventually results in cell leakage. (Van Zyl et al., 2020).

Bacteriocins can inhibit the capacity of pathogen cells to colonize the gastrointestinal tract and fight against antibiotic-resistant bacterial strains. By reducing luminal pH, probiotic microorganisms can survive with harmful pathogens. *Bifdobacterium breve* can reduce the luminal pH and higher quantities of *Bifdobacterium breve* can induce the generation of acetic acid (Lajis, 2020). Through strengthening the function of the gut barrier, probiotics can influence the way bacteria and their host communicate with each other and preserve cellular consistency. The phosphorylation of tight junctional and cytoskeletal proteins is modulated to achieve this consistency. *Lactobacillus* can live on the chicken ileum's epithelial cells. By strengthening this gut communication system, probiotics can effectively exclude harmful microbes from the host gut through competitive means. This anti-pathogenic process is known as competitive exclusion, shows that species of bacteria tenaciously compete for connecting to receptors at specific binding sites in the intestine and it may also incorporate the release of antimicrobial compounds and competition for readily available nutrients(Aziz et al., 2022). Probiotics have the ability to modify the immune system, which enhances the host's immunity. Supplementation of probiotics are essential for inducing a signal network and activating the mucosal immune system (MIS). Using various experimental techniques, the impact of several probiotic microorganisms on dendritic cells (DC) has been studied. Antigen-presenting cells called dendritic cells are essential for innate as well as adaptive immunity. In addition to initiating basic immune reactions that directly result in the formation of B- and T-cell responses, dendritic cells have the ability to recognize and respond to components of bacteria. Probiotics have the ability to directly control gut dendritic cells with pathogen recognition patterns (PRPs) on their surfaces that allow them to accurately identify the pathogen-associated molecular patterns (PAMPs) on the bacterial species. By upregulating co-stimulatory molecular expression, this therapeutic technique promotes maturation of DC. Secretion of cytokine stimulates activation of T-cells when the immune system is activated. B-cell responses towards pathogenesis are determined by T-cell reactions, like T regulatory response or T helper cell polarization, which are determined by DC-originated signals (Kapsenberg, 2003).

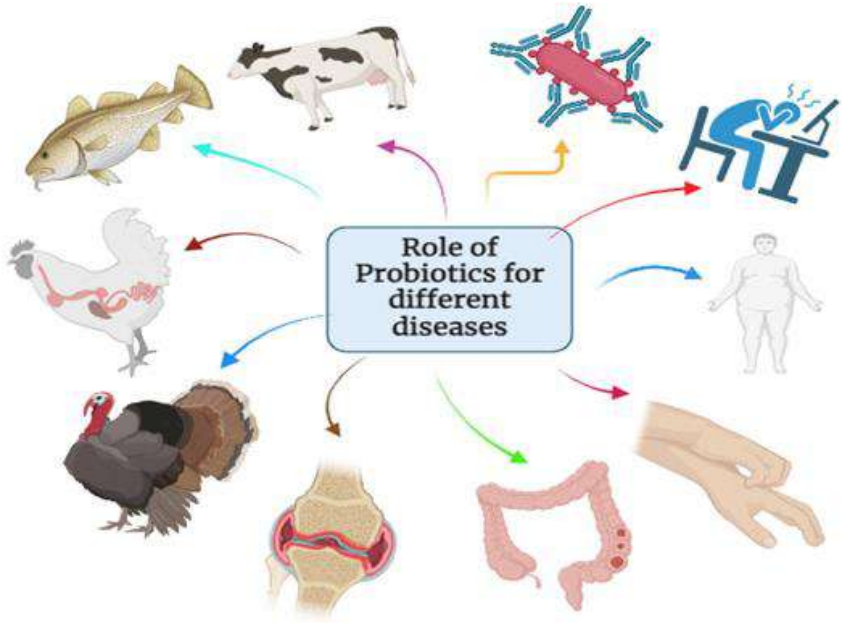

Fig: Use of probiotics against different diseases of animals and humans.

Role of Gut Immune Cells

Gut immunity is an essential barrier against foreign antigens and infections. This barrier is composed of a dispersed population of innate and adaptive effector cells known as the gut-associated lymphoid tissues (GALT). The effector immune cells of GALT consist of antigen-presenting cells like B-cells, M-cells, T-cells, and dendritic cells that balance tolerance and immunity to prevent inflammation in the intestine (Mowat and Agace, 2014).

The intestinal epithelium layer physically divides the gut microbiota from GALT immune cells. It also separates foreign antigens and bacteria from host immune cells and sends signals to immune cells in response to substances produced by the intestinal microbes, which triggers immune responses (Ding et al., 2021). Through the production of mucus and secretory immunoglobulin A (sIgA), the intestinal barrier carries out its defensive role. The gut flora and other antigens are shielded from harm by mucus that is a physical barrier, and sIgA is essential for mucosal humoral adaptive immunity. Plasma cells produce the sIgA within the lamina propria that are transferred to the gut lumen and attach to antigens, toxins, and pathogens, leading to their immune elimination through a non-inflammatory mechanism (Maldonado Galdeano et al., 2019).

Probiotics and Gut Immune Cells

Upon encountering any foreign agent, the host gut MIS starts immune responses to maintain homeostasis, by inducing inflammation and adaptive immune responses. The immune signals provided by the gut microbiota play a significant role in maintaining this balance and triggering protective responses. Numerous characteristics, including the number and size of germinal centers in Peyer's patches, plasma cells that synthesize IgA, $CD4^+$ T cells in the lamina propria, and intraepithelial $CD8\alpha\beta^+$ T cells that express the αβ T cell receptor, have been discovered to be greatly impacted by the presence of gut microbiota. Consequently, manipulating the gut microbiota through probiotics in dietary supplementation emerges as an attractive way to beneficially regulate the immune system of host. Probiotic dietary supplements reshape the composition of intestinal microbes, which promotes the development of GALT and epithelial cells. This supplementation also positively influences the mucous layer properties and enhances the synthesis of antimicrobial peptides and IgA. Probiotic supplementation not only boosts the concentration of IgA synthesizing plasma cells in the intestinal lamina propria but also in distant sites such as mammary glands and bronchi, demonstrating the broader beneficial effects of probiotics beyond the gut (Bai et al., 2019).

Gut microbiota or probiotics engage in interactions with various components of the host gut, including intestinal lymphocytes, dendritic cells, epithelial cells, and macrophages, with pattern recognition receptors being key players in these interactions and subsequent responses from gut cells. This interaction can occur through direct contact between microbes and intestinal epithelial cells or via the internalizing the microbes or their components through M cells by interactions with DC, triggering immune responses regulated by lymphocytes and macrophages. Probiotics modify the cytokine production within the gut immune system to achieve their immunomodulatory effects. Cytokines play a role in cell-to-cell signal transmission, which controls immunological responses (Guan et al., 2019).

Probiotic administration in humans resulted in elevated cytokine synthesis, particularly TNF-α and IFN-γ through T cells in the lamina propria. *L. casei CRL* 431 supplementation increased production of IL-10 by macrophage and Th2 lymphocyte to preserve intestinal homeostasis. The expression of Toll-like receptor (TLR) 6, nuclear factor (NF) kB-P50, IL-1α and tumor necrosis factor (TNF), in the gut mucosa as well as IL-1β, IFN-α and IL-4, in the serum was found to be down-regulated in piglets when *Bacillus amyloliquefaciens* SC06 was added to their diet, in contrast, IL-8 and IL-6 were found to be upregulated. This work demonstrates that *B. amyloliquefaciens* SC06 can alter immunological response of gut epithelial cells by inducing the TLR signaling pathway (Lemme-Dumit et al., 2018). Furthermore, adding *L. acidophilus* to the diet of laying hens boosted humoral and cell-mediated immunity, decreased the proportion of heterophils to lymphocytes, and increased the proliferation of B and T lymphocytes. Adding the *Paenibacillus polymyxa* 10 and *L. plantarum* 16 in broiler chickens decreased cell apoptosis and increased the gut immunity, as evidenced by the upregulation of IL-10, IFN-γ, and IL-6 gene expressions in the jejunum, as well as downregulated serum concentration of creatine kinase and alkaline phosphatase (Alaqil et al., 2020).

Table: Commonly used microbes as probiotics and their sources

Genus	Species	Sources
Gram Positive Bacteria		
Bifidobacterium	*B. lactis, B. animalis, B. thermophiles, B. bifdum, B. longum*	fermented milk, yogurt, cured meats, buttermilk, fermented foods, certain wines
Bacillus	*B.toyonesis, B. amyloliquefaciens, B. subtilis, B. coagulans,*	Yogurt, Kimchi, Kombucha, sauerkrauts, Miso, sourdough bread
Lactobacillus	*L. gallinarum, L. brevis, L. acidophilus, L. casei, L. plantarum, L. gallinarum, L. reuteri*	Sourdough bread, Yoghurt, Kimchi, Kefir, Sauerkraut,
Leuconostoc	*L. mesenteroides*	Fresh vegetables and fruits, Milk based products
Streptococcus	*S. salivarius subsp, S. faecium, S. thermophilus*	Milk and milk-based products, kefir grains, cheese
Clostridium	*C. butyricum*	Yogurt, miso, kimchi, legumes, honeybees, beans,
Gram negative bacteria		
Prevotella	*P. bryantii*	Fermented foods, fruits, vegetables, legumes, Ruminant animals
Escherichia	*E. coli Nissle 1917*	Fermented foods and Milk products, fruits, vegetables, legumes, fermented beverages
Megasphaera	*M. elsdenii*	Fermented foods and Milk products, fruits, vegetables, legumes, Ruminant animals,
Fungi and Yeast		
Aspergillus	*A. niger, A. oryzae*	Soy sauce, cheese, yogurt, fruits like grapes, fermented beverages
Candida	*C. pintolepesii*	Buttermilk, yogurt, cheese, fermented beverages, miso, kimchi
Saccharomyces	*S. cerevisiae, S. boulardii*	Yogurt, kimchi, wine, beer, fermented soy products, Miso

GIT Diseases and Probiotics

The intestinal microbes are important in immunomodulation and development of the mucosal immune system, and any disruption in its balance can lead to the emergence of microbiota-related diseases. Numerous investigations conducted in both humans and mice have indicated a correlation between certain inflammatory conditions and dysbiosis of intestinal microbial community. Experiments using the germ-free mice have demonstrated the essential roles of intestinal microbes to the development of the mucosal defense system. Germ-free mice exhibited notably impaired growth and development of lymphoid structures like Peyer's patches and mesenteric lymph nodes resulting in diminished T and B cell responses, as well as decreased levels of serum IgA and IgG (Kamada et al., 2013).

In the contemporary era, shifts in dietary patterns have contributed to the rise of new metabolic disorders including irritable bowel syndrome, obesity, cardiovascular diseases, colorectal cancer, rheumatoid arthritis, and inflammatory bowel disease (IBD). Consequently, researchers have turned their attention to exploring the potential of relations between the gut microbes and the mucosal immune system for managing and treating these emerging conditions. Studies have investigated various probiotics, either alone or in combinations, for gastrointestinal disorders such as Crohn's disease, IBD, colitis, ulcerative, and diarrhea. However, it has been observed that probiotics are generally considered safe for use in outpatient settings, but there is a risk of probiotic-related sepsis in hospitalized and immune compromised patients (Su et al., 2022).

Improvement in Immune system by using Probiotics

Probiotics can impact both the adaptive and innate immune systems of the hosts. Various types of immune cells, including dendritic cells, granulocytes, B lymphocytes, T lymphocytes, and macrophages, participate in inflammatory reactions, that are regulated by cytokines such as IL-6, IL-8, IL-15, IL-1β, and TNFα interleukins. Conversely, anti-inflammatory reactions are regulated by cytokines like TGFβ, IL-10, and IL-12 Innate immunity acts as the first line of defense for the host organism, providing chemical and physical barriers against pathogens (Hardy et al., 2013).

For instance, intestinal epithelial cells (IECs) serve as a barrier, inhibiting the proliferation of harmful microbes and preventing infections. Certain *Lactobacillus* bacteria, such as *crispatus*, *Lactobacillus fermentum*, *Lactobacillus salivarius*, and *Lactobacillus gasseri*, can effectively modulate the secretion levels of both anti-inflammatory and pro-inflammatory interleukins, such as IL-6, IL-8, and IL-10. This regulation aids for restoring physiological balance and managing inflammation. Any change in intestinal microbial balance is related to many disorders beyond the gut diorders (Luongo et al., 2013). Furthermore, various components of the gut microbiota, including probiotics, pathogens, and commensals, have the potential to activate neural signaling systems within the body. Consequently, investigating the interplay between the microbiota, gut, and brain through animal models may pave the pathway for potential treatments for mental health disorders in the future (Silva et al., 2012). Within the gut microbial community, specific beneficial species are involved in regulating the immune system. Disruptions to these favorable populations can lead to immune dysregulation, increased levels of pathogenic microbes, and inflammation. Probiotic supplements help restore these beneficial populations. The immune-stimulating impact of probiotics begins with their interaction with Peyer's patches and intestinal epithelial cells, which in turn triggers the activation of plasma cells, secretion of IgA antibodies, and transfer of intestinal T cells (Mir et al., 2018).

The immunomodulatory effects of probiotics operate through two primary mechanisms including gene expression regulation and signaling pathways within host cells. These effects are obtained through direct triggering of immune cells residing in the gastrointestinal tract (GIT), leading to activation of macrophages and increased phagocytosis, and modification of enzyme activity by altering metabolism of microbes (Yan and Polk, 2011). In a study, it was showed that antigenic fragments from *L. casei* CRL 431 and *L. paracasei* CNCM I-1518 were internalized through cells of intestinal epithelial following adherence of whole probiotic cells by Toll-like receptors (TLRs), thereby mediating immune stimulation. Up regulation of TLR expression leads to the release of cytokines such as IL-4, IL-6, IFN-γ, and TNF-α (Ashraf and Shah, 2014).

Role of Probiotics in Animals

Use of Probiotic in Poultry

Poultry farming has significantly increased over the past 50 years, now standing at five times the level observed half a century ago. Research conducted by the Food and Agriculture Organization (FAO) in 2018, using the "Global Livestock Environmental Assessment Model," indicates a global demand for approximately 73 million tons of eggs and 100 million tons of meat. Addressing this substantial demand, probiotics offer a promising solution without adverse effects. Probiotics have been shown to mitigate gastrointestinal ailments such as salmonellosis, necrotic enteritis and coccidiosis in poultry (Fazelnia et al., 2021).

Probiotics have been shown to enhance the host's immune system through various mechanisms. For instance, a combination of *Saccharomyces cerevisiae* and *Lactobacillus fermentum* stimulated T-cell immunity in the gut, leading to increased numbers of $CD8^+$, CD4+, and $CD3^+$ T-lymphocytes in GIT of poultry. In small intestine of infant chicks aged 3 and 7 days, the gene expressions of IL-2, IFN-γ and $CD3^+$ were elevated when probiotics such as *Lactobacillus gasseri* TL2919 and *Lactobacillus jensenii* TL2937 were included in their diet, compared to those without probiotics. Furthermore, in chickens probiotics have been found to elevate levels of serum immunoglobulin.

Probiotics in chickens are involved in increasing the immunoglobulin levels such as IgM and IgA in serum when food was supplemented with probiotics including the *Bacillus subtilis*, *Clostridium butyricum* and *Lactobacillus acidophilus* (Zhang and Kim, 2014)

Feeding the chickens with 1% of *Lactobacillus casei*, *Bifdobacterium*, and *Lactobacillus acidophilus* enhanced the levels of immunity, antioxidants and growth rate (Zhang et al., 2021). In chickens, probiotics can help avoid salmonellosis. Salmonellosis can be avoided by hatching chicks immunized with *Lactobacillus plantarum* LTC-113 strain. The hens will be more resilient to the infection if *Lactobacillus plantarum* is able to suppress the growth of pathogenic bacteria in the intestine and maintain the expression of tight junction genes in intestinal epithelial cells. According to recent research, RASV (recombinant avian *Salmonella* vaccine) and probiotics given together to White leghorn chickens can reduce the infection rate in chickens, which are susceptible to APEC (avian pathogenic *Escherichia coli*) and Salmonella infections (Redweik et al., 2020).

Importance of Probiotics in Ruminants

The ruminants harbor a complex microbiota community. Animals ingest proteins and carbohydrates, that are then broken down by microbes residing in the rumen. In ruminants, commonly utilized probiotics include *Enterococcus, Bacillus*, *Aspergillus oryzae*, *lactic acid bacteria (LAB)*, and *Saccharomyces cerevisiae* all of which contribute significantly to ruminant health and well-being (Elghandour et al., 2020). In ruminants, probiotics have been shown to bolster the immune system. In young calves, supplementation with *Lactobacillus plantarum*, *L. salivarius*, and *L. acidophilus*, has been demonstrated to reduce the incidence of diarrhea (Signorini et al., 2012). Nisin derived by *Lactococcus lactis* is antimicrobial peptide in cows. In intra mammary gland, it has been found effective in treating mastitis caused by *Staphylococcus aureus*. Additionally, utilizing a teat spray based on *Lactobacillus* can enhance the condition of the mammary gland and reinforce the teat sphincter function in dairy cows. Moreover, supplementing with probiotics has been shown to mitigate rumen acidosis in cows and enhance the immune system in young calves experiencing stress (Alawneh et al., 2020).

Use of Probiotics in Aquaculture

Global fish farming in 2018 reached approximately 179 million tons and total estimated sales value of USD 401 billions. In aquaculture antibiotics are commonly utilized to meet the growing demand. However, the widespread usage of antibiotics causes the rise of drug-resistant bacteria, which can be transmitted to humans through the food chain. Probiotics offer numerous beneficial effects for aquatic animals. They promote the reproduction and growth of aquatic organisms, provide protection against pathogens, enhance immunity, aid in digestion, enhance the quality of water, and serve as antibiotic alternatives (Banerjee and Ray, 2017).

In fish farming, farmers employ a diverse array of probiotics in, with *Bacillus subtilis* being a commonly utilized option in aquaculture. *Bacillus* probiotics alone have demonstrated the ability to mitigate diverse harmful microbes in fish, including *Flavobacterium*, *Aeromonas*, *Acinetobacter*, *Streptococcus*, *Pseudomonas, Vibrio*, and *Clostridium*. In aquaculture other strains of bacteria are frequently employed as probiotics include LAB bacteria including *Lactobacillus plantarum VSG-3* and *Lactococcus lactis* and gram-negative bacteria. Additionally, various microalgae species like *Tetraselmis suecica*, *Phaeodactylum tricornutum*, *Isochrysis galbana*, *Dunaliella salina*, and *Dunaliella tertiolecta* have been found to enhance the growth and existence rates of aquatic creatures (Giri et al., 2013).

Yeast, specifically *Saccharomyces cerevisiae*, has been demonstrated to be beneficial for aquatic animals. Farmers typically administer probiotic supplements to fish either by dietary supplements or water circulation. Probiotics can consist of a single bacterial strain or a mixture of multiple strains, often supplemented with other immunostimulants or prebiotics. In fish culture the effectiveness of probiotics depends on the appropriate timing and dosage. Bacterial strains such as *Lactobacillus plantarum* and *Bacillus pumilus* have been shown to improve the health states of Nile tilapia. Moreover, red hybrid tilapia fishes were given a diet containing 1% *Bacillus spp*. orally to counteract the negative effects of the Tilapia Lake Virus (Waiyamitra et al., 2020). It has been demonstrated that this food supplementation lowers the mortality rate brought on by Lake Virus infection. *Pseudomonas I-2* that is a probiotic supplement, has shown itself to be capable of suppressing harmful *Vibrio* bacteria. A probiotic called *Pediococcus acidilactici* can protect white-leg shrimp from vibriosis.

Role of Probiotics in Miscellaneous Animals

The probiotic supplement improves the health of farm animals like turkeys, ducks, pigs, lamb, and sheep. Various probiotic food supplements have been recommended by scientists worldwide to ensure the safe production of eggs, meat, milk, and while preserving the health of these animals. Lambs and sheep can have better health with probiotics. In two-month-old lambs and sheep, *Bacillus subtilis* and *Bacillus licheniformis* can increase body weight, enhance the gut microbiota, increase immunity, and maintain regular metabolic processes (Devyatkin et al., 2021).

Birds' health can be improved by probiotics. When *Lactobacillus casei* and *Lactobacillus acidophilus* were added to white Pekin duck diets, the animals gained more weight overall, had higher protein concentration, bactericidal activity, and gut enzyme activity while experiencing lower levels of cortisol, cholesterol, and glucose (Khattab et al., 2021). In addition to producing lactic acid and lowering pathogenic infection, *Lactobacillus* helps keep the balance of gut microbes in geese. *Bacillus licheniformis* and *Bacillus subtilis* have the ability to decrease fat deposition and lipid contents while also increasing the liver's CYP7α1 and LXRα enzyme activity in Cherry Valley Pekin ducks (Huang et al., 2015).

Use of Probiotics on Humans

Role of Probiotics in Food Allergy

Allergy stands as one of the most prevalent chronic conditions globally and is frequently linked to the over activation of the T helper 2 (Th2) branch of adaptive immune system. Among allergies, food allergy reigns supreme as the most existing form, capable of being stimulated through virtually any food, with "major allergens" including milk, fish, wheat, nuts, and egg(Fujimura and Lynch, 2015). It impacts numerous individuals worldwide and is characterized by an adverse health reaction resulting from a specific immune response upon exposure to a particular food, which can manifest as either IgE mediated or non-IgE-mediated. Research has affirmed the significant role of intestinal microbes in homeostasis maintenance within various interconnected networks of host homeostasis and immunity (Polkowska-Pruszyńska et al., 2020).

The intestinal microbes play a role in the development of structural alteration of the host gut mucosa, vitamin K synthesis, neurotransmission, and immunological responses.

Food allergies have a pathophysiological basis and arise as a result of gut microbes. Furthermore, these microbes might contribute to maintaining the immune system's efficacy. A study found that compared to infants who are not allergic to eggs, the early-life gut microbiota of children with egg allergies is more diversified, distinct, and lower taxa (Fazlollahi et al., 2018). There are more *Lachnospiraceae* and *Ruminococcaceae* in the intestine of allergic children. The association between intestinal microbiota and food allergies was demonstrated by using germ-free (GF) mice. It was found that administering broad-spectrum antibiotics to both GF mice and mice enhanced the number of basophils or IgE levels in the GF mice's blood. The results of the experiment indicated that gut microorganisms were necessary for the host's ability to regulate IgE associated with food allergies and basophil-mediated reactions. There is a close association between food allergies and the gut flora. An additional investigation using GF mouse models showed that the loss of gut flora could promote insufficient GALT formation, which would shift the immune response toward Th2, displaying the close link between gut microbes and food allergy (McKenzie et al., 2017).

Role of Probiotics in Cancer

A malignant tumor of epithelial tissue is the source of cancer, and it is commonly believed that a condition of chronic inflammation in a particular area is secondary to the development of cancer. Immune system deficiencies are directly linked to the incidence of cancer. The connection between cancer and gut microbes has received increasing interest since it was discovered that intestinal flora had a positive impact on immunity. The most common illustration is the connection between colorectal cancer and gut flora. Both the mortality and incidence of colorectal cancer are high. Certain bacterial species impact both the development of pre-existing tumors as well as the chance of colorectal cancer (Peterson et al., 2020). Patients with colorectal cancer have a notably lower diversity of microorganisms in their intestines. More specifically, people with colorectal cancer had higher levels of *Fusobacterium* and *Porphyromonas* and lower overall *clostridia* numbers. The survival rate of patients with colorectal cancer is correlated with the amount of *Fusobacterium nucleatum* in their bodies (Ahn et al., 2013).

The scientists found that intestinal microorganisms can produce dangerous substances that can impact the host's immune system, ejecting genotoxic virulence factors and promoting colorectal carcinogenes in animal models. Stools of patients revealed a significant dysbiosis of the intestinal flora, as evidenced by an increase in harmful pro-inflammatory infectious agents and a decrease in commensal bacteria that create butyric acid (Wieczorska et al., 2020).

In addition to increasing inflammation and damaging the gut epithelial barrier, dysbiosis can also increase immunity and lower the risk of colorectal cancer. In human colorectal cancer, these inflammatory cells have been shown to upregulate chemokines and cytokines. The incidence of colorectal cancer may also be influenced by the metabolites produced by gut microorganisms. Certain metabolites called short-chain fatty acids (SCFAs) are produced by gut microorganisms and have the power to regulate inflammation, apoptosis, and epithelial growth. SCFAs can also reduce the risk of colon cancer and boost immunity. However, microbial metabolites may lead to inflammation and hyperproliferation of epithelial cells, which raises the risk of cancer. Numerous research have demonstrated the importance of intestinal microbes in determining the risk of colon tumors (Sánchez-Alcoholado et al., 2020).

Role of Gut Microbiome in Depression

Probiotics such as *Streptococcus thermophilus*, *Saccharomyces boulardii*, *Bifidobacterium species,* and *Lactobacillus species*, are present in our daily food components and these affect brain development, pain sensitivity mood, and CNS (Central nervous system). Beneficial gut microbes enhance immunity and this helps to prevent depression. Depression is a commonly occurring disorder and there is a significant difference between the activation of cell-mediated adaptive immunity in the general population and the depressed patient (Peirce and Alviña, 2019). The stimulation of innate immune mechanisms, like triggering of proinflammatory cytokines interleukin-1 and interleukin-6, are linked to the establishment of depression. It is evident that the immune system plays a role in the occurrence of depression. Since gut flora has the ability to enhance and refine the immune system, which shows that gut micro flora can improvessss immunity in order to treat depression (Mörkl et al., 2020).

Function of Intestinal Microbiomes in Obesity and Adipose Tissue Local Inflammation

Obesity is a multifactorial and metabolic disorder that is caused by genetic factors, life style and diet and some researchers have found that gut flora is also associated to obesity. Adipose tissues of patients have local inflammation and which can change to systemic inflammation. Gut microbes inhibit inflammation and thus preventing obesity and increasing the immune system. There is a relation between immunity and gut flora. The obese persons have more *Firmicutes* and less *Bacteroidtes* showing that obesity is linked to microbes. When cecal microbes were introduced to GF mice and it was found that 60% fat and insulin resistance was enhanced in body. Thus, gut microbes are important in reducing the inflammation and preventing the obesity (Aoun et al., 2020).

Effects of Probiotics on Skin

Numerous studies have shown that there is a two-way communication between the GI tract and skin, and that certain gastrointestinal illnesses can have skin manifestations. Common skin disorders like psoriasis, atopic dermatitis (AD), and acne are strongly linked to the gut microbiota. Furthermore, probiotics are becoming a more popular option for treating these illnesses in addition to some conventional ones(Salem et al., 2018).

Acne Skin Disease

Chronic skin illness called acne typically comes from inflammation, keratin alterations, reduced immunity, and hormone-induced hyper seborrhea. The back, face, and neck are among the common places where this occurs. Benzoyl peroxide, oral and topical retinoids, antimicrobial agents, and an effective skincare routine are the most often used treatments. Probiotics have been linked to improved acne treatments, according to current studies.

Research has verified that probiotics, namely *Lactococcus sp*. HY 449, can directly prevent *P. acnes* from occurring by producing antimicrobial proteins. The majority of acne patients' issues improved, when they consumed probiotic tablets containing *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*. After years of studying the relationship between the skin, gut, and brain, it has been discovered that oral probiotics significantly enhance skin therapy (Mottin and Suyenaga, 2018).

Psoriasis Skin Disease

Psoriasis is an inflammatory and chronic skin disorder just like acne and many people are affected by this disease in the world. In this disease, thick scaly plaques or erythematous appear on the surface of the skin. Psoriasis is related to intestinal microbiota and in patients the intestinal microbial flora was disturbed severely and diversity of specific taxa of bacteria was decreased and their abundance was changed. In patients, the levels of *Actinobacteria* and *Firmicutes* were significantly high in the intestinal microbes while there were decreased levels of *Bacteroides* and *Proteobacteria* and there was a high ratio of *Firmicutes/Bacteroidetes*. Oral usage of *Lactobacillus pentosus* GMNL-77 can treat inflammation of the skin and also improve erythematous lesions because of imiquimod treatment. Probiotic supplementation provides an effective way to improve quality of the skin and it is a safe and cost-effective way (Chen et al., 2017).

Colon Cancer

Procarcinogens in the intestinal tract convert into carcinogens, leading to colon cancer, but specific probiotic strains like *L. acidophilus* can neutralize these procarcinogens. Several potent strains can metabolize procarcinogens, converting them back into non-carcinogenic substances. This process may involve the activation of metabolites that are precursors to carcinogen production. Beneficial microbes can neutralize certain active enzymes, such as beta-glucuronidase and nitroreductase which transform procarcinogenic substances into carcinogens, by removing their harmful properties (Liu et al., 2023). Research indicates that some probiotic species could potentially be used for cancer treatment, although reliable findings are not widely available (Rajput and Li, 2012).

Diarrhea

Diarrhea is characterized by an increased frequency of bowel evacuation, defecation, and water concentrartion in the stool. Various mechanisms have been identified to prevent diarrhea. One mechanism involves the active blocking of receptor sites which is considered logical if there is sign for competition at certain receptors. Toxins or peptides generated from villous endocrine cells may compete with lactobacilli, blocking the force that causes diarrhea. Another mechanism involves the action of local immunoglobulin A (IgA) antibodies that are effective towards rotavirus. Animal studies suggest *lactobacillus* intake can cause secretory IgA. However, there is a drawback to this theory: large concentrations of the causal agents may reduce the impact of secretory IgA. The third mechanism, on the other hand, is the way that probiotics, or Lactobacilli, communicate with the host to control the intestinal defense system and get rid of secretions that are thought to be harmful (Cadieux et al., 2002).

It has been demonstrated that higher expression of MUC2 and MUC3 mRNAs protects cells against detrimental bacterial adhesion when lactobacillus signaling is present. The last theory posits that probiotics might produce substances capable of inactivating harmful particles. Though more specific antiviral activities have not been ruled out, acid may be the likely cause of the impact. Probiotics may work in different ways depending on the etiology of diarrhea. Cell receptors are essential for initiating and regulating the cellular response that leads to the development of immunity (Reid et al., 2002).

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an inflammatory disorder heavily influenced by genetic factors, where the immune system is involved in the beginning, progression, and exacerbation of the condition. RA is marked by inflammation that primarily impacts the joints, leading to the damages of bone and cartilage. The pathophysiological process involves both innate and adaptive immunity, which includes a variety of cells and soluble mediators. Research continues to be done, although some studies have shown that immunoglobulin A (IgA) anti-cilitinated protein antibody (ACPA) is present before the clinical onset of RA (Firestein and McInnes, 2017).

It is interesting to note that the only bacterium *Porphyromonas gingivalis* known to produce peptidylarginine deiminase, an enzyme linked to the presence of ACPA, is one of the main inducers of periodontal disorders. This bacterium has previously been linked to the onset of RA. Additionally, there was a decrease in *Bifidobacterium* species, *Bacteroides fragilis,* and *rectale–Clostridium coccoides*, in RA patients. Increased levels of the pathogenic bacteria *Prevotella copri* were linked to a decrease in Bacteroides and a loss of helpful microorganisms in RA patient (Scher et al. 2013). Th1 cell differentiation was induced in vitro by Pc-p27, and this could be one of the mechanisms aggravating the symptoms of RA patients. This indicates that intestinal dysbiosis and the manipulation of different microbial populations, especially the high levels of *P. copri*, are the main factors influencing the onset and course of illness (Pianta et al., 2017).

When RA patients took daily capsules of *L. casei* 01 probiotic supplementation for eight weeks, their condition improved more than when they took merely a placebo. Similarly, for eight weeks, RA patients were supplemented with a daily probiotic cocktail made up of a combination of *Lactobacillus acidophilus*, *Bifidum*, and *Lactobacillus casei.* Compared to the placebo group, those taking medication with a probiotic combination showed a substantial reduction in insulin, C-reactive protein, and the disease activity score of 28 joints (Zamani et al., 2016).

Inflammatory Bowl Disorder

The two overlapping phenotypes of Crohn's disease (CD) and ulcerative colitis (UC) that make up inflammatory bowel disease (IBD) mostly affect the colon and/or the distal small intestine (CD). Although the exact cause of the condition is unknown, it is believed that the friendly gut microbiota and a genetic predisposition play a crucial part. Changes to the beneficial microflora's overall makeup and activity may be beneficial in the fight against the illness. Probiotics that have been specifically chosen have been studied for their ability to reduce relapse rates and lengthen remission times. Remarkably, *L. rhamnosus, L. salivarius*, *S. cerevisiae* and a strain of *E. coli* (*Nissle*) have all been found to be beneficial in reducing the symptoms of inflammatory bowel disease (Sales-Campos et al., 2014).

Safety Concerns about the Probiotics

While there has been much debate over the effectiveness of probiotics in preventing or treating ailments, the majority of probiotic strains have been identified by regulatory bodies. This safety profile depends mainly on the history of protected probiotic use in foods and on observations made in clinical trials evaluating probiotics efficiency, instead of safety. Probiotic use has been linked to an increased risk of infection and/or mortality in severely ill adults and infant patients in intensive care units, as well as in hospitalized, postoperative, or immunocompromised patients, partly because of fungemia and bacteremia. However, probiotic use may be safe in healthy adults. However, this relationship between probiotic use and a higher likelihood of infection needs to be causally verified, eliminating trials where the probiotic strain was the cause of the bloodstream infection. It's interesting to note that, in contrast to no intervention following antibiotic treatment, increased colonic colonization through probiotic strains was linked to a constant, long-term dysbiosis induced by probiotics (Suez et al., 2020). This dysbiosis significantly postponed the reconstruction of the GI and fecal microbial communities. It has been proposed that soluble substances released by the supplied Lactobacillus species directly impede the formation of the microbiome of individuals at least in vivo. The use of probiotics in people who have received antibiotic treatment may be associated with an increased risk of communicable diseases, and persistent dysbiosis may also possibly relate to the link between antibiotics and non-communicable diseases. This is because persistent dysbiosis may impair the colonization resistance to pathogens given by the microbiome (Suez et al., 2019).

Conclusion

The probiotic family has benefits in humans as well as animals and their family is increasing with time. Additionally, using probiotics provides a wider range of antibiotic replacement options, preventing the development of antibiotic resistance and serving as a useful tool for maintaining gut homeostasis. Probiotics primarily target intestinal epithelial cells that stimulate underlying immune cells to alter systemic and mucosal immunity. As beneficial microbes, probiotics regulate the composition of gut microflora and boost the immune system. They enhance host immunity through sustaining the epithelial barrier, preventing pathogens from binding to the gut surface and regulating and maturing the immune system properly. Additionally, probiotics can improve immunity by influencing gut flora to treat specific disorders. The close relationship between probiotics, immunity, and gut microflora has been well established. In the future, further elucidation of how probiotics regulate intestinal flora and enhance immunity will likely provide effective ways to improve life quality.

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Chapter 40

Role of Probiotics in Irritable Bowel Syndrome (IBS)

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ABSTRACT

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder that causes symptoms such as abdominal pain, bloating, abnormal bowel movements, and can greatly affect daily life Probiotics are microorganisms that live known as they have health benefits and are currently being studied as a treatment option for IBS. The chapters in this book explore the use of probiotics in the treatment of IBS, focusing on how probiotics work, the supporting clinical evidence, and how they can be incorporated into clinical practice Probiotics to balance gut bacteria , reduced inflammation, strengthened the intestinal barrier, gut -May also help influence brain connectivity, which can help reduce IBS symptoms Studies have shown the effectiveness of certain types of probiotics, particularly Lactobacillus and Bifidobacterium in reducing IBS symptoms, but its effectiveness is limited. Probiotics in combination with dietary modification, pharmacotherapy, and behavioral therapy may provide additional benefits. However, probiotics research faces several challenges, including inconsistent research protocols and the need for standardized protocols. Advances in probiotic research, including new strains and drug development, and technological advances such as microbial sequencing are expected to facilitate the development of effective probiotic therapies in the treatment of IBS greater Future directions could be next generation probiotics and commensal bacteria Prebiotics are combined as alternative therapies. Addressing these challenges and implementing future developments may improve the use of probiotics in the treatment of IBS and ultimately improve patient outcomes and quality of life.

KEYWORDS
Microbiota; Inflammation; Modulation; Symptom Relief; Meta-analysis

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INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic gastrointestinal disorder characterized by regular or uncomfortable abdominal pain associated with changed bowel habits. IBS affects approximately 10-15% of the global population and is characterized by symptoms such as abdominal pain, bloating, flatulence, diarrhea (IBS-D), constipation (IBS-C), alternating episodes of diarrhea and constipation (IBS-M) and the presence of mucus in the stool . IBS is classified into three main subtypes based on predominant bowel habit IBS-C (constipation predominant), IBS-D (diarrhea predominant) and IBS-M (Di Rosa et al., 2023).

IBS significantly affects the value of life of sufferers, leading to psychological difficulties, social limitations, compact work productivity, increased absence and negative effects on personal relationships and daily undertakings. This complexity is due to the multifactorial etiology of IBS, including abdominal visceral hypersensitivity, alter matten involvement; altered gut motility and reactivity post infectious isolation with brain-gut interactions in joint gut symbiosis. The interaction of these factors makes IBS a disease that is difficult to treat and treat efficiently (Nevots et al., 2023).

One area of increasing attention in the treatment of IBS is the use of probiotics. Probiotics are live microorganisms that assistance the host when administered in adequate amounts. They are thought to exert their beneficial effects by controlling the intestinal microflora, strengthening the intestinal barrier function, and interrelating with the host's immune system (Sharma and Bajwa, 2021).

The role of probiotics in managing IBS symptoms has been the focus of many studies, which generally suggest that certain strains of probiotics may be active in alleviating some of the symptoms connected with IBS. For example, Bifidobacterium and Lactobacillus are among the most frequently studied genera of probiotics in IBS investigation. These studies have shown that specific strains can help reduce the incidence and severity of abdominal pain, reduce bloating, and improve stool consistency (Skrzydło-Radomańska et al., 2020).

One of the primary mechanisms through which probiotics are said to benefit IBS patients is through inflection of the gut microbiota. Dysbiosis, or an inequity of intestinal microflora, is involved in the pathophysiology of IBS. Probiotics can help restore a healthy balance of gut bacteria and actually reduce IBS symptoms. In addition, probiotics can improve gut barrier function, reduce gut permeability, and stop the translocation of harmful bacteria and toxins that can trigger inflammation and escapes (J. Mishra et al., 2022).

Another mechanism is the contact between probiotics and the host's immune system. Probiotics can modulate immune responses in the gut and potentially reduce the low-grade inflammation often understood in IBS patients. By influencing the production of anti-inflammatory cytokines and other immune mediators, probiotics may help improve some of the immune characteristics of IBS
(Raheem et al., 2021).

Despite the hopeful potential of probiotics in the treatment of IBS, it is significant to note that not all probiotics are created equal. The efficiency of probiotics in the treatment of IBS is highly strain specific and what works for one individual may not effort for another. Therefore, it is crucial to indicate probiotic strains that have been clinically shown to be effective for IBS symptoms. Additionally, the optimal dosage and duration of probiotic treatment for IBS leftovers an area of continuing research (Xu et al., 2021).

Safety is another important attention when using probiotics for IBS. In general, probiotics are considered nontoxic for most individuals, with few reported side effects. However, individuals with fundamental health problems or a weakened immune system should consult their physician before beginning probiotic supplementation. Probiotics offer a auspicious therapeutic option for the management of IBS symptoms. By modulating gut microbiota, strengthening gut barrier function, and interacting with the immune system, probiotics can help reduce the cruelty and frequency of IBS symptoms. However, more research is needed to identify the most effective strains, doses, and duration of treatment. For individuals suffering from IBS, probiotics can be a valuable adding to their treatment plan, potentially improving their quality of life and decreasing the burden of this chronic disease (Kumar et al., 2022).

Introduction to Probiotics

Probiotics are live microorganisms that, when consumed in satisfactory quantities, offer health benefits to the host. They play a key character in maintaining the natural balance of microorganisms in the gut. This balance is dynamic for overall digestive health and can affect additional areas of well-being, including immune function and mental health (Casertano et al., 2022).

Probiotics include different species and strains, each with exclusive properties and health benefits. Two of the most famous and extensively studied genera of probiotic bacteria are Lactobacillus and Bifidobacterium. Lactobacillus species are often found in inflamed foods such as yogurt and sauerkraut and are known for their aptitude to produce lactic acid, which helps inhibit the growth of harmful bacteria. Bifidobacterium species commonly found in the intestines help digest fiber, prevent infection, and produce vitamins (Ibrahim et al., 2023).

In adding to bacteria, some yeast species such as Saccharomyces boulardii are also documented as effective probiotics. Saccharomyces boulardii has been exposed to help treat and prevent gastrointestinal disorders, including diarrhea and inflammatory bowel disease. It mechanism by supporting gut barrier health, modulating the immune system and antagonizing pathogenic microorganisms (Yeşilyurt et al., 2021).

Consumption of probiotics can take many forms, including dietary supplements and foods that are obviously rich in or have been developed with these microorganisms. Regular consumption of probiotics is connected with a number of health benefits, such as improving digestion, strengthening the immune system, and potentially alleviating conditions such as irritable bowel syndrome and some allergies (Simon et al., 2021).

Continuing research remains to explore the enormous potential of probiotics, investigative their effects on conditions ranging from obesity to mental health disorders. The developing understanding of the human microbiome underscores the importance of maintaining a healthy microbial balance and the role probiotics can play in attaining it. Including probiotics in the diet is therefore a practical approach to support and improve overall health (Olvera-Rosales et al., 2021).

Mechanisms of Action

The beneficial effects of probiotics are supposed to be due to an amount of mechanisms, including the instruction of gut flora. Probiotics can help restore and maintain a healthy balance of gut flora, which is often disrupted in IBS patients. By strengthening the function of the intestinal barrier, probiotics strengthen the intestinal epithelial barrier, prevent the penetration of harmful substances into the bloodstream and reduce inflammation (Zheng et al., 2023).

Immune modulation Probiotics modulate the immune system, strengthen the host's defense mechanisms and reduce intestinal inflammation. Probiotics produce short-chain fatty acids and ferment dietary fiber to form SCFA. SCFAs provide energy to colon cells and have anti-inflammatory effects. By inhibiting pathogenic bacteria, probiotics produce substances that prevent the growth of harmful bacteria and compete with them for adhesion sites in the intestinal lining. Production of neurotransmitters Some probiotics produce neurotransmitters (such as serotonin) that can affect gut motility and pain perception (Akram et al., 2024).

Common Strains used in Research and Therapy

Lactobacillus species, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus roteri. Bifidobacterium species, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum. Probiotics, Saccharomyces boulardi (beneficial yeast), Streptococcus thermophilus, Enterococcus faecium (Anwar et al., 2021).

The Gut Microbiota and IBS

Understanding Gut Microbiota and Composition of Gut Microbiota

Gut flora consists of trillions of microorganisms, including bacteria, viruses, fungi and protozoa, that reside primarily in the intestines. Most are bacteria, the main phyla being Firmicutes and Bacteroidetes. Other important phyla include Actinobacteria, Proteobacteria and Verrucus. Role in digestive health. The gut microbiome plays an important role in digestion and metabolism, helping to break down complex carbohydrates, fiber and protein and producing essential nutrients such as K and B vitamins (Yang et al., 2020).

Improving the function of the immune system, regulating the immune system, strengthening the function of the intestinal barrier and protection against pathogenic bacteria. The gut-brain axis communicates with the central nervous system through neural, hormonal, and immune pathways and influences gut movement and behavior. Production of short-chain fatty acids (SCFA). It ferments dietary fiber to form SCFAs such as acetate, propionate and butyrate, which provide energy to colon cells and have anti-inflammatory properties (P. Liu et al., 2021).

Dysbiosis refers to an imbalance in the composition of the intestinal flora, which is characterized by a reduction in microbial diversity and beneficial bacteria. This imbalance leads to increased intestinal permeability, often referred to as "leaky gut," which allows harmful substances to enter the bloodstream and cause inflammation. Immune Dysregulation This causes chronic, low-grade inflammation and is implicated in a variety of conditions, including IBS. It can cause metabolic disorders, obesity, diabetes and other metabolic syndromes. Digestive system problems. It causes symptoms such as bloating, gas, diarrhea and constipation (De Filippis et al., 2020).

Gut Microbiota in IBS Patients and Differences in Microbiota Composition

Studies have shown that IBS patients often show distinct changes in their gut microbiome, including reduced diversity and overall reduced diversity of gut bacteria. The ratio changes, the balance between Firmicutes and Bacteroidetes is disturbed and varies depending on the subtype of IBS (IBS-C, IBS). -D, IBS-M). Increased pathogens, increased levels of potentially harmful bacteria such as E. coli and Clostridium. Decreasing the amount of beneficial bacteria, the level of beneficial bacteria such as Lactobacillus and Bifidobacterium is reduced (Guo et al., 2020).

Potential Causes of Dysbiosis in IBS

Several factors may contribute to dysbiosis in IBS patients, including a high-fat, low-fiber diet that can negatively affect the composition of the gut microbiome. Taking antibiotics Frequent or long-term use of antibiotics can disrupt the balance of intestinal bacteria. Infectious diseases, gastrointestinal infections, can cause long-term changes in the gut microbiome. Stress, psychological stress, can alter gut microbiota through the gut-brain axis. Genetics, a genetic predisposition, can influence the composition and function of the intestinal microflora (Bubier et al., 2021).

Relationship between Microbiota and IBS Symptoms

Changes in the composition of the gut flora in IBS patients are thought to contribute to symptoms through a variety of mechanisms, including visceral sensitivity and changes in gut bacteria that affect the gut-brain axis and may lead to increased sensitivity to pain. Enjoying a change in motility, dysbiosis affects intestinal motility and can cause diarrhea and constipation (Ballan et al., 2020).

Gas production: Some bacteria produce gas as a byproduct of metabolism, causing bloating and discomfort. Inflammation, dysbiosis can cause mild inflammation and worsening of IBS symptoms. Metabolite production an imbalance in the microbiome can lead to the production of metabolites that negatively affect gut health and function. Understanding the complex relationship between gut microbiota and IBS is important for the development of effective treatments (J. Liu et al., 2022).

Probiotics Mechanisms of Action in IBS and Modulation of Gut Microbiota

Probiotics play a key role in reinstating and maintaining the balance of a healthy microbiome by increasing the population of helpful bacteria while reducing harmful bacteria. This balance is essential for optimal digestive health and overall safety. By increasing the number of beneficial microorganisms, probiotics improve digestion and absorption of nutrients and assistance relieve symptoms such as bloating and irregular bowel movements (Horvat et al., 2021).

One of the main ways probiotics promote gut health is by inhibiting pathogenic bacteria. It competes with these harmful microorganisms for adherent sites in the intestine, thus preventing colonization and multiplication of the pathogen. Furthermore, probiotics are antimicrobial compounds including bacitracin and lactic acid that make an unfriendly environment for harmful bacteria and inhibit their growth (Hill et al., 2014; Tran et al., 2022).

The benefits of probiotics go outside gut health. By supporting a balanced microbiome, they help reinforce the immune system and help the body effectively fight diseases. Probiotics also play a role in modulating the immune system and may be chiefly helpful in managing inflammatory situations and autoimmune diseases (Yeşilyurt et al., 2021).

Table 1: The role of probiotics in the treatment of irritable bowel syndrome

Sr.	Aspect	Description	Example	Mechanisms of Action	Clinical Evidence	References
1	Definition and Types	Probiotics are live microorganisms that confer health benefits when consumed in adequate amounts.	Lactobacillus, Bifidobacterium	Modulation of gut microbiota, production of SCFAs, inhibition of pathogens	Reduction in abdominal pain, bloating	(Defaye et al., 2020; S. Mishra and Acharya, 2021)
2	Mechanisms of Action	Probiotics modulate gut microbiota, enhance gut barrier function, and influence the gut-brain axis.	Restoration of microbiota balance	Reduction of gut inflammation, enhancement of gut barrier integrity	Improved stool consistency, quality of life	(Barraza-Ortiz et al., 2021; Mörkl et al., 2020)
3	Efficacy Across Strains	Different probiotic strains vary in their effectiveness in alleviating IBS symptoms.	Bifidobacterium infantis, Lactobacillus rhamnosus	Inhibition of pathogenic bacteria, modulation of immune responses	Reduction in IBS severity, frequency of symptoms	(Kumar et al., 2022; Raheem et al., 2021)
4	Clinical Trials and Meta-analyses	Randomized controlled trials and meta-analyses provide evidence for probiotic efficacy in IBS.	Meta-analyses pooling RCT data	Improvement in global IBS symptoms, abdominal pain reduction	Variability in outcomes across studies	(Horvat et al., 2021; McFarland et al., 2021)
5	Personalized Probiotic Therapy	Tailoring probiotic treatments based on individual patient profiles and microbiome characteristics.	Individualized probiotic formulations	Optimization of gut microbiota, symptom-specific relief	Enhanced treatment response in specific patient profiles	(Grumet et al., 2020; Schupack et al., 2022)
6	Safety and Side Effects	Probiotics are generally well-tolerated, with mild gastrointestinal symptoms reported occasionally.	Gas, bloating, abdominal discomfort	Minimal systemic side effects, transient symptoms	Rare severe adverse reactions reported	(Anadón et al., 2021; Depoorter and Vandenplas, 2021)
7	Prebiotics and Synbiotics	Prebiotics stimulate Growth of beneficial gut bacteria, while synbiotics combine probiotics with prebiotics.	Inulin, fructooligosaccharides (FOS)	Enhanced probiotic survival, synergistic health benefits	Improved gut health, additional symptom relief	(Costa et al., 2022; Martinez et al., 2015)
8	Combination Therapies	Probiotics used in conjunction with diet modifications, medications, or behavioral therapies.	Low FODMAP diet, antispasmodics	Synergistic effects on symptom management, comprehensive treatment approach	Enhanced overall treatment outcomes	(Fikree and Byrne, 2021; Staudacher et al., 2023)
9	Guidelines for Use	Recommendations from medical societies regarding probiotic use in managing IBS.	American College of Gastroenterology (ACG), World Gastroenterology Organisation (WGO)	Consideration of probiotics as part of comprehensive management strategies	Emphasis on evidence-based approaches	(Compare et al., 2022; Sundaram and DM, 2023)
10	Challenges in Research	Inconsistencies in study designs and a need for standardized protocols in probiotic research.	Variability in probiotic strains, dosages	Difficulty in comparison across studies, lack of consensus on optimal protocols	Addressing research gaps for clearer conclusions	(Lawal et al., 2024; Tremblay et al., 2021)
11	Advances in Probiotic Research	Development of new probiotic strains and formulations, leveraging microbiome sequencing technologies.	Next-generation probiotics, live biotherapeutic products (LBPs)	Enhanced therapeutic properties, targeted treatment approaches	Potential for improved treatment efficacy	(Shyam et al., 2021; Singh and Natraj, 2021)

12	Future Directions	Exploration of personalized medicine approaches, integrating microbiome research with clinical practice.	Tailored probiotic therapies based on gut microbiota profiling	Improved patient outcomes, precision medicine applications	Optimizing probiotic use for individualized care	(Ratiner et al., 2024)
13	Economic Considerati ons	Cost-effectiveness and affordability of probiotic treatments in long-term management of IBS.	Healthcare system perspectives	Evaluation of benefits versus costs, impact on healthcare expenditures	Considerations in healthcare resource allocation	(E. D. Shah et al., 2022; Tarricone et al., 2020)
14	Public Health Implications	Potential impact of probiotics on population health and healthcare policy recommendations.	Public health strategies, regulatory frameworks	Integration into dietary guidelines, health promotion initiatives	Role in promoting gut health on a broader scale	(Merenstein et al., 2024; Snetselaar et al., 2021)
15	Patient Education	Importance of educating patients on probiotic use, expectations, and potential benefits.	Patient-centered care, informed decision-making	Empowerment through knowledge, adherence to treatment regimens	Enhancing patient-provider communication	(McFarlane et al., 2023; Nguyen et al., 2020)

Collective probiotics are available from a variability of sources. Enflamed foods such as yogurt, kefir, sauerkraut, and kimchi contain many beneficial live bacteria. In addition, probiotic supplements are available in a variety of forms, including capsules, tablets, and powders, providing a suitable option for individuals looking to increase their probiotic consumption (de Sire et al., 2022).

Current research into probiotics lasts to reveal their potential to report many health issues. Studies have shown promising results using probiotics to treat conditions such as irritable bowel syndrome and even some allergies. There is also developing indication that probiotics can have a positive effect on mental health, possibly through the gut and the brain, highlighting the strong link between gut health and expressive safety (Mörkl et al., 2020).

Addition probiotics to your diet is a way to improve your health. As our empathetic of the microbiome strengthens, the role of probiotics in maintaining and improving health is becoming increasingly deceptive. By supporting the balance of beneficial bacteria in the gut, probiotics provide a natural and effective method to support digestive health, boost the immune system and can improve health (Jiang et al., 2017; Ranjha et al., 2021).

Anti-inflammatory Effects

Probiotics decrease intestinal inflammation by moderating pro- and anti-inflammatory cytokines. They can help reduce inflammatory markers and minor symptoms associated with chronic eczema, such as pain and discomfort. Effects on the immune system Probiotics interact with the lymphoid fluid associated with the gut and improve immune function. These stimulate regulatory T cells and other immune cells that help maintain immune tolerance and prevent excessive inflammation (Topol et al., 2022).

Gut Barrier Function

Strengthening the integrity of the intestinal barrier Probiotics strengthen the intestinal barrier by inducing the formation of a complex of cross-linking proteins that form fibers between epithelial cells. This improvement prevents harmful substances from entering the bloodstream from the intestinal tract and reduces the risk of contamination. Preventing Permeable Gut Syndrome By maintaining the morality of the intestinal barrier, probiotics can help prevent Leaky Gut Syndrome, where symptoms include inflammation of the intestinal lining through it more, permitting toxins and pathogens to enter the bloodstream (Aleman et al., 2023).

Impact on the Gut-brain Axis

Probiotics have an effect on the developed of key neurotransmitters consisting of serotonin, gamma-aminobutyric acid and dopamine, all of which play a important role in modifiable bowel actions, feelings and mood. By modulating these neurotransmitters, probiotics can relieve symptoms such as abdominal pain and improve bowel behavior. This interaction underscores the importance of the gut-mind axis, a reciprocal announcement implement between the gut and the brain (Aghamohammad et al., 2023).

The gut-mind axis is essential for expert information of probiotics can positively influence mental health. Probiotics may help reduce stress and anxiety by moving this axis through multiple mechanisms. They can reduce the production of stress hormones, which include cortisol, and increase the production of positive neurotransmitters that give to overall intellectual health. This dual movement of gut health and mental health is especially cooperative for people with irritable bowel syndrome, a condition often impaired by stress and anxiety (Aghamohammad et al., 2023).

Probiotics offer a complicated technique to discourse IBS symptoms by targeting numerous fundamental factors. They modulate the intestinal microflora, multiply the population of useful bacteria and at the same time decrease the dangerous ones. This balance eases the reduction of inflammation and supports intestinal barrier function, which is essential for maintaining a healthy digestive system. By moving the gut-brain axis, probiotics may also improve stress-related symptoms and offer a complete healing technique (Bassotti, 2022).

We will delve into the unique indication supporting the efficiency of probiotics in the treatment of IBS, as well as the safety aspects of their use. This evidence includes clinical trials and studies that have shown the benefits of changed probiotic strains in relieving IBS symptoms. It is important to observe that whilst probiotics are typically taken into thought safe for most human beings, a limited persons may additionally enjoy mild facet effects (Kumar et al., 2022).

Safety factors of probiotics include the possibility of mild feature results inclusive of fuel and bloating, especially. when first beginning supplementation. These side outcomes are generally temporary and go away as the body adjusts. However, people with weakened immune structures or extreme underlying health conditions need to consult a fitness care provider before starting probiotic supplementation (Kothari et al., 2019).

Probiotics offer a promising treatment alternative for IBS via addressing multiple aspects of the situation. Their capacity to modulate intestine microflora, reduce irritation, improve gut barrier function, and influence the intestine-mind axis makes them a complete and powerful technique to treating signs and symptoms. By analyzing the proof and know the protection elements, individuals with IBS could make informed choices approximately incorporating probiotics into their remedy regimen, doubtlessly improving their high quality of life (Black & Ford, 2021).

Clinical Evidence of Probiotics in IBS and Overview of Clinical Trials

Randomized controlled trials (RCTs), these studies are taken into consideration the gold preferred in scientific studies. Participants had been randomly assigned to remedy with probiotics or placebo, allowing evaluation of consequences. RCTs may additionally help decide the effectiveness of probiotics in handling IBS signs. These research, meta-analyses, integrate information from a couple of RCTs to provide a more entire analysis of the efficacy and safety of probiotics. Meta-analyses can offer stronger evidence due to large pattern sizes and extra statistical electricity. Important findings and effects endorse that probiotics can significantly lessen IBS signs and symptoms inclusive of stomach ache, bloating and abnormal bowel moves (Dale et al., 2019).

Efficacy of different Probiotic Strains

The specific bacterial stress studied, a species of Bifidobacterium, Bifidobacterium infantis, has shown great development in stomach ache, bloating and bowel regularity in numerous studies. Bifidobacterium longum is indicated to lessen the overall severity of IBS signs and symptoms and improve pleasant of lifestyles. Lactobacillus rhamnosus is effective in decreasing stomach pain and bloating. Lactobacillus plantarum has been suggested to enhance universal IBS signs together with stomach pain and bowel frequency. Saccharomyces boulardii, a beneficial yeast that has been proven to lessen diarrhea and improve usual signs in IBS-D sufferers (Qing et al., 2023).

Differences in Effectiveness among Strains

The effectiveness of probiotics can range greatly relying on the stress. Certain strains may be more effective towards positive subtypes of IBS or precise symptoms (stomach ache, bloating, and many others.). Multi-strain formulations may additionally provide a much broader variety of benefits and might deal with a wider range of situations than unmarried-pressure probiotics (Puvanasundram et al., 2021).

Probiotic Formulations and Single-strain vs Multi-strain Probiotics

Single-strain probiotics contain a specific strain of probiotic bacteria. Although effective for targeted symptoms, they do not address the full spectrum of IBS symptoms. Multi-Strain Probiotics Contain multiple strains of probiotics and may offer a more comprehensive approach to symptom relief. These can improve overall gut health and have wide-ranging benefits for IBS sufferers. Dosage and Duration of Treatment The optimal dosage and duration of probiotic treatment will vary by strain and patient. Typical doses range from 10^8 to 10^11 colony forming units per day. The duration of treatment in clinical trials is usually 4 to 12 weeks, and some studies have shown that longer durations of treatment may have longer-lasting effects (Dale et al., 2019; Kutylowksi and Yahia, 2019).

Safety and Side Effects and Adverse Effects Reported

Probiotics are generally well tolerated and most side effects are mild and transient. Common side effects include gas or bloating, abdominal discomfort, diarrhea or constipation (as the gut initially adjusts to the probiotics). Long-term safety considerations Long-term use of probiotics is generally considered safe for most people. However, certain populations (such as immunocompromised individuals and those with serious underlying diseases) should use probiotics with caution and under medical supervision. More research is needed to better understand the long-term safety and potential benefits of long-term probiotic use (Pammi et al., 2024).

Probiotics in Clinical Practice and Guidelines for use
Recommendations from Medical Societies

Various medical suggestions such as the American College of Gastroenterology (ACG) and the World Gastroenterology Organization (WGO) provide rules for the use of probiotics in IBS. These commendations are based on indication from clinical trials and meta-analyses. However, they highlight that the special of probiotic strain, dose and duration should be based on the specific symptoms and subtype of IBS. WGO guidelines recommend that probiotics be considered as part of a comprehensive IBS treatment plan, especially in patients with mild to moderate disease (Makharia et al., 2022).

Indications for probiotic therapy are commonly used in patients with IBS who experience symptoms such as abdominal pain or discomfort, bloating or flatulence, and irregular bowel movements (diarrhea, constipation or intermittent). Probiotics are especially suitable for patients who prefer non-drug treatment or for whom traditional treatment has not been effective enough. Personalized probiotic therapy, tailoring probiotics to the patient profile, personalized probiotic therapy including individual patient symptoms, gut microflora composition, generally this involves choosing specific probiotic strains and formulations based on your health condition (Singh and Natraj, 2021).

Future Directions in Personalized Medicine

The future of personalized probiotic therapy lies in the integration of microbiome research and clinical practice. The goal of this approach is to develop targeted therapies that optimize gut health and reduce IBS symptoms (Su et al., 2020).

Combination Therapies and Probiotics in Conjunction with other Treatments

The effectiveness of probiotics can range greatly counting on the pressure. Certain traces can be greater effective closer to advantageous subtypes of IBS or precise signs and symptoms (stomach ache, bloating, and many others.). Multi-stress formulations may additionally moreover provide a far broader type of blessings and can address a much wider variety of situations than unmarried-stress probiotics (Gendi and Jahan, 2020).

Synergistic Effects

Relating probiotics with other remedies could have a synergistic effect, growing the advantage of every treatment and lowering signs and symptoms. For instance, probiotics and dietary interventions can paintings synergistically to reduce intestine inflammation, improve intestine motility, and restore a healthy microflora balance. Combination cures can also cope with elements of IBS pathophysiology, along with dysbiosis, intestine barrier disorder, and visceral hypersensitive reaction. Probiotics have emerge as a treasured device within the remedy of IBS and provide potential blessings for symptom relief and basic intestine health (Chlebicz-Wójcik and Śliżewska, 2021).

Challenges and Future Directions and Research Gaps and Limitations

Inconsistencies in Study Designs

Numerous research on probiotics and IBS have had differences in have a look at design, consisting of variations in sample length, remedy period, probiotic strains, and doses. These inconsistencies make it tough to attract clean conclusions and evaluate effects among studies. Some research lacked strict placebo controls or used subjective outcome measures, which may additionally introduce bias and have an effect on the reliability of the outcomes. There are also variations within the diagnostic criteria used for IBS. There is a want to increase a widespread protocol in probiotic research to improve the best and comparability of studies. Includes standardized meanings of probiotic lines, dosage and duration of remedy (Zhang et al., 2023).

Advancements in Probiotic Research and New Probiotic Strains and Formulations

Current research is attentive on recognizing new strains of probiotics with specific therapeutic properties for IBS. It includes strains with better ability to modulate intestinal microflora, decrease inflammation and improve intestinal barrier function. Multi-strain, multi-probiotic formulations are being developed to provide broader benefits and report different aspects of the pathophysiology of IBS. New technologies, developments in microbiome sequencing, and metagenomic analysis are providing deeper insight into the formation and function of the gut microbiota (Suez et al., 2020).

Potential for Novel Therapeutics and Next-generation Probiotics

Probiotics, also known as live biotherapeutic produces, are a cutting-edge method to improve gut health and treat a variety of conditions. These progressive probiotics are produced using genetically caused strains that have specific therapeutic properties (Adolfsen et al., 2021).

Premeditated for targeted applications such as antibody production and immune monitoring, it delivers complete and effective health benefits more to traditional probiotics. An interesting area of research is the use of beneficial microbial relations that work composed to restore gut health (Simon et al., 2021).

This method takes gain of the commonplace benefits of a couple of lines and probably offers a more complete strategy to gut health issues. In addition to probiotics, prebiotics and synbiotics play a essential position in supporting the microbiome. Prebiotics are indigestible meals materials that selectively stimulate the increase and activity of useful micro organism in the intestine. Common prebiotics consist of inulin and fructooligosaccharides, which function food for probiotics, growing their survival and effectiveness (Nobre et al., 2022).

Synbiotics, mixtures of prebiotics and probiotics, provide a synergistic impact and provide extra advantages for intestine fitness. This combination will increase the survival charge of probiotics as they pass thru the digestive gadget and guarantees that they reach the intestine in sufficient quantities to offer fitness blessings. Research has proven that combining precise prebiotics with probiotic lines can enhance IBS signs together with bloating, ache, and abnormal bowel moves (Simon et al., 2021).

The future of probiotic research and IBS remedy seems promising, with advances in microbiome technology and technological know-how paving the manner for extra powerful and personalized treatments. Addressing current studies gaps and capitalizing on these advances could lead to new therapeutic procedures that drastically improve the quality of lifestyles of IBS sufferers (Gulliver et al., 2022).

Personalized probiotic remedies, tailor-made to the precise make-up of an individual's microbiome, are at the horizon and offer wish for extra effective treatment of IBS and different gut-related issues. By focusing on the improvement of next-generation probiotics and the strategic use of prebiotics and synbiotics, researchers and fitness specialists are making strides toward greater complete and effective solutions for gut fitness. This integrated approach not only promotes the growth of beneficial bacteria, but also improves overall digestive health, immune function and safety (Simon et al., 2021).

Conclusion

Clinical evidence supports their effectiveness in reducing symptoms such as abdominal pain, constipation, and irregular bowel habits as probiotics hold great promise for the management of irritable bowel syndrome respond by modulating gut bacteria, reducing inflammation, increasing gut barrier function, and affecting the gut brain tissue. However, inconsistencies in study design and the need for standardized designs make it difficult to draw definitive conclusions. Despite these challenges, the potential for future advances in probiotic therapy is high. Other options could include new probiotic strains tailored to specific health needs, personalized probiotic therapies based on a person's unique bacteria, and combination therapies with prebiotics and synbiotics for maximum therapeutic benefit Our continued research with technology emerging trends such as microbiome sequencing will improve our understanding of how strongly probiotics can be modified to treat IBS. Addressing current challenges and adopting future directions will improve the efficacy of probiotic therapies and improve the quality of life of patients with IBS. By advancing research and improving treatments, the potential of probiotics in the overall management of IBS can be realized, bringing hope and relief to many suffering from this condition.

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Chapter 41

Lactobacillus casei: Effects of its use against Pathogens (Parasites, Bacteria and Viruses) of Veterinary and Public Health Importance

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ABSTRACT

Bacteria of the genus *Lactobacillus* and their application in both humans and animals have become very important. The different species of this bacterium, but especially *Lactobacillus casei*, has proven to be a promising strategy for the control of pathogens, as the different routes of administration have demonstrated the ability to stimulate a good humoral and cellular immune response in infected hosts both naturally and experimentally. In addition, *Lactobacillus casei* in humans, rodents and production animals can protect against certain parasitic, bacterial and viral infections, decreasing pathogen loads, establishment and colonization, as well as intestinal lesions, and increasing weight gain and survival. This chapter presents evidence of the above, concerning the study of highly relevant issues related to the use and administration of *Lactobacillus casei* in production animals, humans and animal models for the control of protozoan parasites and helminths, as well as against bacteria and viruses.

KEYWORDS
Bacteria, Helminths, *Lactobacillus casei*, Probiotics, Protozoa, Viruses.

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INTRODUCTION

In recent decades there has been an interest in the role of probiotic bacteria in the prevention of digestive disorders (Elmer et al., 2001; Pereg et al., 2005), therefore different agents have been used among which lactic acid bacteria, particularly *Lactobacillus* species, are the commonly used probiotics (Mombeli and Gismondo, 2000). This bacterium has been shown to be an immunostimulant (Bautista-Garfias et al., 2005), as it has a protective response against numerous infections in both animals and humans (Ashraf et al., 2005; Bautista-Garfias et al., 2002; Bautista-Garfias et al, 2005; Hori et al., 2001; Maldonado and Perdigón, 2006; Sato, 1984; Vercruysse et al., 2007), which is why it has been proposed as an alternative for disease control due to its capacity to increase non-specific immunity (Bautista-Garfias et al., 1999; Masihi, 1994). In addition, probiotics can provide benefits to both animal and human health when administered in adequate amounts (Boros et al., 2022; Hill et al., 2014).

Bacteria of the genus *Lactobacillus* comprise about 180 Gram-positive bacteria (Haakencen et al., 2009), and one of the main mechanisms of action is related to their ability to compete with pathogens for adhesion sites, improve the activity of the intestinal mucosal barrier, produce microbial agents and regulate host immune responses (Butell, 2014; Donelli et al., 2013). These lactic acid microorganisms are used in the dairy industry, as they provide a better taste in dairy products and increase their nutritional properties (Martínez-Gómez et al., 2006), as well as improve intestinal microflora when administered to animals and humans.

Lactobacillus casei (*L. casei*) and its Effects on Immunity

It has been demonstrated that the administration of *Lactobacillus casei* (*L. casei*) in mice stimulates an immuno-protective response against several parasites (Bautista et al., 2008; Bautista-Garfias, 2004;), in addition to stimulating the production of interleukin IL-12 and interferon-gamma (INF-γ) (Kato et al., 1999), thus promoting the expression of cytokines and the maturation of surface markers on the surface of dendritic cells (Christensen et al., 2000) through the stimulation of Toll-like receptors 2 (TLR2) (Matsuguchi et al., 2003), which similarly occurs in *Babesia bovis*-infected cattle, generating a Th1-type immune response associated with the production of INF-γ, interleukin IL-12, nitric oxide, and immunoglobulin IgG2 (Brown and Palmer, 1999; Shoda et al., 2000).

In mice treated with *L. casei*, an increase in the number of mononuclear cells in the stroma of the intestinal villi was observed (Bautista-Garfias et al., 1999), although it was not determined whether these cells were lymphocytes or macrophages, the findings suggest that *L. casei* treatment enhances the local immune response, also improving the amount of major histocompatibility complex class two antigens (MHC-II) on peritoneal macrophages (Kato et al., 1988). Regarding INF-γ in serum from *L. casei* treated animals showed that it is a potential activator of macrophages (Suzuki et al., 1988) and stimulates antigen presentation to enhance MHC gene expression (Gaszynska et al., 1993).

In addition, colonization of *L. casei* in the gut and processing of dead *Lactobacillus* by macrophages in local immune tissues and antigen presentation to Th1 cells may produce IL-2 to activate B cells and T cells, as well as INF-γ, which probably activates macrophages in a pathway in which these cells rapidly process antigens enhancing the acquired immune response, as these macrophages also produce nitric oxide and probably promote an inflammatory response in the gut (Bautista-Garfias et al., 2001).

Intranasal administration of *L. casei* in mice has been shown to induce the production of cytokines such as INF-γ, interleukin IL-12, and tumor necrosis factor-alpha (TNF-α) (Hori et al., 2001), suggesting that inoculation with *L. casei* enhances cell-mediated immunity in the respiratory tract and protects against viral infections such as influenza. It has been proposed that *L. casei* is involved in antibody production, however, some of the mechanisms have not yet been elucidated. It is proposed that the dendritic cells activated by *L. casei*, futherly process the antigens of some protozoa and induce the production of specific IgG1 and IgG2 antibodies (Bajer et al., 2003), and this probably may occur due to the presence of an increased number of memory B cells (Bautista-Garfias et al., 2015). *L. casei* is known to stimulate the production of Toll-like receptors (Maldonado and Perdigón, 2006; Vizoso et al., 2009), as well as modulate adaptive cellular and humoral immunity, leading to an enhanced acquired immune response against particular antigens (Bautista and Mosqueda, 2005; Ferwuerda et al., 2010).

Studies have suggested that probiotics can decrease the pathogenicity of parasites and, as a consequence, influence the course of parasitic infections (Berrili et al., 2012). In this regard, the main mechanisms of action of probiotics are related to their ability to compete with pathogens for adhesion sites, enhance the activity of the mucosal-intestinal barrier, produce antimicrobial agents and regulate host immune responses (Butel, 2014; Donelli et al., 2013). In addition, it regulates anti-inflammatory cytokine (IL-10) levels and increases the number of mucus-producing epithelial cells (McClemens et al., 2013). The mechanism behind immunomodulation involves interactions between *L. casei* and gut-associated lymphoid tissue (GALT), which is an important local immune compartment, thus probiotics such as *L. casei* can modulate the activity of several cells, such as erythrocytes, dendritic cells (DCs) and T-cells, and increase protection against intestinal infections (Boros et al., 2022; De Le Blanc et al., 2007; Friedrich et al., 2017; Randazzo and Contamagna, 2005; Sanchez et al., 2017).

In young animals (bovines) of different ages, *in vitro* studies, have shown that *L. casei* has the ability to produce nitric oxide in bovine monocytes, and specially shows higher production of nitric oxide within 4-6-month-old animals. Studies also suggested that *L. casei* can be used in *in vivo*, to stimulate innate immunity, specifically in young animals (Bautista-Garfias et al., 2016).

Chemical Properties of *Lactobacillus casei*

The hydrophilic nature of the genus *Lactobacillus*, regardless of species, has been reported in several studies (Andreu et al., 1995; Cuperus et al., 1993; Harty et al., 1993; Reid et al., 1992). In addition, it has a maximum affinity for an acidic solvent, such as chloroform, and a low affinity for a basic solvent, such as ethyl acetate, confirming the hydrophilic properties of its cell surface (Pelletier et al., 1997).

Lactobacillus casei produces biosurfactants, which are surface-active microbial compounds with antimicrobial and antioxidant activities with a wide range of physiological properties including methyl palmitate (2,5-O methyl rapmnofuranosyl palmitate) (Mouafo et al., 2021). Although there is little work on the structural characterization of *L. casei* biosurfactants, they have been reported as a mixture of proteins, polysaccharides, phosphates and lipids (Ferreira et al., 2017; Madhu and Paprulla, 2013; Sharma and Saharan, 2016).

Effects of *L. casei* on Parasites Affecting Animal Health

L. casei against *Babesia bovis* (*B. bovis*) and *Babesia bigemina* (*B. bigemina*)

Bautista-Garfias et al, (2008) evaluated the effectiveness of *L. casei* in conjunction with a vaccine against *Babesia bovis* and *Babesia bigemina* resulting in an increase in the agglomerated cell volume and a better rectal temperature in those animals where *L. casei* was applied intramuscularly, also the level of anti-*Babesia* antibodies was found higher after 10 days of treatment, as well as a better production of INF-γ compared to the control groups, indicating that the inoculation of *L. casei* two days before vaccination improves the efficiency of the bivalent vaccine. Subsequently, the same research group conducted a second study evaluating the simultaneous vaccination of cattle with *L. casei* and the bivalent vaccine against bovine Babesiosis under field conditions. A decrease in rectal temperature was recorded 13 days after exposure to *Babesia*-infected ticks, as well as an increase in the average percentage of agglomerated cell volume was recorded between 13 and 15 days. Also, a lower percentage of parasitized erythrocytes was observed 12-14 days after exposure to infected ticks, while anti-*Babesia* IgG antibody levels were higher 20 days after confrontation (Bautista-Garfias et al., 2012). Finally, a third *in vitro* study was developed by Bautista-Garfias et al, (2015), which evaluated the levels of specific IgG1 and IgG2 antibodies against *B. bovis* and *B. bigemina* in cattle co-immunized with *L. casei*, observing that the levels of IgG1 and IgG2 antibodies were found higher in animals co-immunized with *L. casei* and the bivalent vaccine between 15-30 days of post-confrontation, in addition, the rectal temperature remained within normal parameters, and the percentage of parasitized erythrocytes was found lower after 24 hours in vitro.

L. casei against *Eimeria acervulina, E. maxima* and *E. tenella*

So far, there is only one work available in the scientific literature on the use of *L. casei* against coccidia of the genus *Eimeria* by Bautista-Garfias et al., (2003) who compared its effectiveness with that of a commercial vaccine in chickens. The results showed that the daily weight gain was equal to that produced by the commercial vaccine compared to the control groups (untreated-infected; untreated-infected-untreated). In addition, the average number of oocysts was lower and very similar to that of the vaccinated group after 5-8 days of post-infection. Similarly, the average number of intestinal lesions at necropsy (33 days of post-infection) was lower in the duodenum, jejunum, and cecum.

L. casei against Haematobia irritans (*H. irritans*)

As with the previous parasitic genus, there is only one study carried out by Bautista-Garfias et al., (2004), in which *L. casei* was used in conjunction with incomplete Freund's adjuvant (IFA) and immunized with intestinal antigens of the horn fly (*H. irritans*). The results showed that the percentage reduction of oviposited eggs of each fly was lower compared to the immunized group without *L. casei*, and IgG antibody levels were higher in the group immunized with *L. casei* and IFA.

Table 1: *L. casei* against parasites of concern in production animals

Parasites	Species	Authors	Results
B. bovis	Cattle (*Bos taurus taurus*)	Bautista et al., 2008; Bautista-Garfias et al., 2012; Bautista-Garfias et al., 2015	Increased serum IgG1 and IgG2 levels.
B. bigemina	Cattle (*Bos taurus taurus*)	Bautista et al., 2008; Bautista-Garfias et al., 2012; Bautista-Garfias et al., 2015	Increased serum IgG1 and IgG2 levels.
E. acerbulina	Broiler chickens (*Gallus gallus domesticus*)	Bautista-Garfias et al., 2003	Increase in weight gain; decrease in oocyst excretion; decrease in intestinal lesions; increase in chick survival.
E. maxima	Broiler chickens (*Gallus gallus domesticus*)	Bautista-Garfias et al., 2003	Increase in weight gain; decrease in oocyst excretion; decrease in intestinal lesions; increase in chick survival.
E. tenella	Broiler chickens (*Gallus gallus domesticus*)	Bautista-Garfias et al., 2003	Increase in weight gain; decrease in oocyst excretion; decrease in intestinal lesions; increase in chick survival.
H. irritans	Cattle (*Bos taurus taurus*)	Bautista-Garfias et al., 2004	Reduced oviposition of adult flies; increased serum IgG levels.

Effects of *L. casei* on Parasites Affecting Public Health

L. casei against *Babesia microti* (*B. microti*)

Oral and intraperitoneal administration of *L. casei* against the intracellular protozoan *Babesia microti* (*B. microti*), which affects humans, was evaluated using mice as an animal model, and it was observed that mice treated with *L. casei* showed a significant reduction in the percentage of parasitized erythrocytes compared to untreated mice. Infection with *B. microti* and treated with *L. casei* orally or intraperitoneally, seven days before infection, was lower from 17 days post-infection and remained so until the end of the study (day 31). The protective response showed better results when *L. casei* was administered three days before or the same day of infection, demonstrating that the percentage of parasitemia, according to the number of infected erythrocytes, was less than 5% throughout the study, especially when the *L. casei* bacteria were viable (Bautista-Garfias et al., 2005). Subsequently, a study was conducted to evaluate the capacity of viable and dead *L. casei* in mice challenged with erythrocytes infected with *B. microti*. The results showed that mice treated with *L. casei* had a lower average number of parasitized erythrocytes compared to the control group (untreated), and reported low (19-59kDa) and high (63-111kDa) molecular weight *L. casei* components. The results suggest that *L. casei* can induce a protective immune response with both live and dead *L. casei* probiotics (Bautista et al., 2008).

***L. casei* against *Cryptosporidium parvum* (*C. parvum*)**

One of the first studies evaluating the use of probiotics for the control of cryptosporidiosis in humans was carried out by Pickerd and Tuthill (2004), using daily treatment with *L. casei* (Shirota) for 10 days, in which nausea, diarrhea and abdominal pain were reduced, allowing the patient (12-year-old girl) to return to normal activities.

Subsequently, to evaluate the effect of *L. casei* against *C. parvum*, rats were used as a model for this purpose, administering a conjugate of *L. casei* two days before infection, where they measured weight gain, parasite load, damage to the intestinal mucosa and expression of muco-intestinal cytokines. However, the results showed that the daily administration of a conjugate of *L. casei* was ineffective in eradicating the parasite compared to the biological model. One of the possible explanations for the lack of success in this study could be that the conjugate contained in addition to *L. casei*, *L. bugaricus*, *L. acidophilus*, *L. plantarum*, *B. longum*, *B. breve*, *B. infantis*, *S. thermophilus*, which probably could have led to bacterial antagonism, thus reducing the effectiveness of the conjugate (Guitard et al., 2006).

One of the applications of *L. casei* against *C. parvum* was carried out in mice using *C. parvum*-P23 protein inserted into *L. casei* (Zhang strain). The results showed that oral administration of this recombinant protein increased the levels of cytokines IL-6 and interferon gamma (INF-γ), in addition to increasing IgA antibody levels during days 28-35 days, it also increased the IgG antibody levels during 21-42 days of post-infection compared to the control groups, making clear its immunogenic capacity (Geriletu et al., 2011).

***L. casei* against *Entamoeba invadens* (*E. invadens*)**

The effectiveness of the use of *L. casei* against *Entamoeba* protozoa was tested against *E. invadens*, which is very acceptable model for carrying out the evaluations against *E. histolytica*. The results showed that the survival rate of cells infected with *E. invadens* trophozoites was higher in the group where *L. casei* was used, achieving 95% survival in vitro (Sarjapuram et al., 2016).

***L. casei* against *Giardia lamblia* (*G. lamblia*)**

There are few evidence on the application of *L. casei* for the control of *G. lamblia*, however, in a first study in mice infected with trophozoites, it was observed that the oral application of *L. casei* decreased the number of cysts produced and eliminated in the feces by *G. lamblia*, and the number of trophozoites in the small intestine of mice was lower 3-7 days of post-infection. Necropsy findings showed that mice treated with *L. casei* had fewer atrophied villi and fewer infiltrating cells in the small intestine compared to controls. These results demonstrated that *L. casei* minimized *G. lamblia* infection by preventing the adhesion of trophozoites on the intestinal mucosal surface, suggesting that *L. casei* is effective and safe for preventing and treating *G. lamblia* infection (Shukla et al., 2008).

Subsequently, biochemical and histopathological parameters were evaluated in malnourished mice infected with *G. lamblia* and supplemented with *L. casei*. Histological, morphological and cell membrane alterations of the intestinal microvilli showed that *L. casei* supplementation decreased intestinal mucosal damage in the malnourished mice compared to the lesions produced in the control group. Serum total protein, albumin and globulin levels were higher during 7-17 days of post-treatment compared to the malnourished mice infected with *G. lamblia* but not supplemented, and the number of cysts sheds in the feces, as well as the number of trophozoites established in the small intestine was lower in the supplemented and infected animals compared to the controls. The results make it clear that the administration of *L. casei* has an anti-giardiasis effect in vivo, as it modulates and prevents the colonization, multiplication and encystation of *G. lamblia* trophozoites, thus reducing the duration and severity of giardiasis in the murine model (Shukla and Sidhu, 2011).

Subsequently, supplementation was carried out for 7 days with different probiotics of the *Lactobacillus* genus, to counteract the effects of Giardiasis in mice infected with *G. lamblia* trophozoites. The results indicated that mice treated with *L. casei* and infected mice showed a lower number of cysts eliminated in the feces from the first-day post infection until the end of the study, and that the groups treated with *Lactobacillus* eliminated a higher number of colony-forming units (CFU/mL) in the feces. Similarly, mice treated with *L. casei* showed a significant reduction in the number of trophozoites colonizing the small intestine, suggesting that the use of this type of probiotic is effective for the control of murine Giardiasis (Goyal et al., 2011).

Recently, the effect of the use of *L. casei* on parasitological and pathological parameters of hamsters experimentally infected with *G. lamblia* was evaluated. Parasitological parameters showed that, in animals treated with *L. casei*, the number of cysts was reduced by up to 55% after three days of treatment, achieving 100% cyst reduction after 21 days, while animals treated with metronidazole showed 49% reduction three days of post-treatment, achieving a maximum of 80% cyst reduction up to 30 days post-treatment. Pathological parameters showed marked improvement of intestinal villi with mild duodenitis and mild edema compared to moderate active duodenitis in terms of loss of villus structure, with edema of the lamina propria with moderate inflammation and cellular infiltration, including plasma cells and lymphocytes and moderate numbers of neutrophils present in the metronidazole treated group. These results demonstrate the potential therapeutic effect of *L. casei* against experimental giardiasis in hamsters (Shady et al., 2023).

***L. casei* against *Giardia intestinalis* (*G. intestinalis*)**

A group of researchers from India conducted several studies on the use of *L. casei* against protozoa of the genus *Giardia*, specifically against *G. intestinalis*. In a first study, they used daily administration of *L. casei* as a supplement for 7 consecutive days to control infection in mice, evaluating the integrity of the intestinal microvilli membrane, demonstrating that those animals supplemented with *L. casei* and infected with *G. intestinalis* showed less histological and morphological damage to the intestinal mucosa, thus reducing the damage caused by the infection (Shukla et al., 2012).

Subsequently, the use of *L. casei* alone as well as in conjunction with *G. intestinalis* anti-protozoal drugs was evaluated in mice infected with trophozoites and treated at 24 hours of post-infection. The results showed that in animals infected and treated with *L. casei*, as well as in those infected + *L. casei* + albendazole reduced the number of oocysts and trophozoites and restored the intestinal mucosal architecture, with an increase in crypts and villi, and showed moderate inflammation in the lamina propria, suggesting the effectiveness of *L. casei* alone and albendazole in reducing the effects of this parasitosis (Shukla et al., 2013). In addition, oral administration of *L. casei* was evaluated to assess the intestinal physiology and morphology of malnourished mice infected with *G. intestinalis*. The findings indicate that the use of *L. casei* in malnourished and infected animals decreased the number of cysts 24 hours of post-infection, increased small intestinal mass, increased small intestinal enzyme activity (sucrase, lactase, maltase, alkaline phosphatase) and improved intestinal microvilli morphology (Shukla et al., 2013).

Finally, the symbiotic effect of *L. casei* + Inulin was evaluated in malnourished mice infected with *G. intestinalis*. The findings reported showed that those infected animals in which the symbiotic effect of *L. casei* + Inulin was evaluated presented a better intestinal mass and a lower amount of trophozoites. Moreover, the same group of animals presented higher levels of IL-10 and IL-6, nitric oxide, IgG and IgA in both serum and intestinal fluid; in addition, they presented better morphology and orientation of intestinal microvilli. However, further studies were suggested to validate its use in patients (naturally infected humans due to the difference in the intestinal microbiota of mice and humans) (Shukla et al., 2019).

Table 2: *L. casei* against protozoan (intestinal) parasites of public health concern

Parasites (protozoa)	Species	Authors	Results
C. parvum	Humans (*Homo sapiens*)	Pickerd y Tuthill, 2004	Reduction of nausea, diarrhea and abdominal pain.
	Rats (*Rattus norvegicus albinus*)	Guitard et al., 2006	No significant effects (weight gain, parasite load, intestinal mucosal damage and cytokine expression).
	Mice (*Mus musculus*)	Geriletu et al., 2011	Increased IgA and IgG levels, as well as IL-6 and INF-γ levels.
E. invadens	*In vitro* cell culture	Sarjapuram et al., 2016	Increased survival of infected cells.
G. lamblia	Mice (*Mus musculus*)	Shukla et al., 2008	Decrease in atrophied villi and infiltrating cells.
	Mice (*Mus musculus*)	Sukla y Sidhu, 2011	Decreased intestinal damage; increased total protein, albumin and globulin in serum; decreased cysts in feces and trophozoites in intestine.
	Hamsters (*Mesocricetus auratus*)	Shady et al., 2023	Decreased cysts; moderate inflammation and cellular infiltration in intestine; moderate numbers of plasma cells, lymphocytes and neutrophils.
G. intestinalis	Mice (*Mus musculus*)	Shukla et al., 2012	Decreased histological and morphological damage to the intestine; increased membrane integrity of microvilli.
	Mice (*Mus musculus*)	Shukla et al., 2013	Reduction of cysts and trophozoites; restoration of intestinal mucosa with increased crypts and villi; moderate inflammation of lamina propria.
	Mice (*Mus musculus*)	Shukla et al., 2013	Decrease of cysts; increase of intestinal mass and enzyme activity; improvement of microvilli.
	Mice (*Mus musculus*)	Shukla et al., 2019	Improved intestinal mass; decreased trophozoites; increased levels of IL-6 and IL-10, nitric oxide, IgA and IgG in serum and intestinal fluid.

***L. casei* against *Plasmodium chabaudi* (*P. chabaudi*)**

Martínez-Gómez et al., (2006) evaluated the ability of *L. casei* to increase resistance to the protozoan *P. chabaudi* in mice inoculated with previously infected splenocytes. The results of the study showed that mice treated once or twice with *L. casei* prior to infection had a lower percentage of infected erythrocytes compared to groups that were only infected with splenocytes and not given *L. casei*. The authors concluded that administration of *L. casei* to mice increases resistance to *P. chabaudi* infection, resulting in low parasite loads, decreased viability of the protozoan, and increased serum nitrous oxide.

L. casei against _Plasmodium berghei_ (_P. berghei_)

Recent studies evaluated the effect of *L. casei* probiotic combined with chloroquine therapy to reduce the adverse effects of *P. berghei* malaria in the mouse model (Mahajan et al., 2021). The results of this research showed that the group of animals treated exclusively with *L. casei,* reduced the percentage of parasitemia compared to the control group; however, the group treated with *L. casei* + Chloroquine and infected with *P. berghei* showed a greater reduction in the percentages of parasitemia from the first-day of post-infection until the end of the study. When liver histology was performed, a reduction in periportal inflammation and hemosiderosis was also observed when the animals were treated with *L. casei* alone, however, in those animals treated with *L. casei* + Chloroquine, there were fewer liver lesions. The above results show that, when *L. casei* is applied together with a chemical therapy (chloroquine), a synergistic effect was achieved for malaria control in a mouse model, reducing parasite counts and improving the pathological changes that appear after *P. berghei* infection.

Subsequently, a further investigation was carried out to evaluate the effects of the use of probiotics *L. casei* and *B. longum* separately and together, evaluating the level of parasitemia, the composition of the intestinal microbiota, expression of regulatory T lymphocytes, INF-γ and TNF-α in mice infected with *P. berghei*. The results of the study showed that there was a significant difference in the level of parasitemia in animals treated with probiotics compared to the positive control group.

The degree of parasitemia was lower in the groups where the probiotic *L. casei* or *L. casei* + *B. longum* was applied intraperitoneally during the first 5 days of post-infection compared to the control group. The survival rate remained constant (100%) in the *L. casei* + *B. longum* group throughout the study, while in the *L. casei*-only group, the survival rate was 60-100%, compared to 40% survival in the positive control group. The ring-shaped parasites of the protozoan *P. beghei* were observed from day 2 in the control group, while in the treated groups they appeared 4-6 days of post-infection. The level of expression of regulatory T-lymphocytes was higher in the *L. casei* and/or *B. longum* treated animals, either together or separately; however, the expression levels of cytokines INF-γ and TNF-α, and the histological changes (ulceration, erosion and inflammation) in the colon of the mice were not different compared to those of the positive controls. The mechanism involved in the reduction of parasitemia has so far not been fully elucidated. However, immuno-modulatory properties such as enzymes, antimicrobial peptides, and short-chain fatty acids have been attributed, which may play an important role against *P. berghei* infections (Fitri et al., 2023).

L. casei against _Trypanosoma cruzi_ (_T. cruzi_)

Inoculation of *L. casei* to evaluate its oral and intraperitoneal effectiveness against *T. cruzi* infection in experimentally infected mice was carried out by Bautista et al., (2008). A marked reduction in the number of blood parasites (trypomastigotes) was recorded in both the oral and intraperitoneal *L. casei* treated groups compared to the control group from day 6 to day 28 of post-infection. The average total number of blood trypomastigotes recorded between days 10-28 of post-infection was 3,820 for the group treated with *L. casei* orally, while an average of 1,842 was obtained in the group treated with *L. casei* intraperitoneally.

This indicates that intraperitoneal treatment with *L. casei* was more effective in generating resistance to *T. cruzi* infection in mice. The protection conferred against *T. cruzi* was due to the activation of the innate immune response by *L. casei*; although the intraperitoneal route of application was more effective than the oral route, both showed resistance against infection when compared to the control group (saline).

L. casei against _Toxoplasma gondii_ (_T. gondii_)

During the first decade of this century, Martínez-Gómez et al., (2009) evaluated the protection against the formation of brain cysts produced by the protozoan *T. gondii* in mice immunized with cytoskeleton proteins of the parasite in question and the application of *L. casei* as an adjuvant. The percentage reduction in brain cysts was 77% for the group treated with the cytoskeleton proteins and *L. casei* as adjuvant, while the group treated with *L. casei* alone reduced the percentage of brain cysts by 44%, compared to a 6% reduction in brain cysts in the animals treated with phosphate-buffered saline (PBS) alone. The results suggest that the administration of cytoskeletal proteins, using *L. casei* as an adjuvant, is a good vaccine candidate for the control of toxoplasmosis in mice (Martinez-Gomez et al., 2009).

Very recently, a second study evaluated the potential immunobiotic and paraprobiotic effect of *L. casei* in a murine model of systemic toxoplasmosis (Salas-Lais et al., 2020). Among the results of the aforementioned work, a reduction in parasite load (tachyzoites/mL), activation of peritoneal macrophages, as well as inflammatory cytokines (INF-γ, IL-6, TNF-α), and an increase in the expression of monocyte chemoattractant protein-1 (MCP-1) were recorded. Moreover, an increase in the percentage of B-lymphocytes, lymphocytes, natural killer cells (NKC), TCD4+, and TCD44+ lymphocytes were also observed. The survival rate remained constant at 90-100% for the first nine days of post-infection. The authors concluded that the application of viable (immunobiotic) and dead (paraprobiotic) *L. casei* bacteria demonstrated stimulation of the immune system, leading to the destruction of tachyzoites by producing intracellular oxide (Salas-Lais et al., 2020).

L. casei against _Trichinella britovi_ (_T. britovi_)

Recently, the effect of *L. casei* against *T. britovi* was evaluated, as until then there were no reports on the effect of probiotics on *Trichinella* species other than *T. spiralis*. For this purpose, mice were infected with 100 larvae per animal. The results recorded showed that in animals treated with *L. casei*, fewer larvae and adults were recovered both at nine-and thirty-two-days of post-infection. These findings clearly show the potential negative effect on the development of this intestinal nematode, although the exact mechanisms behind this process need to be further investigated, however, the administration of *L. casei* is effective in reducing the parasite load, especially in adults of *T. britovi* (Boros et al., 2022).

Table 3: *L. casei* against haemoprotozoan and brain parasites of public health significance

Parasites (protozoa)	Species	Authors	Results
B. microti	Mice (*Mus musculus*)	Bautista-Garfias et al., 2005; Bautista et al., 2008	Reduction of parasitized red blood cells.
P. chaboudi	Mice (*Mus musculus*)	Martínez-Gómez et al., 2006	Reduction of infected red blood cells, parasite loads and viability of protozoa; increase in serum nitrous oxide.
P. berghei	Mice (*Mus musculus*)	Mahajan et al., 2021	Decreased percentage of parasitemia; reduction in periportal inflammation.
	Mice (*Mus musculus*)	Fitri et al., 2023	Decreased parasitemia; increased regulatory T-lymphocytes, as well as INF-γ and TNF-α; reduced intestinal histological changes.
T. cruzi	Mice (*Mus musculus*)	Bautista et al., 2008	Reduction of blood parasites (trypomastigotes).
T. gondii	Mice (*Mus musculus*)	Martínez-Gómez et al., 2009	Reduction of brain cysts.
	Mice (*Mus musculus*)	Salas-Lais et al., 2020	Reduction of parasite load; activation of peritoneal macrophages, Il-6, INF-γ and TNF-α, increase of B lymphocytes, natural killer cells (NKC), CD4 and TCD44 T lymphocytes.

***L. casei* against *Trichinella spiralis* (*T. spiralis*)**

Bautista-Garfias et al., (1999) conducted the first study to evaluate the effect of viable *L. casei*, administered intraperitoneally, to induce resistance in mice infected with *T. spiralis*. Their results showed that the percentage reduction of adult nematodes in the intestine at 5 days of post-infection was 70-88%, while the reduction of larvae per gram of muscle tissue at 30 days of post-infection was 46-84% in those animals treated with *L. casei*, as well as an increase in intestinal villi size, a higher number of mononuclear cells in the duodenum, and an increase in INF-γ.

Subsequently, De Waard et al., (2001), administered *L. casei* to rats infected with *T. spiralis* two weeks before infection and for 5 days of post-infection, evaluating immunological parameters, and immunoglobulins. Oral administration of *L. casei* increased IgG2b concentrations, concluding that IgG2b is associated with Th1 immune activity, thus playing an important role in immunomodulatory effects in animals with oral administration of *L. casei* and infected *T. spiralis*.

A second study was conducted by Bautista-Garfias et al., (2001), evaluating the ability of orally administered *L. casei* live and dead probiotics, in which adult parasite reduction percentages of 53-58% were obtained when the *L. casei* probiotics were alive, while adult parasite reduction of 44% was obtained when the *L. case*i probiotics were dead. The percentage of larvae recovered in muscle tissue was 70% in mice treated with live *L. casei*, while 65% of larvae recovered were obtained in those animals treated with dead *L. casei* at 30 days of post-infection.

Martínez-Gomez et al., (2009) evaluated the effects of intraperitoneal administration of *L. casei* on the establishment of adult parasites and the production of anti-*T. spiralis* IgA. The results reported show that, in mice treated with *L. casei*, a significant reduction (86%) of adult parasites was established throughout the study (28 days), compared to the control group (without *L. casei*). Likewise, anti-*T. spiralis* IgA levels increased significantly in the group of animals treated with *L. casei*, indicating that inoculation with this probiotic induces protection and increases IgA production in intestinal fluid in mice infected with *T. spiralis*. A couple of years later, the same group of researchers evaluated intraperitoneal inoculation of *L. casei* to induce total protection against infection with low doses (10, 25, 50, 100 and 200 larvae) of *T. spiralis*. The results showed a decrease in the number of adult parasites in all groups treated with *L. casei*, and the percentage of reduction was higher in those animals treated with the lowest doses (10, 25 and 50 larvae).

Similarly, IgG and IgA levels were higher in the *L. casei* treated groups compared to the control groups, however, the highest serum IgG and intestinal IgA levels were obtained in those animals infected with doses of 50 and 200 larvae at both 4- and 10-days of post-infection. Finally, IL-4 levels were higher in all groups treated with *L. casei* and infected with *T. spiralis*, however, the highest IL-4 levels were obtained in the groups infected with 25 and 50 larvae, while at 10 days of post-infection, IL-4 levels were similar in the groups infected with 25, 50 and 200 larvae). All these results suggest that frequent treatment with *L. casei* in mice infected with low doses of *T. spiralis* induces total protection against infection (Martínez-Gómez et al., 2011).

The most recent study on the effects of *L. casei* against *T. spiralis* was carried out by (El Temsahy et al., 2015), administering *L. casei* orally against experimental intestinal trichinellosis and evaluating parasitological, immunological and histological parameters. The results obtained show that oral administration of *L. casei* was able to decrease the establishment of adult parasites in the intestine by 36, 23 and 31% after 5-, 12- and 17 days of post-infection, respectively. In addition, a higher weight was achieved in those animals treated with *L. casei* during the first 6 days of post-infection, compared to the control group.

In terms of immunological parameters, there was a significant increase in serum gamma interferon (INF-γ) levels during the first 12 days of post-infection in the group of animals treated with *L. casei* compared to the control group. Histological results showed that the intestinal villi were larger and the number of goblet cells increased, while tissue damage and inflammation were reduced in animals treated with *L. casei* orally, thus demonstrating the protective capacity of *L. casei* probiotics in mice experimentally infected with *T. spiralis*.

L. casei against *Trichuris muris* (*T. muris*)

Although *L. casei* found to be effective against a wide range of parasites, there are reports in which it has generated susceptibility, such as the nematode *T. muris*, where oral administration to experimentally infected mice showed an increase in parasite load 22 days of post-infection. In addition, the application of viable *L. casei* reduced fecal IgA antibody levels, while the application of dead *L. casei* significantly decreased levels of INF-γ, TNF-α, IL-4, IL-5 and IL-13. The mechanisms of such evidence could be related to the deactivation of TNF-α-dependent Th2 effector response against *T. muris* due to a decrease of this cytokine that is induced by *L. casei* (Dea-Ayuela et al., 2008).

Table 4: *L. casei* against helminths of public health significance

Parasites (helminths)	Species	Authors	Results
T. britovi	Mice (*Mus musculus*)	Boros et al., 2022	Reduction in the establishment of larvae and adult nematodes.
T. spiralis	Mice (*Mus musculus*)	Bautista-Garfias et al., 1999	Reduction of larvae and adults in muscle tissue; increased size of villi; increased number of mononuclear cells; increased INF-γ.
	Rats (*Rattus norvegicus albinus*)	De Waard et al., 2001	Increased IgG2b levels.
	Mice (*Mus musculus*)	Bautista-Garfias et al., 2001	Reduction of larvae and adults in muscle tissue.
	Mice (*Mus musculus*)	Martínez-Gómez et al., 2009	Decreased adult parasites; increased IgA in intestinal fluid.
	Mice (*Mus musculus*)	Martínez-Gómez et al., 2011	Decrease of adult parasites; increase of IgA and IgG in serum and intestine; increase of IL-4.
	Mice (*Mus musculus*)	El Temsahy et al., 2015	Decreased adult parasites; increased weight gain; increased INF-γ; increased intestinal villi size; increased goblet cells; reduced intestinal tissue damage.
T. muris	Mice (*Mus musculus*)	Dea-Ayuela et al., 2008	Increased parasite load; reduced levels of fecal IgA, as well as INF-γ, TNF-α, IL-4, IL-5 and IL-13.

Effects of *L. casei* on bacteria affecting animal health

L. casei against *Brucella abortus* (*B. abortus*)

Mohammadi and Golchin (2020), evaluated the protective effect of the OMP19 antigen of a virulent strain (544) of *B. abortus* as a vaccine candidate and produced within *L. casei* as a vaccine vector. The results of this study showed that application of the antigen in conjunction with *L. casei* increased IgG and IgA levels in the intestinal contents of mice, as well as increased serum levels of cytokines IL-2, IL-4, IL-10, INF-γ and decreased colony-forming unit counts, which was similar to findings produced by the vaccine strain IRIBA produced in calves.

L. casei against *Escherichia coli* (*E. coli*)

To date, there are two studies available in the scientific literature on the use of *L. casei* against bovine mastitis caused by *E. coli*, using in vitro mammary epithelial cell culture and mouse models. Zheng et al., (2021) demonstrated *in vitro* that *L. casei* inhibits *E. coli* adhesion, as well as decreasing cellular desmosome damage, as well as decreases the lactate dehydrogenase enzyme and inflammatory cytokine expression (TNF-α, IL-1β and IL-6). Moreover, *L. casei* increased claudin-1, claudin-4, occludin and zonula occludens expression. Meanwhile, Li et al., (2024) demonstrated that, *L. casei* reduced cell apoptosis and the expression of TNF-α, IL-1β and IL-6; moreover, it suppressed enzyme phosphorylation. With respect to the mouse model, both studies showed that the use of *L. casei* by intramammary infusion reduced histological damage as well as the expression of inflammatory cytokines and increased the expression of claudin-3, occludin and ZO-1 proteins.

L. casei against *Staphylococcus aureus* (*S. aureus*)

A group of researchers in Brazil conducted the first *in vitro* study, to evaluate the invasion capacity of *S. aureus* in bovine mammary epithelial cells, by Bourchard et al., (2013), in which *L. casei* was used as an antagonist to prevent such invasion. The results showed that the CIRM-BIA667 strain of *L. casei* reduced the cell internalization capacity of *S. aureus* by 60-80% during the first 2 hours of post-incubation, without affecting the morphology and viability of bovine mammary epithelial cells.

Subsequently, Souza et al., (2017) conducted a couple of in vitro studies using *L. casei* to prevent *S. aureus* internalization in bovine mammary epithelial cells. In the first study, the results demonstrated the inhibitory potential of *L. casei* (strain BL23) during the first 30 minutes of post-incubation, reducing cell internalization by more than 50%, generating an antagonism with *S. aureus*, thus preventing the production of adhesion proteins towards bovine mammary epithelial cells.

Finally, they evaluated the ability of *L. casei* strain BL23 to modulate the innate immune response of bovine mammary epithelial cells during *S. aureus* infection. The recorded results showed that *L. casei* strain BL23 decreased the expression of proinflammatory cytokines, including interleukins IL-6, IL-8, IL-1α and IL-1β, and TNF-α at 8 hours of post-infection, thus demonstrating the anti-inflammatory properties of *L. casei* (Souza et al., 2018).

Effects of *L. casei* on bacteria that affect public health

***L. casei* against *Mycobacterium bovis* (*M. bovis*)**

In order to reduce the risk of transmission of tuberculosis caused by *M. bovis* in humans, the effect of *L. casei* was evaluated in milk fermented with kefir grains from bovine tuberculosis-positive animals. The results obtained demonstrated the ability of *L. casei* to reduce the viability of M. bovis from 24 hours of post-fermentation, resulting in zero viability of *M. bovis* bacteria after 60 hours of post-fermentation (Macuamule et al., 2016).

Table 5: *L. casei* against bacteria of animal and public health importance

Bacteria	Species	Authors	Results
Brucella abortus	Mice (*Mus musculus*)	Mohammadi and Golchin, 2020	Increases IgG and IgA in intestinal fluid; increases serum IL-5, IL-4, IL-4, IL-10 and IFN-γ levels.
Escherichia coli	*In vitro* culture (Bovine mammary epithelial cells)	Zheng et al., 2021	Inhibits adhesion, decreases cellular desmosome damage; decreases lactate dehydrogenase and expression of TNF-α, IL-1β and IL-6.
	Mice (*Mus musculus*)	Zheng et al., 2021	Reduces histological damage and inflammatory cytokine expression; increases claudin-3, occludin and ZO-1 protein expression.
	In vitro culture (Bovine mammary epithelial cells)	Li et al., 2024	Reduces cell apoptosis and expression of TNF-α,, IL-1β and IL-6.
	Mice (*Mus musculus*)	Li et al., 2024	Reduces histological damage and inflammatory cytokine expression; increases claudin-3, occludin and ZO-1 protein expression.
Staphylococcus aureus	*In vitro* culture (Bovine mammary epithelial cells)	Bourchard et al., 2013	Reduces cell internalization (60-80%), does not affect cell morphology and viability.
	In vitro culture (Bovine mammary epithelial cells)	Souza et al., 2017	Reduces cell internalization (50%), prevents production of adhesion proteins.
	In vitro culture (Bovine mammary epithelial cells)	Souza et al., 2018	Decreases proinflammatory cytokines IL-6, IL-8, IL-1α and IL-1β, and TNF-α.
Mycobacterium bovis	Fermented milk	Macuamule et al., 2016	Decreases bacterial viability 24 h post infection.

Effects of *L. casei* on viruses affecting animal health

***L. casei* against Bovine Viral Diarrhea Virus (BVDB)**

There are few studies on the effects of the application of *L. casei* to control BVDV infections, however, the first study related to this topic was conducted by Bhuyan et al., (2018), who demonstrated in mice that *L. casei* containing recombinant pELX1-E2 antigen, and administered orally and intranasally induced significantly higher levels of intestinal mucosal IgA and serum IgG against E2 antigen, as well as a higher level of cellular immune response (INF-γ and IL-12) compared to intramuscular administration and controls.

L. casei strain W56 was later used to evaluate the effectiveness of the recombinant BVDV-E2 protein. This study demonstrated the effectiveness of *L. casei* in activating dendritic cells in Peyer's patches, as well as T-cell differentiation, enhancing B-cell proliferation, and promoting IgA differentiation by secreting specific anti-E2 antibodies, thus neutralizing BVDV activity. In addition, *L. casei* (strain W56) was able to induce cellular immune responses, and significant levels of IL-2, IL-12 and INF-γ (Th1), as well as IL-4 and IL-10 (Th2), and IL-17 (Th17) (Jia et al., 2020; Wuang et al., 2019). The above studies demonstrate that *L. casei* exhibits protection against BVDV, representing a promising control strategy.

***L. casei* against Newcastle virus**

Several studies on the effects of *L. casei* against Newcastle virus in broilers have shown that *L. casei* administered in the diet of broilers increases humoral immune response (IgG) (Alizadeh et al., 2017; Ogawa et al., 2006), increases body weight (Bautista-Garfías et al., 2011; Ju et al., 2021) and decreases mortality (Bautista-Garfías et al, 2011), reduces organ injury (lungs, liver, spleen, thymus and bursa of Fabricius), and improves serum IL-2 and INF-γ concentrations, as well as elevates IgA levels in intestinal fluid (jejunum) (Ju et al., 2021).

Effects of *L. casei* on Viruses Affecting Public Health

***L. casei* against Influenza viruses (H1N1, H3N2)**

The first report on the use of *L. casei* (Shirota strain) was carried out by Hori et al., (2001), administering *L. casei* intranasally to activate the immune system of the respiratory tract of mice infected with the influenza virus (H1N1). The results showed that *L. casei* is able to induce the expression of IL-12, TNF-α and INF-γ in mediastinal lymph node cells and increase the survival (69%) of mice infected with influenza virus and treated with *L. casei*. These early findings suggested that intranasal administration of *L. casei* enhances the respiratory tract´s cellular immune response and protects against influenza.

Jung et al., (2017) evaluated the effectiveness of heat-killed, intranasally administered *L. casei* probiotics (strain DK128) to protect against influenza virus (H1N1 and H3N2) infection in mice. Protection against both influenza virus subtypes was recorded, with an increase in alveolar macrophages in the lungs and airways and early induction of specific antibodies, as well as a reduction in the levels of proinflammatory cytokines and innate immune cells. Moreover, increased body weight and survival rate (80-100%) of mice treated with *L. casei* intranasally were also observed.

Very recently, Spacova et al., (2023) evaluated the effect of a probiotic-based *L. casei* throat spray in human volunteers intending to reduce the negative effects of viral infections, including H1N1 and H3N2. Their results indicate that the administration of *L. casei* was able to colonize the throat of the patients, in addition to increasing the levels of nuclear factor (NK-κB) activation in monocytes and interferon regulatory factors (IRFs), demonstrating that *L. casei* could act as a therapeutic strategy against viral diseases of the respiratory tract, such as influenza.

Table 6: *L. casei* against viruses of animal and public health importance

Viruses	Species	Authors	Results
Bovine Viral Diarrhea Virus	Mice (*Mus musculus*)	Bhuyan et al., 2018	Increases IgA and IgG levels; increases cellular immune response (INF-γ and IL-12).
	Mice (*Mus musculus*)	Wang et al., 2019	Activation of dendritic cells; production of IgA and IgE; proliferation of lymphocytes; expression of INF-γ and IL-4.
	Mice (*Mus musculus*)	Jia et al., 2020	Dendritic cell activation; T-lymphocyte differentiation; B-lymphocyte proliferation and IgA differentiation; increased IL-2, IL-12, INF-γ, IL-4, IL-10 and IL-17.
Newcastle virus	Broiler chickens (*Gallus gallus domesticus*	Ogawa et al., 2006	Increases IgG levels.
	Broiler chickens (*Gallus gallus domesticus*	Bautista-Garfías et al., 2011	Increases body weight and decreases mortality.
	Broiler chickens (*Gallus gallus domesticus*	Alizadeh et al., 2017	Increases IgG levels.
	Broiler chickens (*Gallus gallus domesticus*)	Ju et al., 2021	Increases body weight; reduces organ damage (lungs, liver, spleen, thymus, and bursa of Fabricius); increases IL-2, INF-γ and IgA levels.
Influenza virus (H1N1, H3N2)	Mice (*Mus musculus*)	Hori et al., 2001	Induces IL-12, TNF-α and INF-γ expression in mediastinal lymph node cells, increases survival.
	Mice (*Mus musculus*)	Jung et al., 2017	Increased pulmonary alveolar macrophages and airways; induction of specific antibodies; reduced levels of proinflammatory cytokines and innate immune cells; increased body weight and survival.
	In vitro culture (Human cells)	Spacova et al., 2023	Throat colonization; increased levels of nuclear factor (NK-κB) activating monocytes and interferon regulatory factors (IRF's).

Conclusions and Perspectives

Since the first study twenty-five years ago, several researchers have used *L. casei* as a strategy for the control of some parasitosis, bacterial and viral diseases related to veterinary and public health. The results of all these studies have demonstrated the effectiveness of *L. casei* in regulating the immune response, reducing parasite loads and/or the establishment of adult parasites, reducing tissue damage in various organs, increasing the weight gains and animal survival.

Concerning the ability of *L. casei* to induce immune responses, *L. casei* stimulates both innate and acquired immunity against parasites, bacteria and viruses. However, the number of investigations for the control of different diseases in production animals is scarce, while they have been evaluated only in animal models or cell cultures. Therefore, it is still necessary to design more studies on the use of *L. casei* in animal production infected naturally and/or experimentally, but, above all, to increase the parameters to be evaluated and which are related to animal welfare and food quality.

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Chapter 42

Bacteriophage Applications in Poultry Production and Health Management

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ABSTRACT

The poultry market, particularly chicken, contributes the most protein and macronutrients to the global diet without any religious or cultural taboos associated with it. Infectious agents such as *Salmonella, Campylobacter, Listeria, Staphylococcus spp. and Escherichia coli* are a threat to the poultry industry. There are 18 to 90% of poultry flocks in European countries that are infected with Campylobacter. A severe risk to health of human is posed by antibiotic use and misuse in the livestock and poultry industries that had led to the development of multi-drug-resistant pathogens in animals and transmission of antibiotic resistance genes (ARGs) from animals to humans by the ingestion of animal products. Phage therapy is successful when used at the right time, in the right amount, with the right delivery system, and in combination with other therapies. Bacteriophages are being used in poultry production for the first time, but it will take time to gain a deeper understanding. This book chapter discusses Bacterial Challenges, Bacteriophages' roles in control, food security and safety, molecular applications, antibiotic resistance, and the future of poultry production.

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INTRODUCTION

Bacteriophages, often identified as phages or BPs, are viruses which particularly target and infect arachea or prokaryotes. Frederick Twort as well as Felix d'Herelle made discoveries of bacteriophages in 1915 as well as 1917, individually (Koskella et al., 2022). Through an approximate total no. of 1031 phage molecules in the biosphere 10X more than the estimated number of bacterial cells on Earth—they are incredibly commonplace worldwide (Gómez-Gómez et al., 2019). Bacteriophages are generally considered safe for humans, but their safety is not universally accepted without reservation. Since then, phages have been applied in clinical settings. With the exception of many Eastern Bloc nations, phage treatment was completely replaced in the Western world with the discovery of penicillin, which signaled the start of the antibiotic era. Lately, there has been a renewed focus on antibiotic-resistant bacterial species due to their rising prevalence (Chopra, Hodgson et al., 1997; Sulakvelidze, 2004).

The long-term efficacy of traditional antibiotics as well as human health are seriously threatened by germs that are resistant to many drugs (Cars et al., 2008) Thousands of people die from illnesses brought on by bacteria resistant to antibiotics per year in the European Union alone. Gram-negative bacteria, for example *Acinetobacter baumannii, Pseudomonas aeruginosa,* as well as *Enterobacteriaceae,* that includes *Klebsiella pneumonia* as well as *Klebsiella pneumonia*, are responsible for two-thirds of these deaths.

The speed at which bacteria are evolving and becoming resistant to antibiotics has led to a concerning state of affairs worldwide. But as a result, there is now less interest in the study and creation of new antibiotic chemicals for the pharmaceutical industry. Let's say, the Food and Drug Administration in the United States approved sixteen novel antibiotics between 1983 and 1987; between 2010 and 2016, this number dropped to only six (Luepke et al., 2017). Due to the negative health effects of the carbapenem class of antibiotics, only two antibiotics have been approved for commercialization by FDA and European Medicine Agency (EMA) in the last 20 years, and there is a global need for new antimicrobials. Consequently, the majority of big pharmaceutical corporations no longer want to invest in the creation of novel antibiotics.

Antibiotic resistance was deemed "the greatest and most urgent global risk" in a conference called by the UN General Assembly on September 1, 2016, given the gravity of the problem (Mattar et al., 2020).

Viruses known as bacteriophages exclusively infect bacteria. In contrast to filamentous and temperate phages, lytic phages proliferate inside the bacterial cell and lyse it at the conclusion of their life cycle to release freshly generated phage particles. After attaching itself to the surface of a vulnerable host cell, the phage virion injects its genome, taking over most of the host metabolism as well as assembling the molecular machinery needed for phage replication and assembly (Clark and March 2006; Skurnik and Strauch 2006). Bacteriophages differ in their structural makeup. Phage virons can have filamentous, pleomorphic, polyhedral, or tails. The majority have single- or double-stranded RNA (ssRNA, dsRNA), double-stranded DNA (dsDNA), and single-stranded DNA (ssDNA) in lower amounts. Tailored phages make up around 96% of all phages and are the most common form of therapeutic phage (Ackermann 2001) shown in Fig. 1.

The potential of phages to destroy dangerous bacterial strains in situ and reproduce exponentially might be crucial for the treatment of infectious illnesses, as well as allow for shorter delivery times. While bacteriophage treatment offers several benefits, this method is not without its restrictions (Specificity of phage, phage-bacteria co-evolution and regulatorily hurdles). The limits of phage treatment resulting from the advent of phage resistance and the occurrence of bacteriophage insensitive mutants were also covered by (Hyman and Abedon, 2010). Like antibiotic resistance, phage resistance develops at a similar rate, and lytic, virulent, broad-spectrum phages that are ideal for treatment are hard to identify and cultivate. Phage treatment relies on a clear bacteriological diagnosis. However, there are concerns about potential side effects and unfavorable immune responses, particularly after repeated exposure.

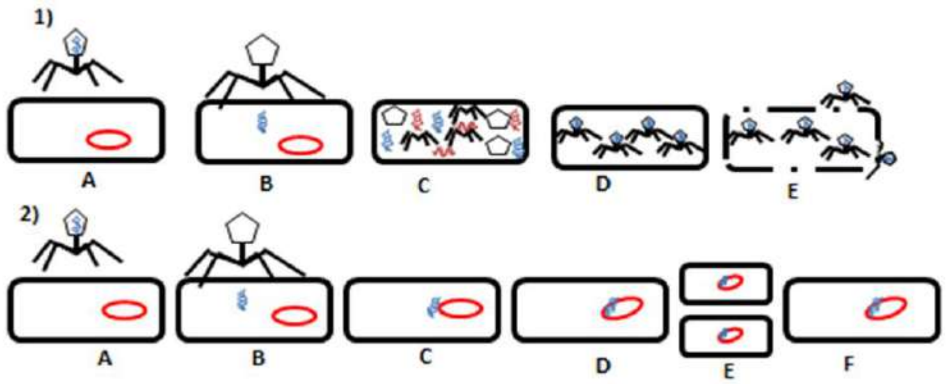

Fig. 1: Bacteriophage life cycle (1) lysogenic Cycle (2) Lytic Cycle

Understanding Bacterial Challenges in Poultry Production

The world's food production and sustainability are presently confronting an unprecedented challenge because of the expanding human population. Chicken health and safety continue to be serious problems that require prompt attention, even if it is acknowledged that the chicken industry is one of the maximum effective and quickly expanding food businesses to solve this challenge. Bacterial illnesses such necrotic enteritis, colibacillosis, and salmonellosis have become more prevalent in chicken farming. Similar to this, underdone poultry adulterated with zoonotic bacterial illnesses like Campylobacter, Salmonella, plus Listeria can cause outbreaks that are extremely dangerous for the public's health.

Bacteriophages are becoming more and more acknowledged as a desirable natural antibacterial substitute in light of the subject of antibiotic resistance or the limited usage of antibiotics in animals raised for food. Recently, bacteriophages have demonstrated encouraging results in the treatment of poultry illnesses, the reduction of carcass contamination, and the improvement of chicken product safety. Technologies that are crucial for bacteriophage interaction with bacterial hosts have been effectively used to precisely illustrate bacteriophages as well as its genes/proteins. This chapter explores the possibility of utilizing lytic bacteriophages to reduce the risk of main bacterial infections associated with poultry. The difficulties in getting companies to embrace this technology are also covered in this paper.

Public health concerns had drained more consideration to pathogens for example due to the risk that poultry poses as a source of such pathogens, *Salmonella enterica* subspecies enterica serovar *Enteritidis (S. Enteritidis*), *Salmonella enterica* subspecies of enteric serovar *Typhimurium (S. Typhimurium), Eschericha coli (E. coli), Listeria monocytogenes (L. monocytogenes*), as well as methicillin-resistant *Staphylococcus aureus* (MRSA) have been identified (Mor-Mur and Yuste, 2010). Most researches had examined the effectiveness of bacteriophages in reducing bacterial counts and controlling bacterial illnesses in poultry, that are zoonotic and ensure a substantial impact on public health (Żbikowska et al., 2020). As per the latest report by the European Food Safety Authority (EFSA) as well as the European Centre for Disease Prevention and Control (ECDC) (2019), and most commonly reported zoonosis in the European Union (EU) were campylobacteriosis, salmonellosis, yersiniosis, and E. coli infections that produce Shiga toxin (STEC) (EFSA, 2015). Individual the lytic bacteriophages are appropriate for phage therapy, which is used to treat bacterial illnesses, due to their limited capacity to destroy bacteria. Antibiotics are not nearly as specific as bacteriophages. It is important to remember that antibiotic therapy alters the normal gut microbiota in addition to eliminating harmful bacteria, which may cause dysbiosis, immunosuppression, moreover ensuing infections (Lin et al., 2017). Therefore, new bacteriophage therapies are a great way to treat bacterial infections in chickens because these therapies' have greater specificities, reduce antibiotic resistance and also involve in food safety (Fig.2).

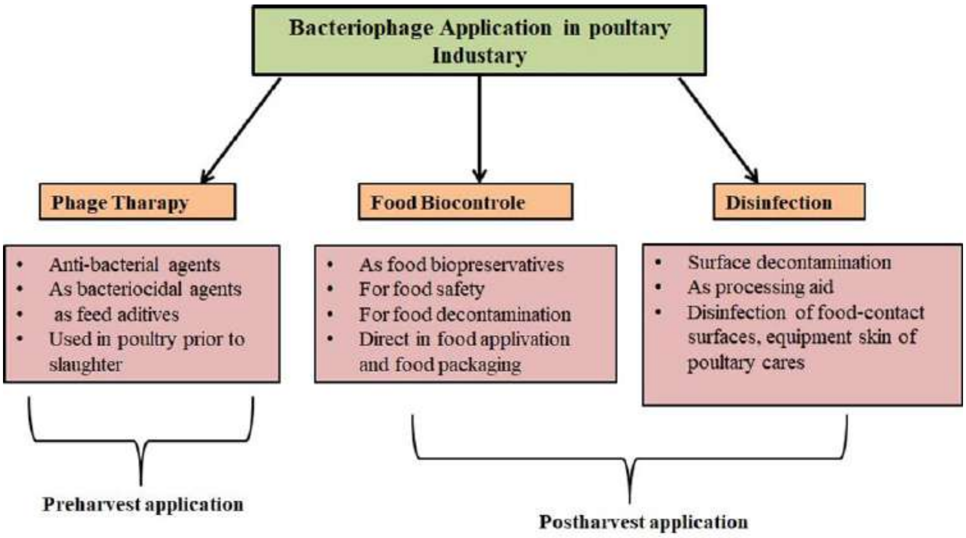

Fig. 2: Bacteriophage application

Role of Bacteriophages in Controlling Poultry Production

A phage attaches itself with a bacterium during infection, then introduces its genome into the cell. Resulting that, a phage usually goes through either the lytic (virulent) or lysogenic (temperate) life cycles. In order to produce phage components, lytic phages commandeer the cell's machinery. Afterwards, they lyse, or kill, the cell, releasing fresh phage particles. Lysogenic phages proliferate as a unit with the host cell by integrating their nucleic acid within its chromosome, all without causing the cell to die. It is possible to cause lysogenic phages to adhere to a lytic cycle in specific conditions (Dennehy and Abedon, 2021).

Here are more life cycles, for example persistent infection besides pseudolysogeny. A bacteriophage enters a cell during pseudolysogeny, but it neither permanently integrates into the host genome nor hijacks the machinery responsible for cell replication. When a host cell experiences unfavorable development conditions, pseudolysogeny takes place. Because it permits the phage genome to be maintained until the host's growth circumstances are favorable once more, this procedure appears to be essential for phage survival. Long-term, continual production of new phage particles occurs in chronic infections, yet there is no discernible cell death (Elois et al., 2023).

Bacteriophages are formed of basic genetic material, which could be either single- or double-stranded, then wrapped in a protein capsid. The three main phage structural forms are a filamentous form, an icosahedral head which has a tail, and an icosahedral head lacking a tail (Naureen et al., 2020).

The most popular meat consumed worldwide is poultry, especially chicken, which is also a significant source of high-quality protein and macronutrients without being associated with any social, religious, or cultural taboos. The demand for beef and pork can rise by 66 and 43%, respectively, between 2005 and 2050, but poultry is predicted to grow at the fastest rate—121%—becoming the most consumed meat globally over the next five years, corresponding to the Organization for Economic Cooperation and Development/Food and Agricultural Organization of the United Nations" (OECD/FAO, 2016).

While the poultry industry has seen impressive growth, this close relationship between humans and birds can also increase the risk of food-borne illnesses like salmonellosis and campylobacteriosis due to bacterial contamination. This contamination also shortens the shelf life of poultry meat, making it highly perishable.

The presence of pathogenic microorganisms in the animal prior to its slaughter at the farm of origin and cross-infection from the processing and production settings that come into touch with the contaminated animal or corpse can easily lead to contamination Moreover, inadequate pathogen control strategies and imprecise pathogen detection methods now in use at farms and/or processing facilities may be the root cause of the majority of diseases associated with poultry (Fister et al., 2019).

Furthermore, it is common to find pathogenic or spoilage bacteria residing on a variety of biotic and abiotic surfaces as sessile colonies embedded in biofilms. In industrial settings for poultry, biofilm development on work surfaces poses a severe risk, since the spread of these structures may allow dangerous germs to be released, which might contaminate and ruin carcasses.

As a result, the poultry sector has several difficulties in ensuring the safety of its products. The chicken industry has used a variety of pathogen-reduction intervention techniques over the past 20 years. Many compounds, such as chlorine and cetylpyridinium chloride (CPC), are rarely classified as generally recognized as safe (GRAS). Other tactics, like organic acids, while frequently successful, can have detrimental organoleptic effects (Hashem and Parveen, 2016).

Physical methods have been utilized extensively in the processing and production of chicken because they are successful in reducing the bacterial load on broiler carcasses. These methods rely on thermal treatment, ionizing irradiation, ultraviolet (UV), and high-pressure processing. They may, however, alter the meat's chemical and physical characteristics as well as bring about unfavorable alterations to its texture, flavor, and color.

To guarantee microbiological food safety, several biological treatments have been tried along with chemical and physical ones for inactivating harmful bacteria in chicken. Alternative approaches are emerging that utilize natural preservatives. These preservatives can be derived from plants or animals and can be either naturally occurring or artificially altered. One promising option is bacteriocins, produced by lactic acid bacteria. Bacteriocins offer an antibacterial effect without compromising food quality (Han et al., 2022).

The poultry industry faces a challenge: balancing food safety with consumer concerns. The overuse of antibiotics in animal production has led to the emergence of new strains of antibiotic-resistant bacteria. This, coupled with consumer anxieties about residues from detergents and disinfectants used in food processing, has driven the search for safer alternatives. In response, the industry is increasingly turning to natural antimicrobial agents for decontamination. These agents, derived from plants or animals, offer a promising solution. They can be as effective as traditional methods while addressing consumer concerns and potentially mitigating the rise of antibiotic resistance. Phages provide a unique chance to attack pathogens in a variety of foods without altering the typical microbiota, physicochemical properties, or organoleptic qualities because of their uniqueness. As a result, phages have received a lot of interest for their potential use as bio-preservatives and as an antibiotic substitute for the management of food-borne bacterial infections (Fig. 3).

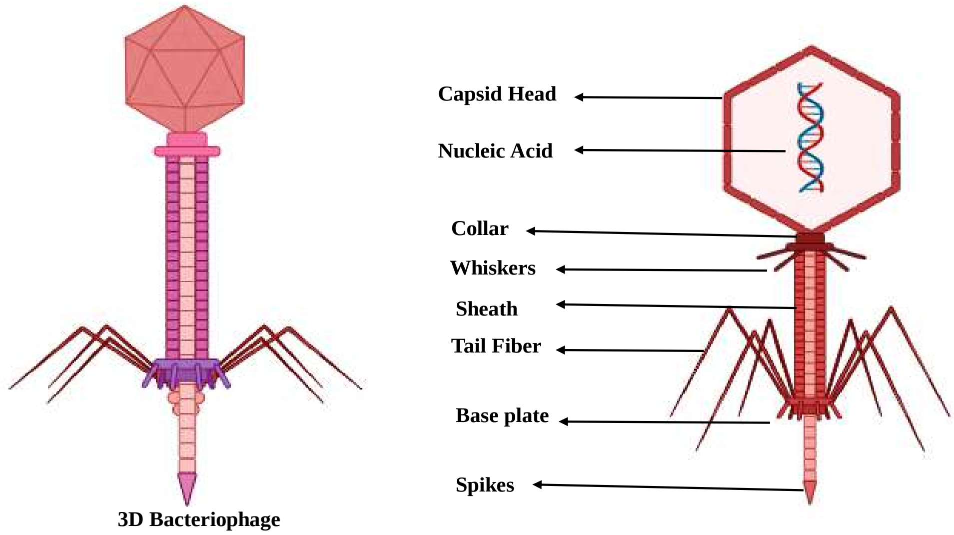

Fig. 3: Structure of bacteriophage.

Bacteriophage and Food Security/Safety

Foodborne infections found in the stomach and hide of agricultural animals that produce food are the main cause of cross-contamination in the food chain. Before cattle are slaughtered, target disease populations on and within them can be effectively decreased using on-farm phage-based control techniques. On the other hand, a target pathogen's incidence and persistence might change between animals, within a herd, or in various parts of the same animal.

In grill birds intended for slaughter, *C. jejuni* is common, while *E. coli* O157 super-shedders have been found in up to 20% of cow herds. It can be difficult to distinguish between transitory shedders and super-shedding animals within a herd, and it may take several significant sample episodes spread out over a lengthy time before phage therapy. A target pathogen may colonise the rumen, cecum, colon, and rectum, among other settings in the cow gut. The location of the colony within the digestive system affects the effectiveness of phage-based therapies.

This revision eliminates redundancy while still conveying the need to understand how phages remain stable throughout their use. It is necessary to optimize individual phages or cocktails in order to infect specific bacteria in a variety of settings and biofilms (Bumunang et al., 2023). Before phage application, comparative genomics may distinguish, monitor, and offer important information about likely bacterial variations within a specific pathogen population.

For phage applications to be used in agriculture and human therapy, a deeper comprehension of phage viability, stability, and survival in a variety of challenging conditions is needed. The Myoviridae or Siphoviridae families of phages were all employed in the experimental investigations. The majority of phages were extracted from their intended host and demonstrated to be stable and infectious during the course of therapy.

It is well known that certain phages belonging to these families exhibit exceptional resilience in harsh conditions, including desert surface sand that is subjected to extreme heat and cold (Zampara et al., 2017). The majority of research on phage-based L. monocytogenes management in food items has been on beef, pig, and poultry that are ready to eat. Food safety may be threatened by L. monocytogenes enrichment from cold storage. A potential remedy for managing Listeria monocytogenes in food items is the use of phages. Since silage is a frequent source of L. monocytogenes, using phages to target this source may be a useful strategy for stopping transmission of the infection on farms. It may be less expensive to target feed rather than to directly manage L. monocytogenes in cattle. Phage cocktails should be made with phages that are resistant to a broad variety of pH levels and temperatures for optimal effectiveness (Table 1).

Table 1: An overview of research on bacteriophages used to manage food-borne infections in/on animals used for food

Target specimen	Target Bacteria	Phage/Family	Phage/Mixture	Phage Dose	Phage Delivery Route	Efficacy	References
36-day-old chick	*C. jejuni*	NCTC12672, 12673, 12674, and 12678/Myoviridae	Cocktail	7.2 and 7.9 PFU/mL	Oral	A 3.2 log_{10} CFU/g	Kittler et al,2013
25-day-old chick	*C. jejuni*	CP220/*Myoviridae*	Single	10^7 and 10^9 PFU/mL	Oral	A 2.0 log_{10} CFU/g reduction 2 days post-treatment	El-Shibiny et al, 2009
24-day-old chick	*C. jejuni*	CP20 and CP30A/*Myoviridae*	Cocktail	10^7 PFU/mL	Oral	A reduction of up to 2.4 log_{10} CFU/g 2 days post-treatment	Richards et al,2019
4-day-old chick	*S. enterica* serotype Enteritidis	CNPSA1, CNPSA3, and CNPSA4/Nd[1]	Single	10^{11} PFU/mL	Oral	A reduction of 3.5 orders of magnitude of CFU/g 5 days post treatment	Fiorentin et al, 2005

The Reduction of Salmonella in Chicken Skin

The purpose of the study was to find out how bacteriophages and sanitizers affected chicken skin that had been experimentally infected with *S. enteritidis*. A randomized full block design with repetition was used in the trial, where treatments were organized into ten blocks, each including three duplicates. Using sterile, disposable spreaders, the chicken skin portions were equally disseminated over both sides after being injected with 105CFU/cm2. The parts were split into six batches, each including thirty portions, once they had dried. Three batches of samples were submerged in decontamination solutions, including lactic acid, peracetic acid, and sodium dichloroisocyanurate. In a separate batch, samples were submerged in sterile distilled water, phage cocktail, or untreated control (Oliveira et al., 2009). In order to replicate industrial settings, all decontamination chemicals were chilled to 6°C. Following treatment, slices of chicken skin were placed in sterile stomacher bags, placed in solutions designed to inactivate each agent, mechanically agitated (stomaching), and serially diluted. Sections treated with lactic acid were put to phosphate buffered saline, while sections handled with sodium dichloroisocyanurate or peracetic acid had been added to buffered peptone water. Saline was used to dilute skin samples. *S. enteritidis* counts were quantitatively determined using the droplet technique, which involved depositing successive dilutions onto XLT4 agar plates and incubating them for six to eight hours at 37°C. The numbers on S. enteritidis plates were given in CFU/cm^2.

Phage Sensitivity of Salmonella Recovered from Chicken Skin

S. enteritidis colonies were removed from chicken skin that had been phage-treated in order to determine whether there was any resistance to the five phages used in the decontamination treatment. The drop-on-lawn technique described before was used to evaluate the phage resistance of S. enteritidis isolates. After an 18-hour incubation period at 37 °C, the plates were examined for the presence of phage plaques, which shows phage sensitivity (Hungaro et al., 2013).

Bacteriophage Applications in Poultry Production

Food sustainability and safety are crucial challenges in the global food industry, as Western countries increasingly consume organic foods. The demand for food rises as a result of the expanding world population, which is predicted to reach 9.7 billion by 2050 and 11.2 billion by 2100. This puts pressure on the food business to adhere to food safety laws. Every year, 600 million individuals are afflicted with foodborne diseases, which lead to 420,000 fatalities and more than $110 billion in economic losses. Despite advances in technology, manufacturing practices, and hygiene, microbial safety problems persist, such as emergence of antibiotic resistance in bacteria and food borne illness. The industry is further burdened by the restricted use of specific antibiotics during the production of food animals and the dearth of novel antimicrobials. The FDA has approved food safety products from many commercial firms that use phage-based solutions to combat major food-borne diseases. The industry's faith in the effectiveness and security of phage-based preparations is demonstrated by this advancement.

Since bacteria, including phages, are naturally occurring, benign, and widely distributed in the environment, they are excellent choices for pathogen identification and management in the food production process. Salmonella serovars, Escherichia coli, and other major food-borne pathogens have recently been profitably controlled with phage-based products. These products have been approved by the FDA for food safety, and several commercial phage businesses have obtained the classification of Generally Recognized as Safe (GRAS). This study focuses on the most current developments in phage biocontrol in the food industry (Fig. 4).

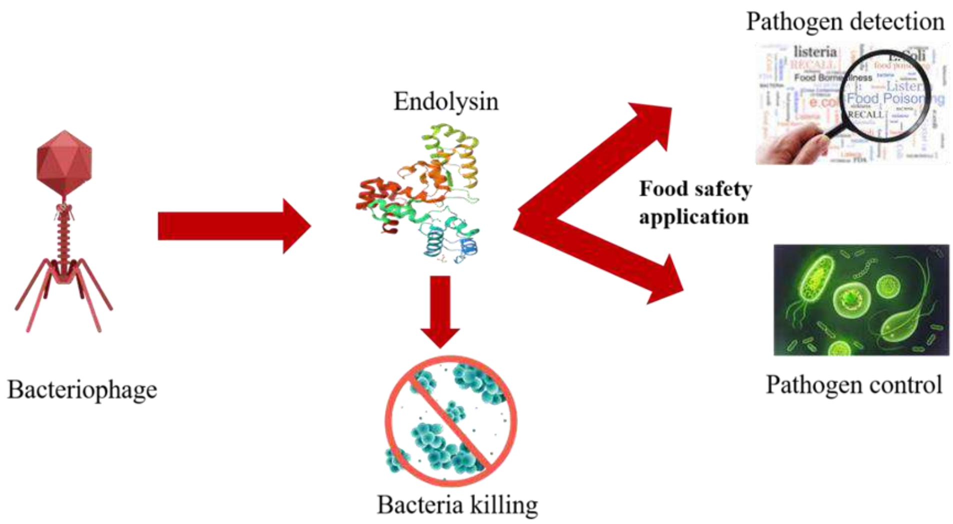

Fig. 4: Role of bacteriophage in food safety.

Since their discovery in 1915, bacteriophages—harmless viruses that infect bacteria—have found extensive application in both veterinary and human medicine as well as agriculture. They have the ability to incorporate genetic material into a bacteria's chromosome without causing cell death or lysis of cells to release viral particles. Because of their high specificity, replication by itself, self-limiting nature, ability to adapt continuously to modify host infrastructure, low inherent toxicity, ease of isolation and propagation at a low cost, resistance to environmental stresses during food processing, and extended shelf life, phages present advantages as biocontrol agents. They are widely distributed in food and have been shown to be absent from a number of processed, raw, fermented, and seafood goods. Phages are found in the same settings that their bacterial host(s) currently reside in or were formerly present, and people eat them on a regular basis. However, due to their potential to contribute to the decreasing effectiveness of antibiotics utilized for treating bacterial infections in humans and the development of superbugs like Salmonella DT104 or methicillin-resistant as well as multidrug-resistant Staphylococcus aureus, the use of antibiotics in farm animals has become a serious concern. Therefore, phages are a potential solution for food safety (Guenther et al., 2012).

Phage treatment, also known as minimizing pathogen colonization in livestock, is a key production technique that lowers the risk of cross-contamination with animal feces during food processing. It can be applied either during animal growth or prior to animal slaughter. For example, it is predicted that a two-log reduction in the quantity of Campylobacter in poultry intestines will be sufficient to lower the frequency of campylobacteriosis associated with chicken meat consumption by a factor of thirty. For a number of infections, phage treatment in animals has previously proven effective. Phages can be sprayed on to target pathogenic E. Coli in poultry, orally/rectally applied to control E. Coli in ruminants, orally administered to treat Salmonella and Campylobacter within poultry, and mixed into drinking water or food (Fig. 5) (Goodridge and Bisha, 2011).

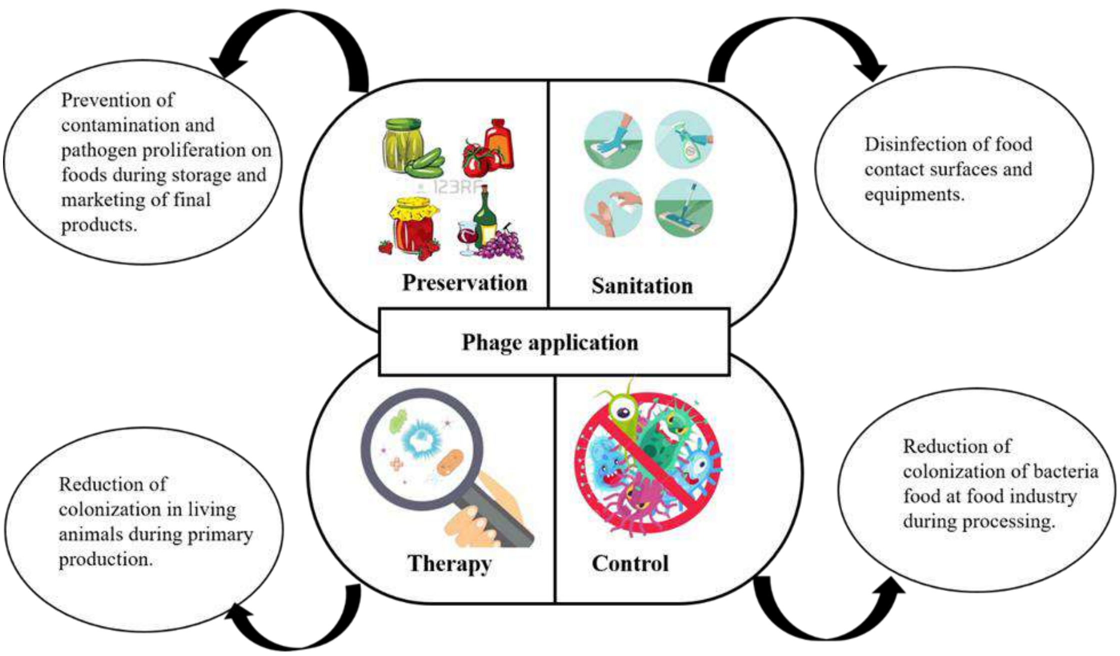

Fig. 5: Uses of phages to enhance food safety across the food chain.

Phages could be used to reduce colonization on food contact surfaces during industrial food processing. Their efficacy diminishes when applied to non-growing bacteria, but they remain powerful against actively developing ones. Phage titers that are high can be used to suppress infections that replicate as soon as food starts to warm up or that use "lysis from without" methods. Biofilms are frequently observed on surfaces used in the handling, storing, and processing of food, especially in hard-to-clean or sterilize locations, including tiny pipe systems, uneven surfaces, and complicated machinery crevices. Phages have demonstrated potential in mitigating viable cells against in vitro biofilms produced by spoilage and pathogenic bacteria in optimal conditions, which are defined as controlled environments with optimal temperature, pH, and nutrient availability; these attributes are indicative of those that facilitate biofilm formation in real-world scenarios. However, because bacteria vary widely in different environments, using them for bio-sanitation is difficult (Premaratne et al., 2021).

Phage lysing systems have been demonstrated to lyse hosts at as low as 1°C, making them suitable agents for food bio-preservation since they prevent the development of harmful and spoilage bacteria, especially psychrotrophic bacteria, on chilled foods. Phages can further regulate the growth of these bacteria once the meals are brought to room temperature (Sillankorva, Oliveira and Azeredo, 2012).

Bacteriophage Applications in Poultry Production

Viruses known as "bacteria eaters," as their name roughly translates, are known to attack and infect bacteria. Twort and D'herelle independently discovered bacteriophages in 1915 and1917, respectively (Duckworth, 1976). The most prevalent creatures on Earth are bacteriophages. Similar to other viruses, bacteriaphages need a host cell in order to multiply. Most phages are extremely selective and only have the ability to infect a small number of closely related bacteria. While bacteriophages are capable of killing bacteria, they are unable to utilize any resources from a deceased organism. Rather of being thought of as predators, bacteriaphages are really parasites. The bulk of gut viral genomes (97.7%) are composed of phage viruses, with eukaryotic (2.1%) and archaeal (0.1%) viruses following in order of prevalence (Abd-El Wahab et al., 2023).

Poultry, particularly chicken, is the most widely eaten meat in the world. It is also a substantial source of high-quality proteins or macronutrients and is not taboo in any religious, social, or cultural context.Most cases of Campylobacter and Salmonella are in chicken (Han et al., 2022).

Bacteriophages as Antibiotic Resistance

These bacteria *Salmonella enterica, Campylobacter jejuni, E. coli*, as well as *Staphylococcus aureus* causes diseases in poultry. In the chicken business, bacteriophage-based treatments have also been studied as an antibiotic substitute. Because of their extreme selectivity, bacteriophages could only be able to target a particular bacterial infection within the afflicted animal. However, in typical scenarios with many clinical strain infections, a specially blended complex cocktail of various bacteriophages might increase their antibacterial efficacy. Bacteriophages could be utilized as safe sterilizers in industrial settings to lessen adulteration on contact surfaces that come into contact with food or on chicken carcasses, in addition to their application in decreasing bacterial contamination in animals (Abd-El Wahab et al., 2023).

Examples in Industrial Use

Salmonella

Concerns from the public about strains that are resistant to antibiotics, particularly in zoonotic infections such as Salmonella, have prompted the chicken industry to find alternate forms of management. Because many of the ensuing food-borne illnesses are connected to chicken goods, minimizing microbial contamination during the manufacturing of poultry is essential. Salmonella Enteritidis recovery in broiler chicks treated with bacteriophage mixtures may be temporarily reduced; however, 48 hours later, there was no difference between the treated or untreated groups. Furthermore, there was no difference in the amount of Salmonella Enteritidis between the bacteriophage cocktail and a probiotic culture as compared to bacteriophages alone. A thorough investigation has been conducted to determine if bacteriophages in chickens have the ability to suppress paratyphoid Salmonella and cause illnesses associated with the bacteria.

Phage *S. Typhimurium* strains F98 [type 14], Beauville (type 40), and 1,116 (type 141) are examples of bacteriophages. birds challenged with *S. Typhimurium* at a dosage of 1012 plaque forming units (PFUs)/mL and found that the death rate linked to *S. Typhimurium* could be reduced to 20% compared with 56% in the untreated group. Six hours after therapy, S. Typhimurium returned to its pre-disease levels, but it was not completely eliminated. Moreover, if Salmonella was present, the bacteriophages do not survive in the gastrointestinal system. Bacteriophages often only survived as long as they were added to feed orally. For bacteriophages to be successful, they need to be administered in high amounts right away following S. Typhimurium infection. When bacteriophages are administered in excess, they have the potential to kill *S. Typhimurium*. The hens' death rate was lower when they received phage therapy, but not when they were subjected to the Salmonella challenge.

Numerous times, it was shown that using the bacteriophage combination in drinking water was safe. The behavior of the birds remained unaffected, as did the production metrics. In contrast to the control henhouses, where Salmonella was still found, the proportion of Salmonella in cloacal swabs at the end of the fattening phase (33 day) was nil.

Campylobacter

A prevalent cause of recorded food-borne enteritis is infection with campylobacter (Chinivasagam et al., 2020). Rarely, campylobacter is seen in birds under the age of two to three weeks. There are significant differences in the prevalence of Campylobacter species in chicken flocks, with values ranging from 2 to 100%. The study's findings show that at the time of slaughter, chickens and broiler flocks have a 42.5–100% prevalence of Campylobacter spp. There is an urgent need to reduce the prevalence of Campylobacter because there have been more reports of the bacterial pathogenicity and antibiotic resistance to erythromycin, gentamicin, tetracycline, and fluoroquinolones.

The majority of Campylobacter-specific bacteriophages in poultry are found in the Myoviridae family, with a smaller percentage occasionally found in the Siphoviridae family. This suggests that Campylobacter colonization in poultry can be effectively suppressed by phage therapy, which lowers the likelihood of Campylobacter getting into the food chain. Research indicates that pre-harvest phage treatment is more effective over Campylobacter loads in the feces and intestinal contents of experimentally diseased chickens without having a bad impact on the health of the animals. For instance, 28 hours after treating 47-day-old chicks with a mixture of Campylobacter phages orally, the number of bacteria in the ceca dramatically reduced (1-3 log10 CFUs/g).

In comparison to the negative control, phage CP14 (5 × 108 PFUs) treatment resulted in a reduction in 20-day-old chicks over the course of 31 days. After two days of therapy, the amount of *C. jejuni* or *C. coli* in chicken feces was reduced by almost 2 log10 CFUs/g, thanks to oral gavage and the in-feed administration of a three-phage cocktail. On the other hand, phage-treated hens have reportedly regenerated certain resistant bacterial phenotypes, but the phages did not prevent the decline of Campylobacter. The proper phage selection, optimization of the delivery method and dose, and research on chickens are essential components of an effective phage treatment regimen to cure Campylobacter. (Abd-El Wahab et al., 2023)

Campylobacter spp. successfully colonize the gut after infecting the bird, primarily the mucosa of the cecal crypts. In order to evade clearance, it has the ability to infiltrate the intestinal epithelium and grow quickly in the intestinal mucus. Furthermore, the animal's weakened immune system allows it to survive in commensal settings, allowing the bird to serve as a reservoir for human campylobacteriosis (de Mesquita Souza Saraiva et al., 2022).

Non-antibiotic Substitutes Include Bacteriophages, Antimicrobial Peptides and Bacteriocins

Bacteriocins are proteinaceous substances that only kill the type of bacteria that produce them. Bacteriocin synthesis and activity have, for the most part, only been shown in lab settings. Most of the evidence supporting the function of bacteriocins in natural systems like the digestive tract is indirect. When regularly added to the water supply, a genetically engineered strain of avian Escherichia coli that generates the bacteriocin microcin 24 decreased intestine Salmonella typhimurium levels in chickens. The potential of intestinal bacteria to generate bacteriocins in vitro is supported by the isolation of Fusobacterium mortiferum from chicken ceca. The bacteriocin-producing Enterococcus faecium strain J96 was also isolated from a chicken crop and had some protective effect on chicks infected with S. pullorum, indicating that bacteriocins might be beneficial for the survival of the digestive tract (Joerger, 2003).

Despite this, bacteriophages were superseded by antibiotics in the management of bacterial illnesses. Because bacteriophages are very selective to a particular strain or bacterial species, they safeguard the remainder of the microbiota, making bacteriophage treatment safe. Like "intelligent" or "active" medications, bacteriophages can be administered as a single dosage, proliferate while bacteria are still present and decompose in the same manner as their target bacteria until they are both eliminated from the body. Bacteriophages remain attached with their host bacteria, unlike other antibiotics that might trigger allergies, and the immune system typically identifies and tolerates them without endangering humans or animals. Bacteriophages are cheap and simple to replicate. Co-administration of antibiotics and bacteriophages enables synergistic and optimal therapeutic outcomes. *Salmonella* was also treated when bacteriophage treatment was used to treat bacterial infectious illnesses in poultry. The following are some highlights of the use of bacteriophages against Salmonella.

- Single dosages of high titer bacteriophages are preferable than repeated low titer doses.
- The effectiveness of using bacteriophages to prevent infections may have decreased due to the emergence of resistance.
- The capacity of the bacteria to produce resistance determines the efficacy of bacteriophage treatment.
- Bacteriophage cocktails are preferable to single bacteriophages
- By decreasing bacteria spread and death, the synergy between probiotics and bacteriophages may enhance healing.
- Bacteriophages are employed in food treatment even though they are considered "generally regarded as safe" (GRAS) goods; nonetheless, in order to utilize them in poultry farms, production methods must be in place.

Enzymes that Hydrolyze Peptididoglycans

Bacteriophages have two different types of enzymes:

- Endolysins
- Virion-associated peptidoglycan hydrolases

In order to get bacteriophage genetic material into the bacterial cell, the bacterial cell wall must be broken down by virion-associated peptidoglycan hydrolases, or VAPGHs. Bacteria are lysed by endolysins, which are the enzymes generated during the last phase of bacteriophage replication. Because these enzymes use peptidoglycan as their substrate and function as antibiotics by lysing out bacteria, they are categorized as enzybiotics, hydrolytic enzymes with antibiotic activity.One or more catalytic domains can be used to distinguish between endolysins and VAPGHs; endolysins also have a cell wall binding domain (CWBD), but VAPGHs do not have one.

For Salmonella in Poultry, What Is Left to Consolidate Bacteriophage/Endolysin Therapy?

Within the industry, bacteriophage formulations for commercial feed that may include Salmonella are generally accepted as acceptable for use with chicken by-products and other high-risk feed. Regulating the use of bacteriophages to cure illnesses in people or animals remains unrestricted. Treatment with Bacteriophage/Endolysin Differs A customized treatment should be developed by testing each pathogen isolate for the particular bacteriophages/endolysins. Personalized therapies such as autologous somatic cell therapy and tissue engineering, as well as potential uses like bacteriophage/therapy, are referred to as Advanced Therapy Medicinal Products (ATMPs) by the European legislation. As more clinical studies demonstrate the effectiveness of bacteriophages or their hydrolytic enzymes in treating multidrug-resistant infections of Salmonella, their popularity as pharmaceutical options will undoubtedly grow, and the data they give will help build regulatory frameworks (Ruvalcaba-Gómez et al., 2022).

A. Prevention of Bacterial Infection

Many professions and industries, including food preservation, aquaculture, animal husbandry, plant preservation, and medicine, need control of bacterial presence or populations. Conventional antimicrobial chemotherapy is frequently used to accomplish this. Phage treatment is one potential substitute for antibiotic therapy, which is becoming increasingly required due to the growth in antibiotic resistance. However, because stringent national and international standards would need to be followed, it could be difficult to apply phages in medicine on a large scale. (Rotman et al., 2020).

Eating poultry products typically exposes one to campylobacter, one of the primary food-borne bacteria. The frequent presence of Campylobacter as a component of the microbial population in the gastrointestinal tract of chicken still poses a challenge to optimizing intervention strategies. Immunoglobulin (IgY) is specific to SE on preventing colonization in broiler chickens that have oral infections. Whole cell antigens from SE were used to induce hyperimmunization in commercial Single Comb White Leghorn (SCWL) chickens. The enzyme-linked sorbent assay (ELISA) was used to measure the levels of anti-Salmonella antibodies, IgG and IgY, in egg yolk and serum, respectively (Rahimi et al., 2007).

In poultry medicine, the application of exogenous cytokines against infectious agents has focused on three main areas:

- Using them as adjuvants for vaccines
- Directly preventing infections and/or the undesirable consequences of immune responses that pathogens elicit
- Stimulating the ontogeny and activation of newborn host defences (Kogut, 2000).

B. Treatment of Bacterial Diseases

Phage treatment has a higher success rate and is safer than antibiotics, in part because it is more specific to certain bacteria and can only infect a single species, serotype, or strain. The commensal bacterial flora is not destroyed by this process. Targeted treatments using phages are now being successfully employed to treat infections that recover slowly in both people and animals. In the US, they are also used to remove germs from the surface of meals derived from plants and animals. Bacteriophages can offer an alternate method of getting rid of infections in an era where antibiotic resistance in bacteria is increasing and antibiotic use is being restricted (Tiwari et al., 2011).

Salmonella

In chicken farms, salmonella infection is a serious issue. Phages of Salmonella were isolated from chicken feces. Once the host range of the phages was established, morphological characterization was performed using transmission electron microscopy inspection. One-step growth curves were then used to calculate the replication parameters and adsorption rates. Following that, the phage cocktail was made and evaluated for efficacy in three different settings: shavings, plastic surfaces, and drinking water. The findings show that the phage cocktail can reduce the quantity of Salmonella by up to 2.80 log10 units in drinking water, up to 2.30 log10 units in shavings, and up to 2.31 log10 units in plastic surfaces. It has been discovered that phage combinations are an effective alternative for reducing Salmonella infection in situations including chickens (Evran et al., 2022).

Hundreds of thousands of individuals worldwide suffer from salmonellosis, which can be fatal and cause severe fever and diarrhea. Due to its widespread significance, Salmonella has been the subject of monitoring systems in many nations, which have made it possible to gather crucial data regarding antibiotic resistance. More than 2,650 serovars of Salmonella enterica have been discovered too far, and a number of them are linked to major sources of illness and public health concerns, including chicken meat and eggs. Resistant Salmonella spp. in hens have the potential to induce occupational salmonellosis in farmers and keepers, in addition to causing financial losses.

Phage Treatment

Phage treatment was also found to be effective in preventing horizontal infections in flocks of laying hens caused by strains of *S. Gallinarum*. After being in contact with infected persons, hens treated with bacteriophages added to their feed saw a 5% death rate, but the group not treated with phage treatment experienced a 30% mortality rate (Tiwari et al., 2011).

Antibacterial Treatment

As demonstrated by a significant (about 80%) synergistic antibacterial effect of a commercial oral probiotic preparation applied in conjunction with a bacteriophage "cocktail" of phages S2a, S9, and S11 (5.4 × 106 PFU/0.5ml/bird) at 4, 5 and 6 days of age as well as at 8, 9 and 10 days of age to combat *S. Typhimurium* infections in poultry, bacteriophages may be used in combination with other preparations. Compared to challenged birds who were not given treatment, treated chickens with bacteriophages and a probiotic had ten times fewer bacteria in their spleen, liver, and caecum (Tiwari et al., 2011).

Challenges and Limitations

The commercial poultry business is always looking for innovative ways to fight avian flu. The first week saw an increase in bird mortality due to many bacterial illnesses, including coli septicemia, involving around 10 different bacterial species. Because of the persistent illnesses, this has an impact on the flock's output, consistency, and appropriateness for slaughter. Poultry disease syndromes caused by *Escherichia coli (E. coli)* include septicemia, respiratory tract infections, and infections of the yolk sac (omphalitis). Septicemia is the defining feature of E. coli infections in young chickens. While pericarditis, air sacculitis, and perihepatitis may be symptoms of the subacute type of septicemia, acute septicemia may be fatal. O1, O2, and O78 serogroups comprise a large number of *E. coli* isolates that are often recovered from commercial broiler chicks (Swelum et al., 2021).

Harmful bacterial infections cause significant mortalities, poor weight increase, and poor flock homogeneity, especially in the first week of the birds' life. Producers suffer financial losses as a result. Antibiotics that promote in-feed development and are prophylactic have long been used as a preventive measure to address persistent issues. (Swelum et al., 2021)

1. In addition to causing significant financial losses, these bacteria's pathogenicity also endangers public health. Scientists are now again interested in employing bacteriophages as antimicrobial agents due to the growing incidence of bacterial infections that are resistant to the majority of traditional antibiotics (Rotman et al., 2020).
2. The length of the bacteriophage's in vivo activity is known as the limitation of the therapy. Additionally, the lytic activity of bacteriophages declines. It takes 60 minutes for intravenously administered bacteriophage (T7 phage) to clear, while the half-life of λ-phage was found to be around 6 hours. Delivery mechanisms need to be designed to protect the phage from serum inactivation or acidic/alkaline pH in order to stop in vivo bacteriophage decay. Sustained bacteriophage releases by any biomaterial matrix locally implanted in the place of infection site could increase the effective therapeutic duration by eliminating the requirement for repeated phage infusion. This might be helpful when treating non-topical tissues like bone, where it is generally believed that intravenous phage injection will not result in surgical site closure (Rotman et al., 2020).

Future Perspective

Furthermore, as the world's population is expected to reach 9.7 billion in 2050 and10.9 billion in 2100, there will be a growing need for meat protein worldwide, which is linked to the rising demand for chicken meat. By 2030, South Asia's chicken meat demand is expected to rise dramatically (75%), particularly in nations like India, where intake is expected to rise from 1.05 to 9.92 million tons yearly during the next three decades. (de Mesquita Souza Saraiva et al., 2022)

Concerns over animal care, cleanliness and disease prevention that may arise from strong genetic pressure to increase meat and egg output are now quite high. The natural immunity and consequently illness tolerance of animals are negatively impacted by genetic pressure to increase their productivity. Improved illness prevention, dietary management, and husbandry techniques lead to genetic selection. Reduction of the market age by around 4 weeks, improved growth rate, increased breast yield, increased laying rate, and increased daily egg mass have all been achieved. There is, however, great concern that the above-mentioned selection pressure may have already sparked major animal welfare and disease issues. Increased selection forces also impede the freedom of animals.

Conclusion

Public health is also threatened by antimicrobial resistance, resulting in a reduction in production. Due to the elimination of pathogens by bacteriophages, antibiotics have been replaced as an effective alternative solution to treat infections. As a result, meat production has increased. The use of these phages in general and economically requires a lot of research, since several formats are used. The application of this novel technique has resulted in considerable economic losses being reduced. Some limitations do, however, warrant attention, including adverse reactions, bacteriophage infections themselves, eliminating beneficial bacteria, and dose standardization.

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Chapter 43

Innovative Strategies: Redefining E. coli control in poultry without Antibiotics

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ABSTRACT

The poultry industry has contributed a significant role in bridging the nutritional gap in many countries by producing eggs and meat products that have high level of protein and vital nutrients at a lower cost. Since the ban of antibiotic growth promoters (AGPs), the natural antibiotics alternatives including prebiotics, probiotics, organic acids, symbiotics, immunostimulants, enzymes, essential oils, and phytogenic including botanicals, oleoresins, essential oils, and herbs are most commonly used as feed additives that have gained popularity in organic poultry industry. They are widely utilized across the world due to their distinct features and good influence on poultry production. They are simple to combine with other feed ingredients, leave no tissue residue behind, enhance feed intake, feed gain, feed conversion rate, boost immunity in birds, enhance digestion, increase the availability and absorbability of nutrients, have anti-microbial properties, do not alter carcass characteristics, reduce the need for antibiotics, act as antioxidants and anti-inflammatory agents, compete for stressors, and produce nutritious organic products that are safe for human consumption. Therefore, the current review focuses on a comprehensive description of different natural antibiotic growth promoters' alternatives, the mode of their action, and their impacts on poultry production.

KEYWORDS
E. coli, antibiotics, organic additives, poultry, AMR, health

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INTRODUCTION

Organic farming is one of agricultural industries with the quickest growth. Consumer demand has driven the market since the 1990s for goods produced on organic farms, including animal products. Sales of organic food had been increased from $28.4 billion in 2012 to $35 billion in the United States in 2014. During 2023, it has approached to $70 billion (Okey, 2023). US retailers imported organic foods worth billions of dollars into the American market since demand for them exceeded supply in the past few years. Poultry meat and eggs are among the organic animal products that are readily available and well-liked by customer's worldwide (Ponnampalam et al., 2019).

According to the most recent studies, the organic poultry market will rise from 9.78 billion to 10.34 billion dollars in 2022 and 2023 respectively with 5.8% CAGR. Nevertheless, the sector has yet to be able to keep up with the growing demand for organic poultry, despite having 3.5 million certified layer hens, 400,000 certified organic turkeys, and 9 million certified broilers. This confirms the Organic Trade Association's forecast of record growth for the organic poultry industry in the upcoming years. While organic chicken production has a lot of room to grow. There are uncertainties about the organic eggs and meat that may be contaminated with food-borne diseases could bound this potential (Wan et al., 2019).

Producing broiler chicks is a significant component of the global poultry industry. In order to control pathogens in organic poultry, it is necessary to act quickly to discover alternative and appropriate antimicrobial intervention techniques. To protect animals, human beings, and their surroundings, the National Organic Program forbids extensive usage of herbicides, hormones, pesticides, and antibiotics in poultry as well as agriculture practices. This could increase the sustainability of the industry (Zhang et al., 2021).

If the use of antibiotics is restricted in poultry, colibacillosis will become a more significant problem. So, there is need to search out alternatives to antibiotics usage. So that prevent and treat colibacillosis in poultry. The purpose of the following studies is to control if herbal extracts, bacteriophage, prebiotics, probiotics, and some other things can be utilized to treat *E. coli*.

E. coli Infections in Poultry

Colibacillosis is the term used to describe an infection that is characterized by enteritis, hemorrhagic septicemia, swollen head syndrome, salpingitis, peritonitis, coliform cellulitis, synovitis, orchitis, omphalitis, and colisepticemia. It is distinguished by pericarditis, air saculitis, perihepatitis, and other diseases in its subacute form and septicemia, which can be fatal in its acute form. The features include the presence of exudations, such as serum, fibrin, and inflammatory cells (pus), in the peritoneal (abdominal) cavity. Chickens have an inflammatory reaction that results in fibrin, which is visible, also cover the surface of several organs, such as intestine, liver, lungs, oviduct, and ovary.

According to observations, avian-colibacillosis is a prevalent disease that affects all age birds globally and has significant financial effect on the poultry production. Economic losses occur from the mortality and also due to reduced productivity of infected birds, primarily during the peak period of egg production and also during the late laying period (Linden, 2015). Avian Pathogenic *Escherichia coli* (APEC) infection is also occurred with infectious bursal disease (IBD) virus, mycoplasma, New Castle disease (ND) virus, infectious bronchitis (IB) virus, and environmental influences such as ammonia, humidity, temperature and dust. Additionally, when an egg is contaminated with feces, *E. coli* can enter the shell and disseminate to offsprings during hatching, leading to early chick death and also yolk sac infection. Birds' digestive tracts naturally contain *E. coli* bacteria, and most strains do not spread illness. Yolk sac infection and colisepticemia in early chick occurs. Coli-granuloma and peritonitis of eggs in adults occur (Masud et al., 2020).

Etiology

Escherichia coli is facultative, Gram negative, anaerobic, nonacid fast, non-spore forming, bacillus that is typically 3 × 0.6 µm in size and belongs to the family Enterobacteriaceae. It is usually present in GIT tract of human beings, animals, and poultry. Generally, *E. coli* strains are not harmful to people, but some strains can potentially infect human beings and even commercially raised poultry (Ievy et al., 2022). Enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), entero-invasive (EIEC), and enterotoxigenic *E. coli* (ETEC) are some of the strains that cause food poisoning. Hosts with weakened immune systems are more susceptible to virulent *E. coli* (Lim et al., 2020).

Epidemiology

E. coli is widespread and naturally found in GIT of animals and poultry at conc. of about 10^6 per gram. At the same time, the dust in chicken houses can have concentrations of up to 10^5–10^6 per gram. Both healthy birds and their eggs are susceptible to contamination from garbage and excrement. Newborn chicks may contract the disease through the ovaries. The pathogenic *E. coli* sources include food, rodent waste, and tainted well water. While infective *E. coli* isolates in poultry often have few serogroups, especially O1, O2, and O78. But O15 and O55 can be occasionally identified in small amounts as well (Parin and Simsek, 2023).

Transmission

The gastrointestinal system of chickens and other avian species frequently contains the bacterium *E. coli* as normal bio-flora. It is possible for the same or different poultry species to transmit bacteria by oral-fecal route. It can return to the environment through infected birds' droppings. The most likely places for *E. coli* strains to be detected are in the chickens' intestines and surrounding. The most common way for poultry to get *E. coli* infection is by the inhalation of *E. coli* infected dust particles. The flies, beetle, insects, rats, mites, and wild birds are only a few vectors that can spread *E. coli* to new environments. *E. coli* transmission can take place directly, indirectly, or both horizontally and vertically. *E. coli* can be transmitted vertically and transfer to progeny (Olawuwo et al., 2022).

Clinical Signs and Lesions

Typically, broilers that are around five weeks old can be affected mostly. Due to predisposing circumstances, resistance may be compromised then chicks of any age may be infected. If their resistance has been compromised, chicks under ten days old are particularly vulnerable. In these circumstances, the infection can be moderate or persistent, without any clinical symptoms. Coli-septicemia can cause birds to lose their appetite and stop drinking and eating. The reduced water consumption may be a sign of the disease's severity. Birds who are chronically afflicted display evidence of retarded growth rate and wastefulness.

E. coli infection occurs in two forms: **i)** localized **ii)** systemic.

Infected chicken has swollen appearance of face. Skin with Inflammatory exudate that builds up due to microorganisms, mostly *E. coli*, following the upper respiratory tract (URT) viral infection, as infectious bronchitis virus (IBV), avian metapneumovirus, etc. Loose connective tissue Inflammation beneath the skin is known as cellulitis. In subcutaneous tissues, caseous and serosanguinous exudates are frequently found in the belly or between midline and thigh (Struthers, 2024).

Affected birds show following signs and symptoms:

- Swollen abdomen, depression, and huddling
- Birds are often dehydrated, stunted, vent pasting as well as enlarged gall-bladder
- Hemorrhages on the surface of the intestine and peritonitis
- Distended yolk sac with anomaly in color, smell, and consistency
- Un-absorbed yolk sac

Each type has unique pathogenic factors that influence the signs and symptoms of enteric illness. Oviduct inflammation is called salpingitis caused by *E. coli* infection. It reduces production of eggs, resulting in the mortality of breeders and laying hens. Infection is caused by the cloaca, vent pecking, infected air sacs, and prolapse (Linden, 2015). *E. coli* is most often the cause of egg peritonitis. Egg peritonitis is peritoneal inflammation induced by a cracked egg in the abdomen. Random mortality in laying or breeding hens is caused by it commonly (Rosales, 2019).

Coligranuloma, also known as Hjarre's disease that affects hens, quails, and turkeys. It is a rare type of colibacillosis. Several granulomas are seen in ventricle, pro-ventriculus, liver, small intestine, mesentery, and cecum. The pathogenic *E. coli* in blood of birds is classified as coli-septicemia. Different phases of colisepticemia are: i) sub-acute polyserositis ii) acute septicemia iii) chronic granulomatous inflammation. Air sacculitis of various degrees results in respiratory symptoms such as rales, sneezing, and coughing. Colisepticemia can cause osteomyelitis, arthritis, tenosynovitis, and spondylitis. Air sacs become opaque, enlarge, and may carry caseous exudate on PM inspection. Infected birds seem normal and are frequently discovered dead with a full culture (Panth, 2019).

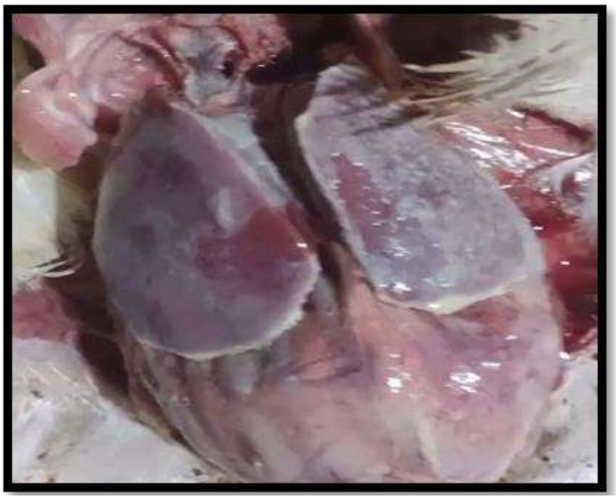

Fig. 1(a, b): Prominent sign of *E. coli* caesious layer on Liver, perihepatitis.

Antibiotic Treatment for *E. coli* in Poultry

Some *E. coli* strains have been effectively treated using antibiotics such as tetracyclines, ampicillin, sulfas, and streptomycin. Early treatment is recommended, with a follow-up that takes antibiotic sensitivity testing, considering the particular isolate. Antibiotic therapy becomes less effective when the organism becomes sequestered or encapsulated in caseous exudate; consequently, chronic phases of infection are less likely to be treated appropriately. The great majority of clinical isolates, however, are tetracycline resistant, with most APEC isolates resistant to 5 or more drugs. Fluoroquinolones are currently prohibited in several nations, including the United States (Dixit et al., 2024).

The spread of AMR is the most challenging topic in human, animal, and environmental health in the following century. AMR emerged as one of the main obstacles to economic growth (Akter et al., 2022). Antibiotic overuse and misuse are significant contributors in formation and spread of *E. coli* with antibiotic-resistant which can transfer to human beings via direct contact with ill animals or with contaminated food. There is antibiotics extensively usage in poultry to treat infectious disorders and as growth promoters (Roy et al., 2022). AMR is unavoidable due to the extensive antibiotic usage in clinical as well as non-clinical settings in countries with limited resources (Hoque et al., 2020). Bacteria such as *E. coli* have evolved multi-drug resistance (MDR) as a result of haphazard antibiotic usage (Islam et al., 2021). The rise of MDR strains resistant to antimicrobial medicines may result in increased morbidity, mortality, and healthcare costs (Dadgostar, 2019).

Between 2010 and 2021, antimicrobial resistance genes have been described in 58.8% (10/17) of the published articles. In poultry samples, resistance genes for tetracyclines, sulfa medicines, fluoroquinolones, beta-lactams, aminoglycosides, polymyxins, and phenicols etc. are found in *E. coli*. All of the resistance-genes have a more significant proportion (about 100%), suggesting a severe problem in the health structure. Furthermore, presence of transposons linked genes and natural plasmids in *E. coli* isolates obtained from poultry and poultry habitats (different bla genes, tet A, B, C, etc.) suggests the presence of many genetic mobile elements (Khruengsai et al., 2023). According to a comprehensive research, *E. coli* from poultry and its environment were resistant to fourteen anti-microbial drugs and forty-five distinct antimicrobial agents. A current study found that isolates of *E. coli* from poultry flesh was resistant to thirteen antibiotic classes, which concerns the medical community. *E. coli* isolates are also resistant to penicillin (Parvin et al., 2020).

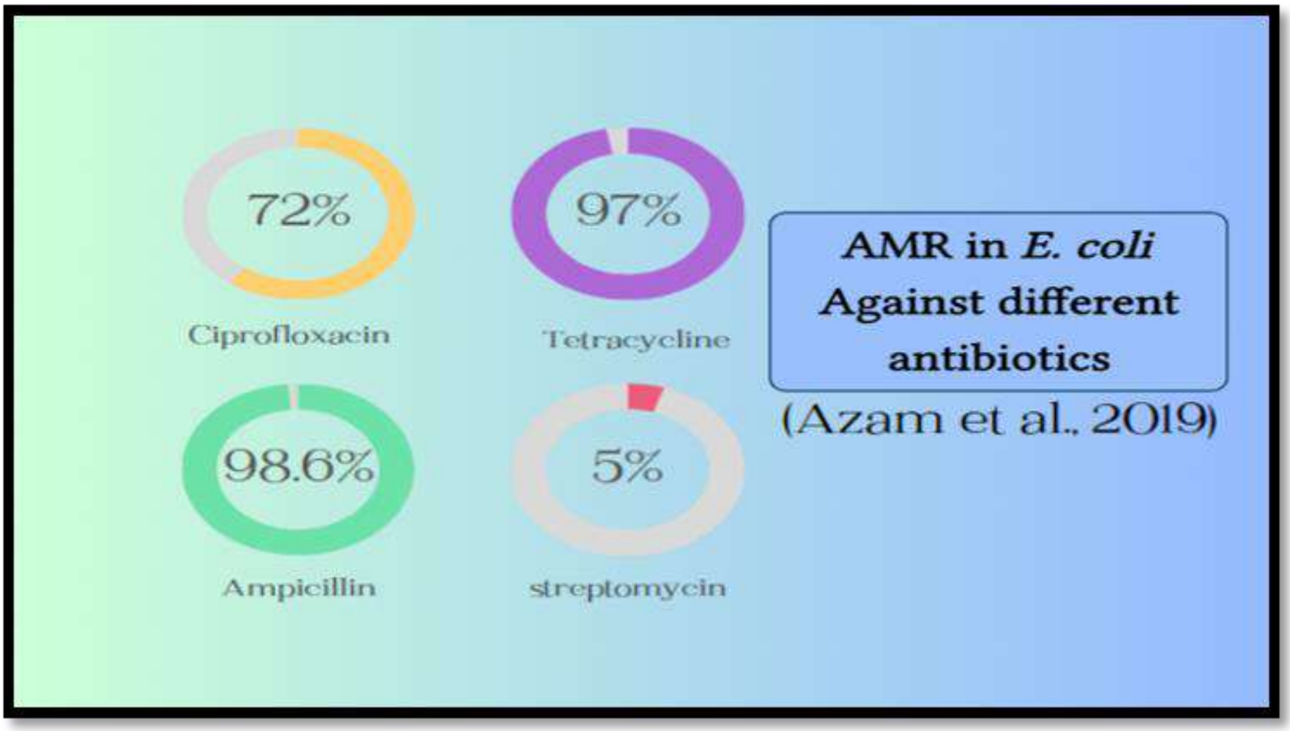

Fig. 2: AMR values against different antibiotics in *E. coli*

Alternative Treatments of *E. coli*

A. Probiotics

Antibiotics have been utilized as growth promoters or feed additives since the 1940s as it was discovered that feeding *Streptomyces aureofaciens* with chlortetracycline residues to birds or animals increased their growth rate. European Union (EU) has prohibited the use of antibiotics as food additives or growth stimulants. The reason for this was the rise of microbial resistance to antibiotics since 2006, which has been used to treat infections in animals and poultry. Furthermore, antibiotics create other problems, as beneficial bacteria in chicken gut being killed (Uzabaci and Yibar, 2023).

Due to the biohazards of antibiotic usage, such as their lingering effects on meat and food products, nutritionists have recently concentrated their efforts on developing innovative and alternatives to therapeutic supplements and growth promotors to avoid illnesses and boost avian immunity (Yadav et al., 2016). In this regard, bacteriophages, avian egg antibodies, cytokines, toll-like receptors, probiotics, and other substances has been investigated for their potential benefits for protecting animals from infections and enhancing production performance. Probiotics protective effects and advantageous uses are evident in a number of ways. Probiotics have the potential to improve growth performance, the quality of eggs, nutrient absorption, and digestibility, thereby increasing production and protecting the health of poultry. The most popular probiotic strains include Streptococcus, Lactobacillus, Bacillus subtilis, and Bifidobacterium strains. These strains not only promote growth but also have the ability to reduce harmful bacteria like *Salmonella typhimurium, Escherichia coli, Clostridium perfringens, Staphylococcus aureus,* and others. Because of its potential to limit pathogen development, the proper selection of probiotic strains might reduce the adverse effects of antibiotic treatment and has several valuable uses (Sugiharto, 2023).

Live bacteria like Lactobacillus, Bacillus subtilis, Bifidobacterium, and Streptococcus are just a few sources of probiotics. Other sources include yeasts like *Saccharomyces boulardii, Saccharomyces cerevisiae*, Candida, and fungus like Aspergillus. Probiotics can work in several different ways to inhibit pathogens, including by producing organic acids and antibiotics like hydrogen peroxide, bacteriocins, and defensins. They can also influence the host immune system's regulatory T cells, effector B and T cells, antigen-presenting cells, and enterocytes. Probiotics can also modulate the function and phenotype of dendritic cells, the production of anti- and pro-inflammatory cytokines, the stimulation of antibody (sIgA) production, the enhancement of macrophages (NK) and natural killer cells activity, the release of nitric oxide, the modulation of apoptosis and many other biological processes (Abun et al., 2024).

Probiotic supplementation resulted in lower counts of *Escherichia coli*, total coliforms and higher lactobacilli counts in the gut of chickens. Additionally, the probiotic combination increases the population of helpful bacteria while reducing *Escherichia coli* in the cecal contents, improving the bacterial count. Dietary *Enterococcus faecium* probiotic supplementation improves growth performance and decreases death rate of the broiler chickens. It boosts the humoral immune response, regulating the release of inflammatory cytokines, increasing the expression of tight junction proteins (TJ proteins), and maintaining the intestinal barrier against *E. coli* O78 infection. *E. faecium* has the power to preserve intestinal integrity and reduce the excessive intestinal permeability brought on by *E. coli* infection (Huang et al., 2019).

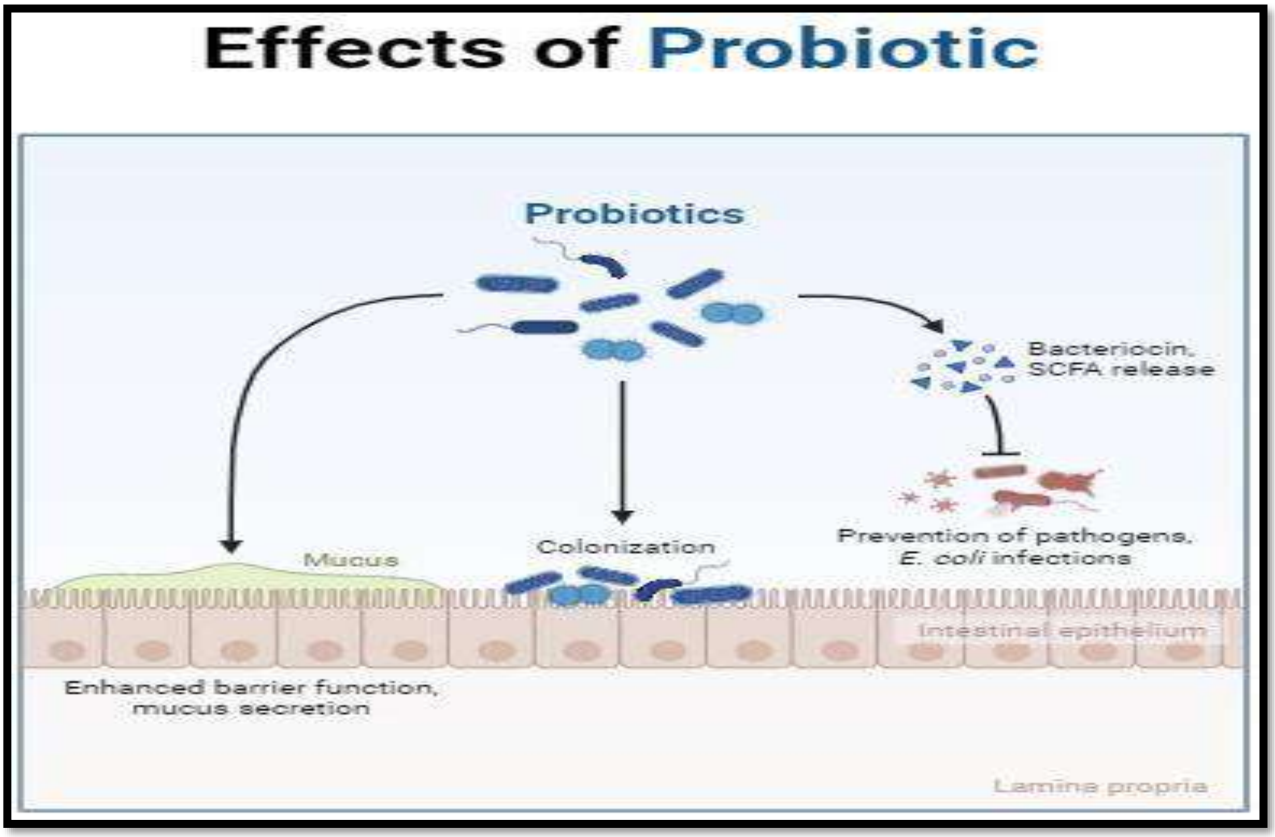

Fig. 3: Probiotic effect on intestinal villi and their mode of action.

Prebiotics

Traditionally, prebiotics were represented by a small range of carbohydrates and related chemicals. Galacto-oligosaccharides (GOS), mannano-oligosaccharides (MOS), and fructo-oligosaccharides (FOS) are among the more often used in animal and poultry research. Fundamentally, the host animal or person ingesting these substances does not make use of them, but some bacteria, such as bifidobacteria and lactic acid bacteria, can use them as substrates. Given that FOS and comparable prebiotics are thought to be largely fermentable and less likely to persist intact in the GIT for extended periods of time, prebiotic variations in pathogen and host responses may also be connected to the chemical composition of the prebiotic. In contrast, the yeast-derived MOS can directly reduce GIT infections by attaching to the flagella of microbes like *Escherichia coli* and Salmonella, reducing their colonization of the GIT by interfering with their attachment to GIT epithelial cells. By attaching to macrophages and dendritic antigen-presenting cells that carry the C-type lectins of the mannose receptor, yeast mannans have also been demonstrated to function as immunological adjuvants and directly induce immune responses (Fomentini et al., 2016).

Isolated alginate from marine brown algae called polymannuronate is thought to be a promising prebiotic. It is known to preferentially colonize helpful microorganisms while excluding pathogenic and dangerous microbes. According to new research, *E. coli* levels were found to decrease and lactic acid bacteria to increase when dietary polymannuronate levels were raised. Furthermore, a higher level of acetic acid was discovered in the broilers. Thus, the synthesis of lactic acid, acetic acid, and volatile fatty acids (VFA) might reduce intestinal pH and produce an environment that prevents the development of dangerous bacteria. In recent studies, species-specific quantitative PCR has been performed, and the results show that FOS (2.5 g/kg feed) enhanced the population of Lactobacillus while limiting the growth of *E. coli* and *C. perfringens* in broilers (Zhu et al., 2015).

Herbal Extracts and Essential oils

The large variety of functionally important secondary metabolites (phytochemicals) that plants generate; has a wide range of therapeutic benefits. Most of these chemicals are employed by plants as a kind of protection against other microbes, herbivores, and rivals. Essential oils (EOs), polypeptides, alkaloids, lectins, phenolic compounds, and polyacetylenes are the prominent phytochemicals found in plants. The complex blend of natural, volatile, and aromatic molecules known as essential oils (EOs) is produced by aromatic plants, many of which have been employed in traditional medicine. It has been noted that EOs work by disrupting cell walls and cytoplasmic membranes, which causes lysis and the leaking of intracellular chemicals. The attachment of cytoplasmic membrane to outer membrane (MO) in Gram-negative bacteria did not result in an increase in resistance to the constituents of EOs. Several substances have now proven they may disrupt the OM by releasing LPS.

Additionally, several phenolic components of EOs (such as carvacrol and thymol) have shown the capacity to interact with OM and exhibit bactericidal action (Chen et al., 2023).

Currently, administering antibiotics to treat infections causes harmful damage to the organs, tissues, and cells of the host. Herbs can prevent, treat, or combat the detrimental effects of antimicrobial agents. Certain plants have beneficial antibacterial and antifungal characteristics that can be used in medicinal settings. Clinical tests on infected birds revealed that *Cassia auriculata* plants possess vigorous microbicidal activity (Alem, 2024).

There is, however, a need for more information on how well EOs improve sanitary conditions. These natural chemical vapors may be used to control undesirable agents, according to data on the efficiency of air-dispersed EOs in lowering fungal and bacterial load in nosocomial environments. Relevant anti-*E. coli* activity is present in *L. cubeba*. Neral (32.5%) and geranial (36.4%) aldehydes are primarily responsible for the hole formation on membranes of *E. coli* cells. *M. piperita* EO has good reactivity against *E. coli* during different tests. Bacteria were tested with *M. piperita* EO; *E. coli* showed the highest level of antibacterial activity. *P. graveolens* and *O. basilicum* EOs demonstrated moderate antibacterial activity, supporting findings from prior investigations that these substances more effectively combated Gram-positive bacteria than Gram-negative bacteria. The combination of *S. aromaticum* and *C. zeylanimcum* may be an effective alternate therapy for *E. coli*. *A. fumigatus* and *E. coli* may both be eliminated by *C. citratus* when applied alone (Ebani et al., 2018).

Cloves' whole essential oil inhibits the growth of *E. coli*. It has been demonstrated in vitro that oregano essential oil is a potent inhibitor of *E. coli*. Carvacrol, a key ingredient of oregano essential oil, was discovered to have less antibacterial activity than the whole oil. Additionally, cinnamon oil has been demonstrated to have an antibacterial effect against *E. coli* in vitro. Another plant with antibacterial capabilities demonstrated in vitro is garlic (*Allium sativum*). Two strains of *E. coli* were effectively inhibited by the aqueous extract of garlic and were susceptible to its effects (Abd El-Ghany, 2024).

Modes of Action

PFAs (phytobiotic feed additives) improve health of gut and also improves its performance by different mechanisms: antimicrobial properties, antioxidative properties, growth promotion, improved digestion, improved palatability, and improved health of gut. Researches on palatability are indecisive, but PFAs can improve quality of feed by its anti-oxidative properties and slow fungal and bacterial growth. EOs breaks cell wall and cell membrane of infectious agents and also increase permeability of membranes of cell which results in release of cell contents including genome (Abd El-Hack et al., 2022).

D. Bacteriophages

The emergence of bacteria with various antibiotic resistance has increased the need to find an alternative of antibiotics to treat bacterial infections. A class of viruses known as bacteriophages is found all across nature and is exclusively linked to the life cycle of bacteria. We refer to them as parasitic bacteria. Bacteriophages are viruses that infect and have the ability to kill bacteria. Twort (1915) and d'Herelle (1917) separately discovered these viruses. Both the intramuscular and aerosol routes can be used to administer bacteriophage. Recent studies have shown that while bacteriophages administered by aerosol method do show some results, they do not yield positive outcomes against *E. coli*. Chickens that are administered bacteriophage aerosol can be protected against *E. coli* infection for up to three days. Additionally, it has been discovered that bacteriophages injected intramuscularly produce excellent and productive outcomes. The idea that bacteriophage can be used as a successful substitute for antibiotics in animal production to prevent and treat bacterial diseases is supported by the fact that bacteriophage can be therapeutic if given with high enough titers (Roth et al., 2019).

Phage treatment is safer and more effective than antibiotics in part because bacteriophages are unique to specific bacteria, meaning they can infect only one strain, species, or serotype. This mode of action does not harm the commensal gut flora. Because bacteriophages replicate themselves during treatment, applying them again is unnecessary. Another benefit of phages is that they cannot destroy eukaryotic cells. This results in drop in their titers and a significant decrease in the quantity of dangerous bacteria which cause infection. Because most phages are mostly made of proteins and nucleic acids, they have the equally significant benefit of not being poisonous (LA and Waturangi, 2023).

Phage therapy has several benefits, but its use is severely restricted, in part due to the fact that individual bacteriophages are not effective against infections with a wide range of symptoms. Complex etiological agent identification and characterization are frequently required. Furthermore, some bacterial viruses particularly lysogenic phages, which encode the genes of bacterial toxins and turn benign bacteria into dangerous ones, do not fit the criteria to be used in therapy. Additionally, they might be implicated in the spread of drug-resistant genes among bacteria. Phage therapy can also have unfavorable effects, such as phages being eliminated by the reticuloendothelial system, which shortens the phages' half-lives in the body and decreases therapeutic efficacy (Lee et al., 2024).

Phage therapy is also helpful in curing bacterial infections in various animal species. Additionally effective in treating poultry infections are bacteriophages. Evaluating the viability of bacterial viruses to control infections that significantly affect the productivity and health of animals is one of the goals of phage therapy. Treatment with phages has successfully treated chicken colibacillosis and avoided infections.

Organic Acids

Chemicals with an acidic pH are called organic acids. The most common kinds are carboxylic acids, which include butyric acid, lactic acid, sorbic acid, acetic acid, formic acid, citric acid, uric acid, oxalic acid, and propionic acid. Organic acids can support the intestinal health of chickens by enhancing feed conversion ratios, livability, weight gain, live weight, and immune responses. This is done only when combined with good management, good nutrition, and biosecurity practices (Saeed et al., 2017).

The impact of organic acids on intestinal health and broiler performance reduces pH, which makes organic acids to inhibit proton donors in aqueous solution and results in weak acids. This activity is associated with organic acids' antibacterial action. Since the dissociation of weak acids is pH-dependent, lower pH values result in increased antibacterial activity (Jadhao et al., 2019). When organic acids are not dissociated, they are lipid-soluble and can be readily incorporated into bacterial cells through both carrier-mediated and passive mechanisms. When there is alkaline environment, organic acids produce protons intracellularly. Then pH reduces to a point where it disrupts microbial metabolism by impeding vital enzyme activity. This forces the bacterial cell to expend energy exporting excess protons, which ultimately leads to starvation and death. Protons H^+ have the ability to denature DNA and proteins that are sensitive to acid in bacteria. Because unlike other bacteria as *E. coli*, Lactic acid bacteria can grow at low pH level. Lactic acid bacteria are more resistant to organic acids. Due to their high internal potassium content, gram-positive bacteria like Lactobacilli are shielded from acid anions (Araujo et al., 2019).

Organic acids lower the quantity of harmful microorganisms because of their antibacterial activity. This leads to decrease level of toxic bacterial metabolites, less bacterial competition for nutrients, better protein digestibility, and enhanced avian growth (Hassan et al., 2014). The histological structure of the gastrointestinal tract is altered by the addition of organic acids, which increases the length of the villus and enhances the intestinal mucosa's capacity to absorb nutrients. As a result, better nutrient absorption, and enhanced growth performance are achieved. pH low level in stomach promotes good bacterial growth while inhibiting harmful micro-organisms. Additionally, these acid anions combine zinc, phosphorus, magnesium, and calcium to increase their digestibility. The decreased pH in the stomach causes an increase in pepsin activity. Peptides generated by the proteolysis of pepsin trigger the release of hormones that control the digestion and absorption of proteins, including cholecystokinin and gastrin (Padmini et al., 2017).

There are many commercially available organic acid products which are micro-granulated feed acidifiers that are based on lactic acid, fumaric acid, formic acid, and ammonium format. These are considered as unprotected organic acid mixtures which are active in foregut and neutralized by bile. 49.0% benzoic acid and 3.0% calcium used in organic acid products. Due to its dissociation in alkaline pH of jejunum, organic acid combinations active in midgut to protect it. Spices and herbal extracts are essential for enhancing the health and productivity of bird. The benefits of plant extracts or active ingredients in bird feed may include:

- Boosting feed intake and appetite.
- Improving the synthesis of endogenous digestive enzymes.
- Boosting immunity.
- Having antioxidant, antiviral, antibacterial, and anthelminthic properties.

Flavonoids, glucosinolates, isoprene derivatives, and other herbal metabolites may have an impact on the gut's physiological and molecular processes in birds. Nutrient metabolism may be intermediate, along with the stabilizing effect on the gut microbiome. Numerous infections are hazardous to livestock and poultry production, causing significant financial losses (El-Saadony et al., 2021).

The herbal feed additives usage is now more popular in the production of chickens because of the restrictions on the use of various antibiotics, their harmful side effects, and their affordability. There are several uses of spices, herbs, and their extracts. These encourage feed intake, boost the immune system, function as antioxidants, and have antibacterial, coccidiostat, and anthelmintic qualities. The herbal feed additives which are mostly used in poultry feed are cinnamon, nutmeg, cloves, coriander, cardamom, cumin, anise, parsley, celery, fenugreek, pepper, capsicum, ginger, garlic, horseradish, mustard, onion, mint, shatavari, rosemary, thyme, shatavari, jivanti, and turmeric (Abou-Kassem et al., 2021).

Enzymes

Chemical reactions can be accelerated by biological catalysts known as enzymes. Enzymes are protein molecules that have significant effects on how stable they are during the digestive tract's passage and the formation of high-temperature meals (pelleting). To break down food, all creatures need enzymes. Adding specific enzymes to the feed increases the nutritional content of the food and speeds up the process of digestion. Ultimately, by lowering the amount of manure produced and the amount of nitrogen and phosphorus expelled. Enzymes in feed are utilized to increase the efficiency of feed, lower feed costs, and enhance environment (Barletta, 2010).

Enzymes are classified into different groups. They can be classified based on the substrate they act on and their origin. Enzymes are categorized based on the substrates they break down into three groups:

- Phytases, which break down phytate.
- Proteases, which break down fiber and starch.
- Proteases which are made up of enzymes that break down proteins.

The sources of enzyme are exogenous and endogenous. Since the 1920s, studies on the role of enzymes in poultry diets have been conducted. Protozyme, an enzyme product derived from *Aspergillus oryzae*, was the first documented application of this product in poultry diets. An un-pelleted poultry meal including wheat and rye at different inclusion levels, increase significantly body weight gain when enzyme cocktails containing ß-glucanase and xylanase was added (Mak et al., 2022).

Exogenous enzyme supplementation has emerged as the gold standard for enhancing nutrient utilization efficiency and digestibility. All creatures require enzymes, which are either produced by the animal itself or by microorganisms in their digestive system, in order to digest food. Enzyme supplementation aids in lowering nutrient excretion, which might incur additional costs for the farmer, feed supplier, and the environment if left unchecked (Ali and Abdelaziz, 2018).

Reduced feed costs are contingent upon the presence of exogenous enzymes. Enzymes can lower production costs by inadequately absorbing other nutrients since feed makes up the majority of production costs. Utilizing enzymes in feed becomes more financially appealing and offers a more noteworthy return on investment as the price of wheat, fat, corn, and inorganic P rises. Inositol and inorganic phosphate are the products of the hydrolysis of phytate by the enzyme phytase (AKDAĞ and KIYMA, 2023). More specifically, phytase is also referred to as myo-inositol hexa-bisphosphate phosphohydrolase. For growth and maintenance, poultry require phosphorus (P) in their diet. As a result, the diet needs to contain a certain quantity. Even with an adequate supply of total P in the diet, chickens cannot digest some of the total P derived from cereal grains. Because it is linked to phytate, roughly 60% of P is inaccessible to non-ruminant animals. Phytate causes significant decreases in nutritional availability by binding to numerous dietary cations, including fat, protein, vitamins, Ca, Mg, Fe, Cu, Zn, and Mn (Bashir and Kumar, 2021).

According to market trends, hydrolytic enzymes have become more popular as feed supplements to help with the digestion and absorption of minerals that are not readily available, including dietary phytate. Because of their low microbial population in the upper part of the digestive tract and the absence of strong endogenous phytase activity, non-ruminant animals can only digest phytate. In poultry, the digestive tract (crop, proventriculus, and gizzard) is the primary location of phytate breakdown by phytases; the distal gastrointestinal system has less degradation in this context (Barletta, 2010).

Conclusion

Large-scale use of the natural antibiotic growth promoter alternatives is advised because they are safe, healthy, and have a positive immune-modulating effect. They also digestion, improve productivity, absorbability, availability of nutrients, intestinal health, and the production of organic chicken meat that is useful, safe, and nutritious for human consumption. These all are beneficial against *E. coli* treatment as alternative of antibiotics to combat AMR.

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Chapter 44

Preventive Approaches for Ruminant Coccidiosis; Probiotics, Prebiotics and Botanicals

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ABSTRACT

The use of synthetic drugs is banned in ruminants for growth and performance due to the development of resistance in many microbial agents. This situation led to the need of an alternative to these synthetic products. Plant based products are considered as the best alternative of synthetic drugs in ruminants. The growth and performance of ruminants have been affected by intestinal diseases, especially coccidiosis which cause severe damage to the ruminal microbiota. The plant based products such as flavonoids, tannins, saponins and many other botanical products are used in ruminants which help to fight against different *Eimeria* species to prevent Coccidiosis. Probiotics have also gained the focus of scientists and used various beneficial bacterial species as probiotics in ruminants. The use of probiotics was limited to bacterial and viral disease but recently they are used to treat various parasitic infections especially the intestinal infections and the outcomes were outstanding. The molecular mechanics of these natural products are still unknown and very limited knowledge is available about the process.

KEYWORDS		
Eimeria, Ruminants, Probiotics, Plant product, Saponins, tannins, Flavonoids	Received: 14-Jun-2024 Revised: 18-Jul-2024 Accepted: 20-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Parasitic infections are known to occur in all vertebrate animals and generally termed as coccidiosis. A large number of species of coccidia are discovered to cause infections in all animals including herbivores, omnivores and carnivores. Some of these coccidian species are known to cause severe infections in their host but some are of less clinical importance. The major sign of coccidiosis is bloody diarrhea noticed by veterinarians and producers involved in the production and healthcare of ruminants. Pathogenic coccidial infections are the major reason for bloody diarrhea also called bloody scours and white scours. The pathogenic agents which cause coccidiosis are commonly single-cell protozoa which present in the intestinal cells of its host. These protozoa develop and multiply inside the intestinal cells so they cause the destruction of these cells. As a result of the destruction, they are called parasitic protozoa besides the fact that they cause an infection or not in their host. The coccidial species that cause infections in ruminants, exhibit no symptoms even though the diagnosis confirmed heavy parasitic infestation in the host. As a result of this situation it is important to differentiate between the major pathogenic species and other bacterial, viral and less important causes of intestinal diseases. The agent which causes coccidiosis belongs to genus *Eimeria* and family *Eimeriidae* so the term *eimeriiasis* or coccidiosis is generally used to represent the infections caused by these species. In ruminants, *Eimeria* species are host specific, they cause infections in the host and complete their development and reproduction in the intestinal tract of their host.

Pathology of Coccidiosis in Ruminants

The outcome related to the pathological and clinical situation may be influenced by many other factors. These factors include species of *Eimeria* which cause present infection, severity of infection, replication rate of related species, inflammatory conditions, immune status, managemental stress and any other infection present at that time. *Eimeria* localizes intracellularly in the intestine of its host, which causes potential damage to the mucosal lining of the intestine (Figure 1). The rate of infection and results of the infection depends on the species of the *Eimeria* which can be different in different hosts and the living conditions. The major destruction caused by the parasite is usually in the late reproduction stages such as during schizogony and gamogony (Friend and Stockdale, 1980). This is because of the multiplication of the parasite in its first schizogony stage which results in an increase in the number of intestinal cells in the further multiplication of the parasite. In the animals, infected with *Eimeria*, major damage happened just before the start of excretion of the oocyst. In goat kids, early infection of *Eimeria* results in haemorrhagic enteritis (Taylor and Catchpole, 1994). Polyps formation in the small intestine is a result of *Eimeria* infection (Koudela and Boková, 1998), it also causes white nodule formation in the mucous visible from serosal surface (Kanyari, 1990).

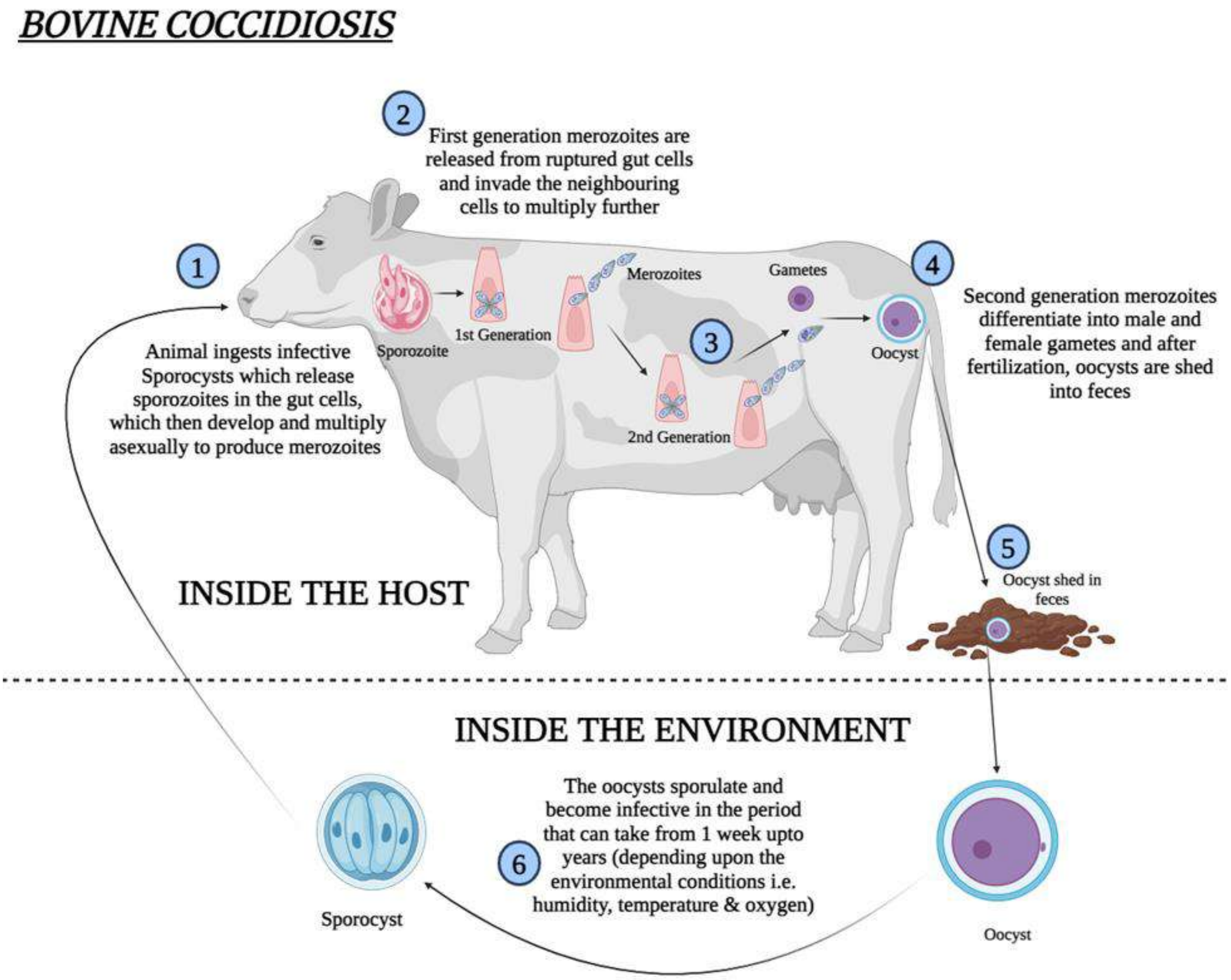

Fig. 1: Cycle of coccidiosis inside the ruminants and the envisronment.

Economic Impacts of Coccidiosis in Livestock Industry

Because of the clinical and subclinical cases of coccidiosis in tropical regions, there is no documented data about the economic losses caused by coccidiosis. The subclinical infection of coccidiosis is not of much importance so it is not usually compared to other diseases (Khodakaram-Tafti and Hashemnia, 2017). In small ruminants, a larger number of animals with high production rates can be a source of high economic loss if they get infected due to coccidiosis (Foreyt, 1990). In animals with mild coccidial infection, the economic losses can be characterized as low production rates and no clinical signs available. According to an estimation, loss of $140 million per annum has been faced due to sheep and goat production globally (Fitzgerald, 1981). The major contributors to the loss include mortality rate, high cost of treatment for diarrhea, increased sensitivity to secondary infection, decrease production rates. The long term effects of coccidiosis include less feed efficiency, decreased growth rate and reproductive performance (Lassen and Østergaard, 2012). It has been seen that subclinical infection of coccidiosis contributes more in economic losses as compared to clinical cases as the animals are affected for a long period of time (Daugschies and Najdrowski, 2005).

Treatment Options for Ruminant Coccidiosis

The best way to treat the animal having coccidial infection is to follow the instruction of the veterinarian that may include treatment with ionophores, use of amprolium or sulpha drugs and any other alternatives. In the past, the best way to treat coccidiosis was the use of antiparasitic drugs which not only gives brilliant results against a wide range of parasites but also are cost effective (Ali et al., 2022; Alvi et al., 2022). The prime way to treat coccidiosis is to use different chemicals and ionophores drugs (Adeyemi et al., 2023). Oral route is mainly used to give medications mixed in water or feed. The immense and irregular use of these drugs to treat infection has become the major reason for the development of various new species which may or may not be sensitive to these drugs (Gray et al., 2021; Ahmad et al., 2023). The major constraints of high production from farm animals are development of resistance in parasites and the control of parasites. This situation alarms the need of some alternative ways which especially includes plant based medications, probiotics and prebiotics which is safer in terms of resistance development and also cost friendly (Saeed and Alkheraije, 2023).

Probiotics; an Emerging Preventive Measure

It has been proved through many in vitro studies that the feed supplemented probiotics reside in the intestine of the ruminants and exhibit characteristics antimicrobial activities against many pathogenic agents which cause enteric infections (Adeniyi et al., 2015; Lin et al., 2020). This ability of probiotics made them a potential factor to use as therapeutic agents to treat many intestinal diseases (Prabhurajeshwar and Chandrakanth, 2019). To support the intestinal epithelial barrier, probiotics helps to enhance the expression of the components of barrier function (Rokana et al., 2016; Bron et al., 2017) which helps in the prevention and control of many gastrointestinal diseases in its host (Lucey et al., 2021). Many probiotics are known for the production of metabolites such as bacteriocins which helps to control growth of the pathogens and assist in defense mechanisms to prevent infections (Osuntoki and Korie, 2010; Meale et al., 2017). Probiotics also compete with pathogenic agents for the attachment to the gut epithelium which also helps to prevent infections (Rokana et al., 2017). The mechanism of probiotics to work against different pathogens to prevent infections is also investigated recently. The rate of parasite-borne diseases is high in dairy animals as compared to bacterial diseases. The therapeutic effects of probiotics against parasites such as *Eimeria* have been proved through many studies using different animal models (Travers et al., 2011). Some studies stated that probiotics can be used to decrease the helminth infection in dairy animals. To find out the mode of action of probiotics against parasitic infections still requires more experimental data.

Compound Probiotics

Compound probiotics consist of more than one beneficial bacterial species which mainly include ECL1.2 strain of Bacillus subtilis, different strains of other bacteria such as *Saccharomyces cerevisiae J, Lactobacillus plantarum R* and *Lactococcus lactis*. Different studies were established to find out the probiotics improvements on growth rate, immune function and gut microbial status of the weaned lambs which were infected with coccidial infection. The drastic impacts of coccidiosis include diarrhea, damage to intestinal epithelium, decrease growth rate and increase rate of mortality (Reeg et al., 2005). Studies show that the animals fed with probiotics supplements showed better performance as the probiotics safeguard the intestine from coccidial damage. It is done by competing with coccidia for the binding sites on the intestinal mucosa which hinder the proliferation and replication of the pathogens thus protecting the intestine up to some extent (Bozkurt et al., 2014). The use of compound probiotics as feed supplements improve the growth performance factors and the effects of probiotics were comparable with the drug diclazuril (Giannenas et al., 2014). Outcomes of another study shows that the supplementation of compound probiotics don't have major beneficial impacts on growth rate, performance level, immune responses and improvement of intestinal microbiota of the weaned lambs. To achieve these effects a long term use of these probiotics required but the supplementation of compound probiotics have produced significant improvements of daily weight gain, decreased fecal score and decreased oocysts of coccidia in the fecal sample of the infected weaned lambs with coccidia.

***L. plantarum* and *B. toyonensis* Probiotics**

After the birth, the first few days of the pre-weaned calves are very significant as they are at high risk of mortality and morbidity (Mee, 2013; Jiang et al., 2020; Hordofa et al., 2021). This is because neonatal calves have an immature immune system and antioxidant systems are also not fully functional so these neonates have a low resistance against infections and diseases. At that time of their life, calves are at high risk of getting respiratory and intestinal infections which affect their growth rate and overall health (Chester-Jones et al., 2017). *Lactobacillus plantarum* has a good reputation due to its characteristics ability to colonize the gastric cells and play the role of beneficial bacteria in the intestine (Le and Yang, 2018), it is known to be present in several fermented feed products including different types of silages (Busconi et al., 2008; Goel et al., 2020). The beneficial effects of *L. plantarum* are quoted as promote digestibility of nutrients, enhance immune responses, and act as a barrier to hinder the colonization of pathogenic agents in the intestine in different species of animal (Wang et al., 2018; Ding et al., 2019). The characteristics qualities of *L. plantarum* which made it a perfect probiotic supplement to be used in pre-weaned calves include robustness, ability to show resistance against bile and acids and its ability to produce antimicrobial compounds (Ahire et al., 2021). *Bacillus* species have been known to be used as probiotics because of their unique ability to produce endospores. These endospores have the potential to tolerate the adverse environmental conditions that made them able to stay in the gastrointestinal tract (Casula and Cutting, 2002). A strain of *Bacillus cereus* known as *Bacillus toyonensis* is naturally present and used as a probiotic due to its non-toxigenic and non-pathogenic characteristics. It is used as a safe feed additive as there are no adverse side effects of this probiotics have been reported on different animal species (EFSA, 2014). The probiotic properties of *B. toyonensis* include development of antimicrobial compounds, enhanced immunity responses and improved gut health (Abd El-Hack et al., 2020; Pechrkong et al., 2023).

Alternative Control Measures for Coccidiosis

Plant-based Products

Natural products usually extracted from plants are the most reliable agents to control and prevent infections. Currently, a lot of research studies reported that plant based products are best to use against coccidial infections. Several plant species are known to be used as an alternative of drugs, used for the production of many drugs such as ionophores and other synthetic compounds. Some phyto compounds are identified with antimicrobial property and used to prevent and control *Eimeria* infections in ruminants (Nahed et al., 2022). Natural products are relevant to use against many infections due to the low efficacy of many synthetic drugs. These products are also used as additives with other classic anticoccidial drugs to enhance the effect of drugs and to achieve long term control of coccidial infections. There is no relevant data about the emergence of parasitic resistance against these natural products however the risk may exist (Quiroz-Castañeda and Dantán-González, 2015). The efficacy of plant based products is considered to be no more than those of licensed anticoccidial drugs (Peek and Landman, 2011).

Herbs or Spices

Herbs and spices such as rosemary, thyme, garlic, oregano, turmeric, peppermint and basil belong to aromatic plants used to enhance flavor and aroma. These aromatic plants consist of phenolic compounds which exhibit antioxidants properties such as thymol (Franz et al., 2010). These compounds are known to protect their host for free radical induced oxidative stress (Madsen and Bertelsen, 1995; Couladis et al., 2003). Turmeric, an aromatic plant, contain curcumin which shows anti-*Eimeria* properties. The lambs infected with *Eimeria* give feed supplemented with turmeric shows decrease weight loss, low output score of oocysts, reduction in inflammation and oxidative stress (Cervantes-Valencia et al., 2016). The essential oils of rosemary are reported to prevent the sporulation of *Eimeria* oocysts in sheep (Figure 2) (Aouadi et al., 2021).

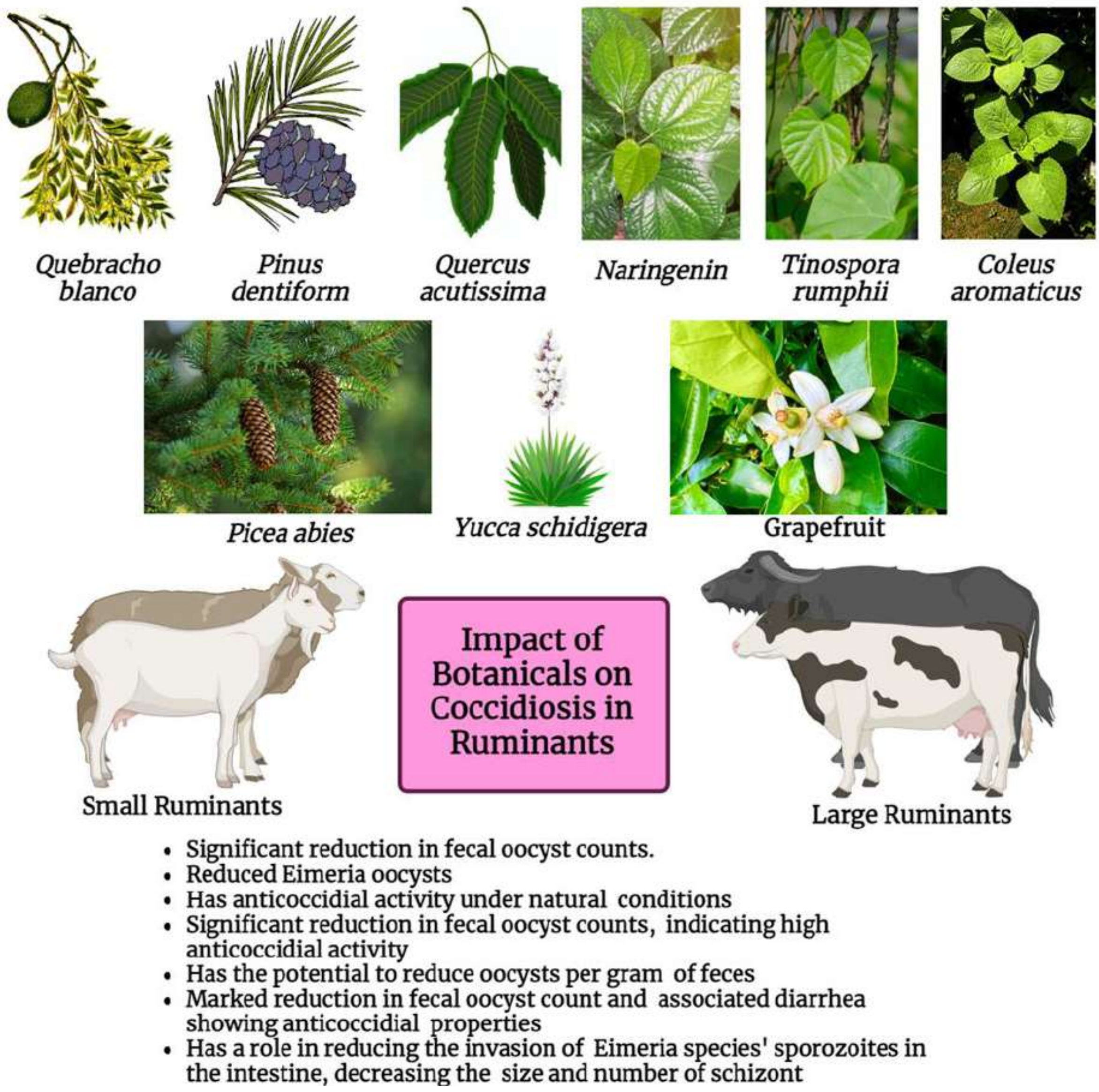

Fig. 2: Impact of different plants on ruminant coccidiosis.

Trace Elements

Trace elements for example zinc, copper, and selenium play vital roles in maintaining the immune system, energy production levels and other physiological functions of animals. Trace elements act as dietary antioxidants and safeguard the ruminants from parasitic infections (A Puertollano et al., 2011). It has been reported in a study that a mineral mixture of zinc, copper, selenium and manganese decrease the excretion of *Eimeria* oocysts in sheep but have no effect on the occurrence of diarrhea (Cazarotto et al., 2018).

Essential Oils and Vitamins

Essential oils are extracted from different parts of plants through different methods. These essential oils and vitamins are known to exhibit several potential properties including immunomodulation, antifungal properties and antioxidant activities (Iordache et al., 2023). Due to these properties These oils and vitamins are used against coccidiosis (Youssefi et al., 2023). Several studies show that essential oils and vitamins are very effective in preventing coccidiosis (Saeed et al., 2023).

Saponins

Saponins are naturally occurring compounds present in several parts of the plants including seeds, roots, fruit, bark, tube, and leaves (Subiono and Tavip, 2023). Attributed to their foam-forming property, they are called saponins just like soap in aqueous solution (Rai et al., 2021). There are several groups of saponins such as glycosylated steroids and triterpenoids (Li et al., 2023). The mode of action of saponins is polarization of cell membrane and vacuolization, through these mechanisms saponins fight against *Eimeria* (Saladino et al., 2022). *Eimeria* are double membrane structures, almost impossible to destroy by the saponins but through the micropyle cap, saponins entering the oocyst wall of *Eimeria* cause serious destruction of sporocyst (Rizwan et al., 2021). Saponins directly bind with the ruminal protozoa and destroy them. Another major function of saponins is the vacuolization in the endoplasmic reticulum of protozoa during all the developmental stages. They cause the disruption of the cell division process of protozoa and mitochondrial activities (Peng et al., 2021). Several plants containing saponins are known to have anticoccidial properties (Trotta et al., 2023).

Flavonoids

Flavonoids are naturally occurring phenolic compounds extracted from plants (Chen et al., 2021). Flavonoids are widely used for their antioxidant properties present in various plant parts including fruit, vegetables and flowers or in whole plants such as *Mangifera indica* (Shen et al., 2022). There are many beneficial effects of these compounds due to which they are widely used in the pharmaceutical and nutraceutical industry (Ayala-Fuentes and Chavez-Santoscoy, 2021). The antioxidant properties of flavonoids (Ashfaq et al., 2021) and there work to prevent damage due to oxygen reactive species (Thenmozhi et al., 2023) made them of great importance to be used against *Eimeria* species (Mounir et al., 2022). Due to these benefits, plants containing flavonoids are widely used against coccidiosis in ruminants (El-Ghareeb et al., 2023).

Tannins

Tannins belong to phenolic compounds and are generally found in seed coat and foliage of plants such as sorghum (Galgano et al., 2021). They are widely used against *Eimeria* in ruminants due to their antiparasitic activities (Choi et al., 2022; Kumar et al., 2022). The mode of action of tannins is that they enter the inner wall of oocysts of *Eimeria* and disrupt the cellular functions (Saladino et al., 2022). They also interfere with the cell components causing thickening of walls of oocysts (Hur et al., 2005).

Conclusion

The use of botanicals, herbs, spices and probiotics has become the need of the livestock industry. Various parts of plant or whole plant, probiotics, herbs and spices have been used for the treatment of coccidiosis in ruminants. These alternatives not only present best treatment options but also are very economical so used widely in the livestock industry. These herbal products have many beneficial properties such as antimicrobial activities, antioxidant properties, immunomodulation, anti-inflammatory and anti-parasitic activities. The basic mechanics of action of these botanical products are still in debate and research is required to find out the exact mode of action of herbal products. The use of the therapeutic nature of probiotics helps to control the infestation of parasites in ruminants and by further understanding of the mode of action of these probiotics led to the development of new ways to fight against these pathogenic agents. In future, herbal products, probiotics and prebiotics would become a powerful tool to treat and control many pathogenic infections including Coccidiosis in ruminants.

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Chapter 45

Prebiotics, Probiotics and the Future of Digestive Wellbeing

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ABSTRACT

The increasing number of studies emphasizing the importance of digestion for overall well-being suggests that this is widely recognized. The diverse community of bacteria, viruses, and fungi that make up the gut microbiome plays a crucial role in immune system development, nutrient absorption, and digestion. Maintaining a healthy gut microbiome requires a delicate balance between harmful and beneficial bacteria in the intestines, highlighting the complexity of gut function and its impact on overall health. Lactobacillus and bifidobacterium species are found in fermented foods and supplements. Probiotics, live bacteria with health benefits, offer various advantages such as improved digestion, enhanced immune response, and reduced gastrointestinal disorders. Prebiotics indigestible fibers present in certain foods are essential for the growth and activity of probiotic microorganisms and the maintenance of a diverse and healthy gut microbiota. Probiotics and prebiotics work together to strengthen the intestinal barrier, reduce inflammation, and boost immunity, resulting in a synergistic effect. Future trends in digestive health include precision therapeutics tailored to individual microbiomes, microbiome diagnostics for argeted treatment and holistic approaches that consider the interplay between diet, lifestyle, and gut health. The field of microbiome research is advancing rapidly with next-generation sequencing and microbiome engineering, offering the potential for more precise and comprehensive strategies to improve gut health and overall well-being, as well as personalized treatments and a greater understanding of the gut microbiome's role in both good and poor health.

KEYWORDS		
Digestive well-being, Probiotics, Prebiotics, Immune response, Gut health	Received: 04-Jun-2024 Revised: 22-Jul-2024 Accepted: 13-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Digestive Wellness

Overview

Digestive wellness is a key component of overall health, with a focus on proactive approaches to antiaging, optimal well-being, and prevention, (Cummings et al., 2004), highlighting the importance of functional testing in addressing metabolic and hepatic function. Technological advancements, such as an ontology-based herb expert system, have been developed to treat digestive diseases and improve public health.

Understanding the Gut Microbiome

The gut microbiome is an essential part of maintenance of an individual's health being a complex population of bacterial, viral and fungal microorganisms residing in the gastrointestinal tract (Alonso and Guarner, 2013). It has been reported to affect digestion, absorption of nutrients and the immune system (Cummings et al., 2004), recent evidence shows its relation to mood and other health disorders (Rogers et al., 2010). The intestinal microorganisms should not be in a state of imbalance and should be balanced and the diet and other ways of functioning also have their impacts. It is thus crucial to comprehend the dynamics of this internal environment so that we can learn how to make the proper choices that will promote health and wellbeing of a person (Redondo-Useros et al., 2020).

Microbial Diversity in the Gut

Gut form and function is absolutely obviously associated with the gut microbial community structure (Panse, 2023).

The diverse diversity of bacteria and other microorganism that is resident in our gut is known to serve a vital responsibility of boosting the immune system, facilitating absorption of nutrients as well as digestion (Bengmark, 2013). Optimised health is linked to a resilient microbiome, which re-emphasises pillars of health such as high fibre diversity dietary habits (Hills et al., 2019)

Importance of Maintaining a Healthy Gut

Studies have enlightened the complex architecture of the gut with a focus on the role of tight junctions in maintaining barrier function (Vancamelbeke and Vermeir, 2017). Nutrition and gut health is now the subject of many articles, emphasizing a strong connection between the choice of food and the possibility to maintain the health of gut bacteria (Power et al., 2014). There is a growing trend in the scientific literature toward identifying "gut health" as specific medical outcome for patients (Baty et al., 2014). Extensive studies explore the interplay between gut microbiota, healthcare, and health outcomes, highlighting the key features that support a healthy gut ecosystem beneficial to health, as depicted in (Figure 1) (Rowland et al., 2018). Additionally, the impact of maintaining a balanced gut microbiota on the balance between health and disease is elucidated, underscoring the importance of incorporating probiotics and prebiotics into one's diet (Ballan et al., 2020).

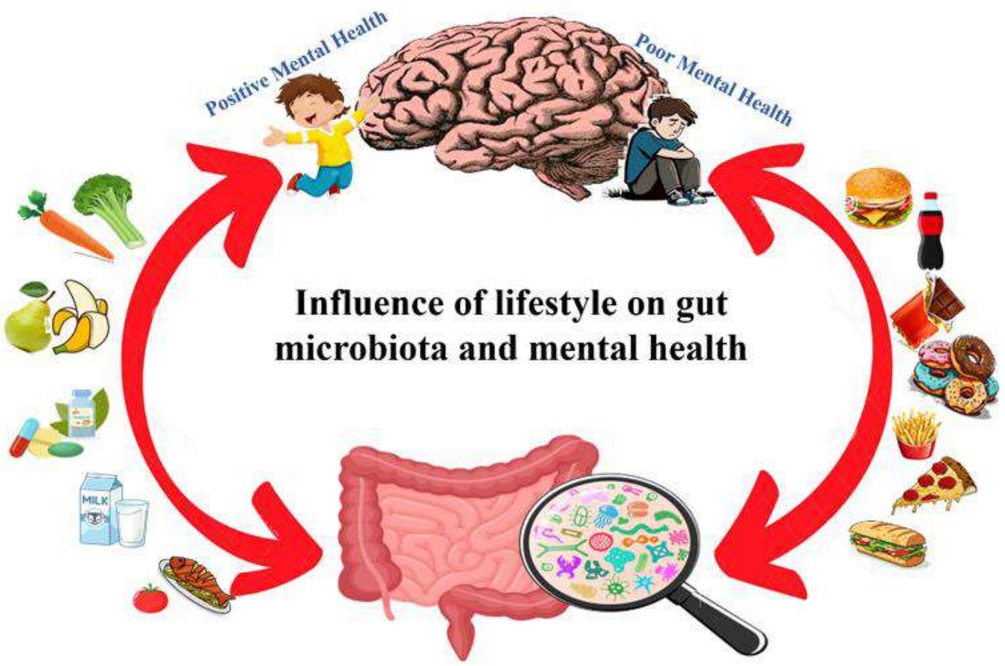

Fig. 1: Shows the Influence of lifestyle on Gut microbiota and Mental health.

The Impact of Gut Microbiome on Overall Health

Research suggests that the gut microbiota plays a crucial role in regulating health and disease by influencing metabolism and the overall well-being of the host (Fan and Pedersen, 2021). Evidence supporting the importance of early-life microbial colonization of the gut in immune balance further underscores the significance of the human gut microbiota (Martin et al., 2010). The relationship between food components, dietary habits, and healthy gut microbiota is explored, emphasizing the interconnectedness that affects bioavailability (Vernocchi et al., 2020). The extensive body of research underscores the intricate connections between gut health, microbial equilibrium, nutrition, and general health, as depicted in (Fig. 2), underscoring the importance of maintaining gut function for optimal health outcomes (Neish, 2009).

Probiotics

The detailed examination of probiotics focuses on their historical importance, particularly as lactic acid bacteria lactobacilli and bifidobacteria that influence gut health (Selle and Klaenhammer, 2013). The research underscores the significance of ensuring both safety and efficacy while showcasing the numerous applications of probiotics in healthcare and food (Sanders et al., 2010). Fig. 3 displays a common list of probiotics. An enormous amount of time is spent reviewing the safety aspects and a very elaborate analysis is made of the safety aspects in more details outlining the toxicity, pathogenicity, and infection risks (Anadón et al., 2021). This stress the importance of using the probiotics in offsetting the impacts of antibiotic treatment and expounds on the evolutionary history of the later (Santacroce et al., 2019).

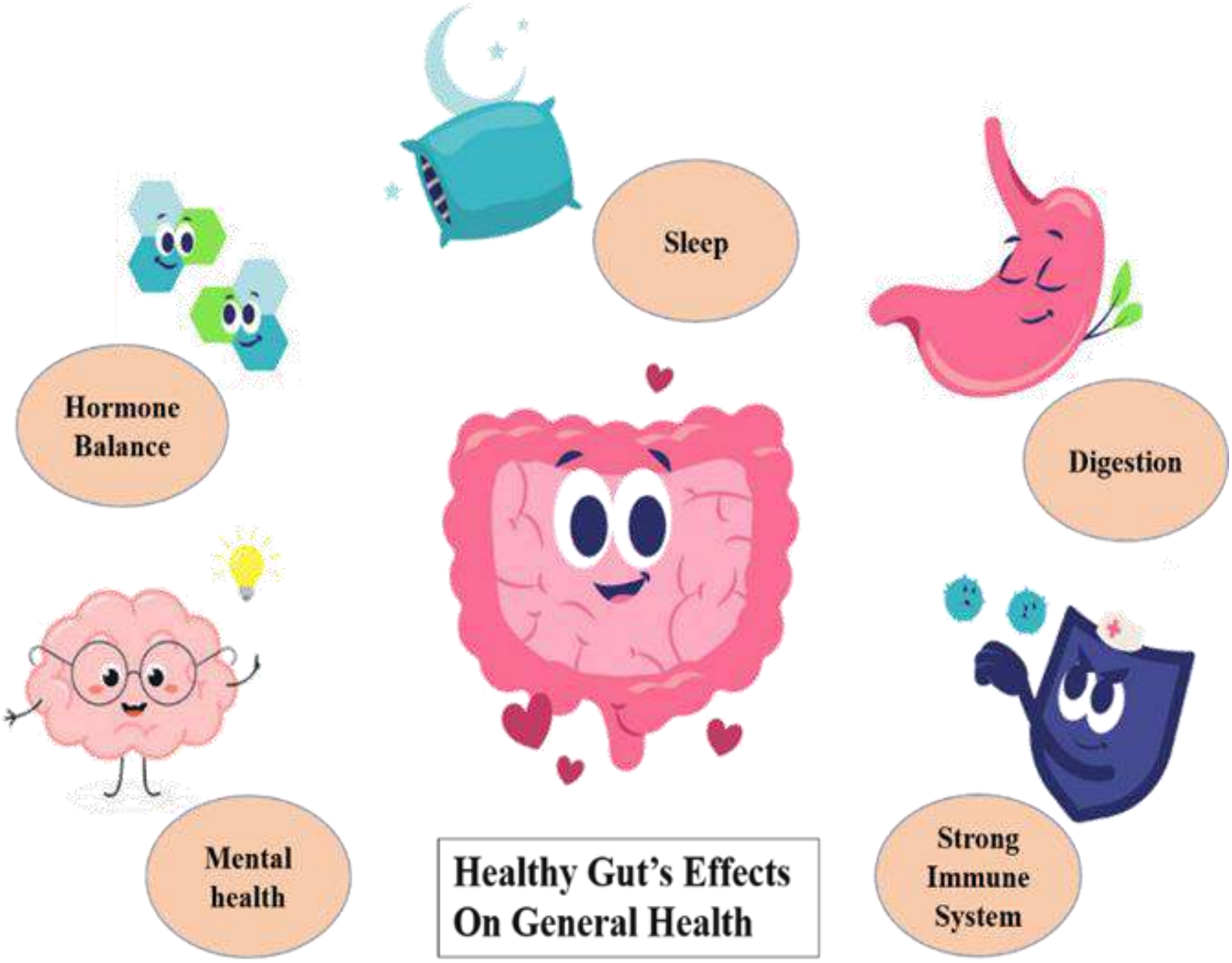

Fig. 2: Shows Healthy Gut's effects on General Health of a Person.

Live Microorganisms with Health Benefits

For wellbeing include probiotics or other live helpful microorganisms that have positive impacts on wellbeing (Mishra and Acharya, 2021). Especially, Lactobacillus and Bifidobacterium help in metabolism and nutrient absorption and maintain the balance of essential microorganisms in the gut (Reyed, 2007). There are several benefits associated with the use of probiotics among them is enhancement of immune functions and decreased cases of gastrointestinal complications (Wang et al., 2021). These microorganisms that exist in supplements and fermented foods such as yogurts, sauerkraut, and kefir, support overall well-being by maintaining interaction between the constituents of the complex human microbiome (Almutairi, 2016).

Saccharomyces boulardii Also known as Probiotic yeast used widely because it can survive in stomach Acids. It helps to enhance the stability of the intestinal barrier, suppress the proliferative activity of undesirable bacteria along with the regulation of beneficial bacteria, which are important for the balance of a healthy gut. Kellogg's brand supplement ingredient base Saccharomyces boulardii as an efficient cure for gastrointestinal problems like diarrhea, hence implying it as a valuable addition to the gut (Sen and Mansell, 2020).

Lactobacillus acidophilus is probiotic bacteria that are usually used in yogurt and other healthy foods supplements. Immunomodulation, lactose breakdown, nutrient assimilation and, in general, gut health is supported by it (Behnsen et al., 2013).

Types of Probiotics

Various types of probiotics offer unique health benefits (Behnsen, et al., 2013). Lactobacillus, Bifidobacterium, and other strains are commonly studied, with recent research yielding over 67,900 results (Iyer et al., 2023).

Lactobacillus

Extensive research has focused on the probiotic properties of lactobacillus strains, renowned for their diverse applications (Zhang et al., 2018). These strains are selected and named based on their distinct characteristics, playing a crucial role in digestive health (Patrick et al., 2007). The investigation looks at their impact on bacterial translocation and injury to the liver (Adawi et al., 2001).

Fig. 3: Shows a list of Probiotics found naturally in food.

Bifidobacterium

The second most common probiotic component is bifidobacteria strains, which have many positive characteristics described in scientific literature (Arboleya et al.: 2011). Identification of antibiotic profiles shows unique information for therapeutic uses (Scavizzi et al., 2002).

Sources of Probiotics

Probiotic supplements are a convenient balanced and healthy gut microbiome (Sanders, 2008).

Fermented Foods

Yogurt, kefir, sauerkraut, and kimchi are some examples.
Contains abundant live bacteria that promote good digestion (Bifidobacterium, Lactobacillus).

Dairy Products

Buttermilk and certain cheeses are included.
Probiotics that assist intestinal homeostasis are present.

Non-Dairy Options

Kombucha, a fermented beverage, is one such example.

Provides Probiotics for those Unable to Consume Dairy

Probiotic Supplements

Available in powders and pills. A simple solution for anyone seeking to enhance their gut health.

Mechanism of Action of Probiotics

Maintaining a balanced gut microbial environment is essential for overall health. Probiotics operate in various ways to deliver their beneficial effects (Hemaiswarya et al., 2013). Figure 4 illustrates these selection criteria for probiotics as follows:

1. **Microbiota Balance:** Inhibiting probiotics help maintaining a balanced microbiota in the digestive tract by inhibiting the growth of harmful bacteria and promoting a diverse range of beneficial bacteria (Butel, 2014).
2. Intestinal Barrier Support: Probiotics enhance the integrity of the intestinal barrier, reducing the risk of inflammation and, infections and acting as a defense against pathogens (Boirivant and Strober, 2007).
3. **Production of Bioactive Compounds:** Probiotics synthesize bioactive compounds like short-chain fatty acids that support various physiological processes and overall health (Indira et al., 2019).
4. **Immune System Regulation:** Probiotics modulate the immune system by influencing cytokine production and promoting a balanced immune response through interactions with immune cells (Sherman and Ossa, 2009).
5. **Overall Well-Beingng:** By strengthening immunity, aiding in digestion, and promoting general well-being. probiotics have a wide range of positive effects on human health (Walker, 2008).

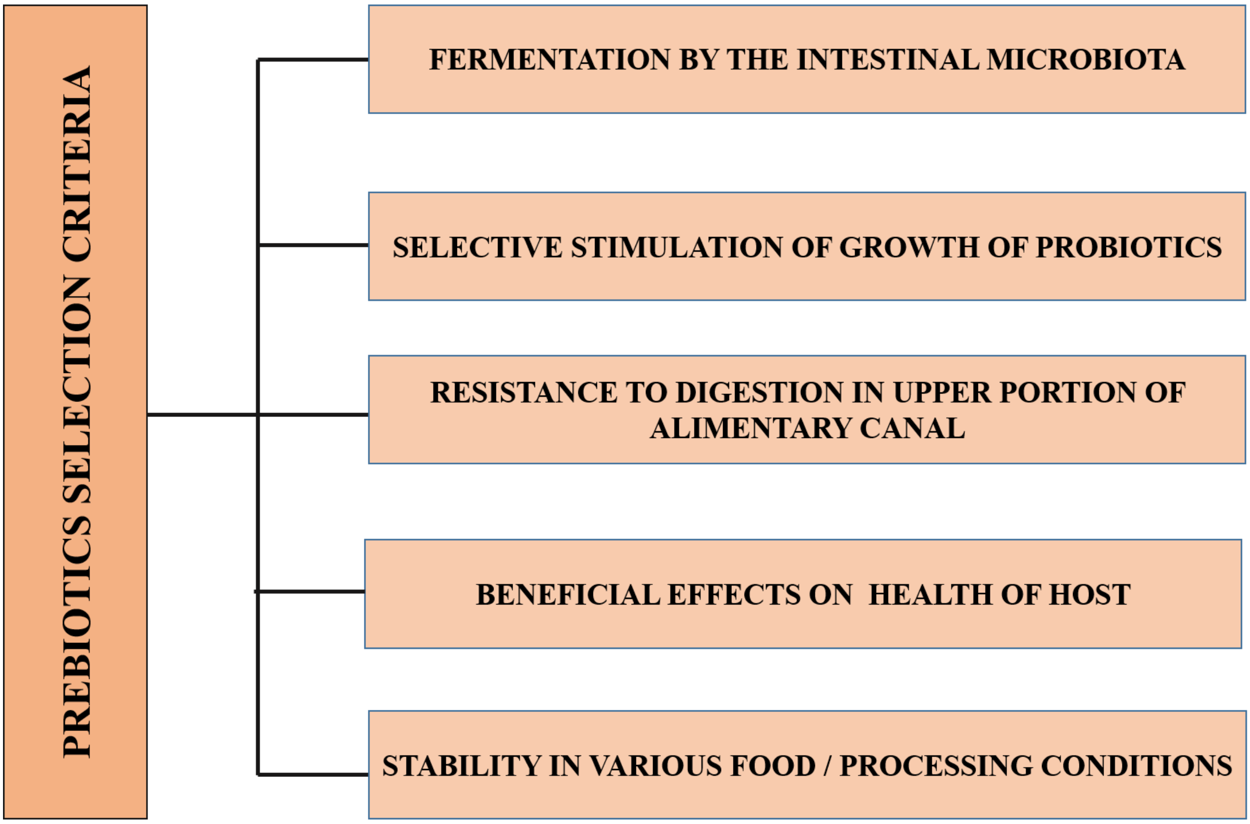

Fig. 4: Shows the Probiotics selection criteria.

Prebiotics

Prebiotics are essential non-digestible fibers present in certain foods that serve asimportant nourishment for the beneficial bacteria residing in the gut (Mohanty et al., 2018). Figure 5 provides a list of naturally occurring prebiotics. Unlike probiotics, which are live microorganisms, prebiotics, act as stimulants that promote the growth and function of these beneficial bacteria, thereby contributing to a healthy and balanced gut microbiome (Nagpal et al., 2013).

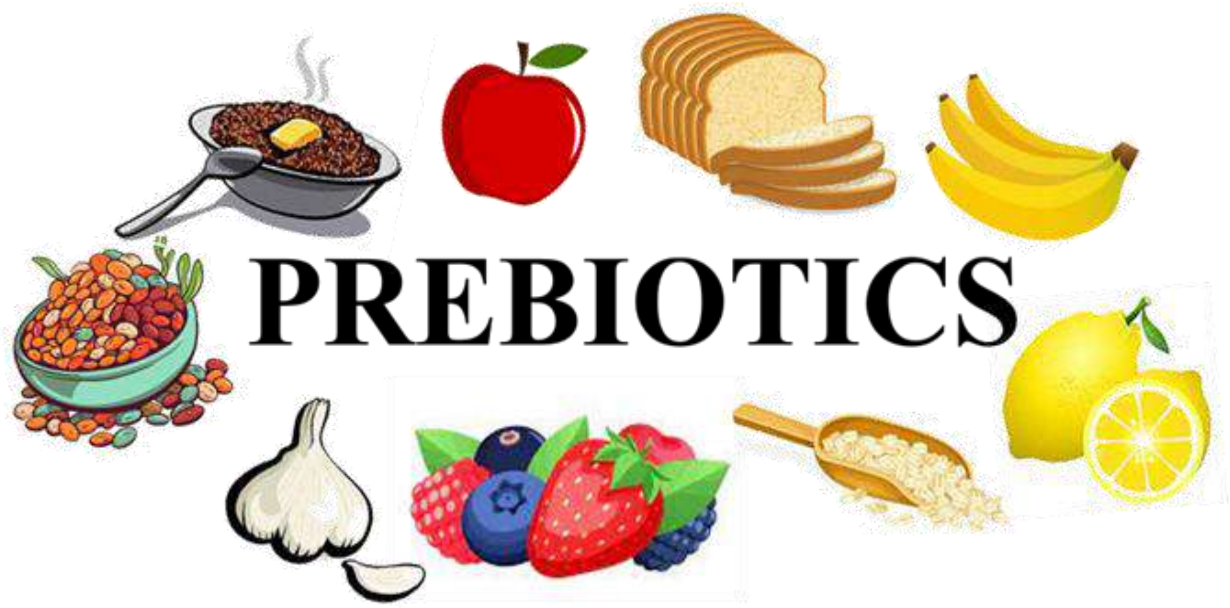

Fig. 5: Shows the list of Prebiotics found naturally in food.

Prebiotics Explained

Prebiotics, derived from fibrous fruits, vegetables, and whole grains, remain intact as they pass through the small intestine and reach the colon without being broken down (Appanna and Appanna, 2018). They serve as a nourishing source for probiotic bacteria in the colon, aiding in their growth and enhancing their beneficial effects (Sekhon and Jairath, 2010).

Types of Prebiotics

The main types of prebiotics include insulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), and resistant starch. Insulin is found in onion, garlic, and chicory root, promoting gut health. FOS, Rich in leeks, asparagus, and bananas, encourages the growth of beneficial microorganisms. GOS, found in dairy products and legumes, supports the growth of probiotics. Resistant starch, present in seeds, whole grains, and green bananas, bypasses digestion to reach the colon and nourish good bacteria.

Prebiotic Sources

A variety of foods naturally contain prebiotics (Al-Sheraji et al., 2013). Here are some common sources of prebiotics:

Chicory Root: Onions and Garlic

- Rich in inulin, which promotes the growth of beneficial bacteria.

Bananas

- Contain fructooligosaccharides (FOS), that support probiotic growth.
- **CELERY AND ONIONS:** High in fructose, which helps cultivate a healthy gut flora.
- **Lentils and Legumes:** Provide galactooligosaccharides (GOS) to support probiotic growth.
- **Whole Grains:** resistant starch, which is unbroken when it enters the colon and feeds good bacteria.

Seeds

- Contains resistant starch, supporting a diverse gut microbiota.

The Role of Prebiotics in Promoting Gut Health

Prebiotics play a crucial role in supporting intestinal well-being by providing nourishment to beneficial bacteria in the gut (Tuohy et al., 2003). Found in foods like chicory root, garlic, bananas, and whole grains, these non-digestible fibers reach the colon intact and serve as vital source for the growth and activity of probiotic microorganisms (Subhashree, 2018). Prebiotics stimulate the proliferation of these beneficial bacteria, aiding in the maintenance of a diverse and balanced gut microbiota (Peng et al., 2020). This symbiotic relationship not only enhances nutrient absorption but also reduces inflammation, strengthens the gut barrier, and enhances overall immune function (Sehrawat et al., 2021). Including prebiotic-rich foods in your diet can help support a healthy gut environment, leading to improved digestion and overall well-being (Ballan et al., 2020).

Interaction between Probiotics and Prebiotics

Probiotics, being live beneficial microorganisms, rely on prebiotics for their survival. Prebiotics, present in various foods, provide probiotics with an undigested energy source, supporting their growth and function in the gut (Zoumpopoulou et al., 2018). This combined action strengthens the intestinal barrier, reduces inflammation, and enhances overall immunity, all of which contribute to maintaining a diverse and healthy microbiome (Allaire et al., 2018). The synergetic effect of probiotics and prebiotics creates a powerful environment for optimal gut health and overall well-being, going beyond individual benefits (Bandyopadhyay and Mandal, 2014).

Definition and Importance of Digestive Wellness

Researchers acknowledge that the idea of a "normal flora" in the digestive system can vary depending on the diet and location (Aimutis and Polzin, 2011). Studies examining the connection between digestive health and overall well-being highlight the importance of digestive health in (Figure 6) (Neish, 2009). In essence, the understanding and significance of digestive wellness have expanded beyond mere substance to encompass enjoyment, entertainment, and all facets of life, emphasizing its comprehensive impact on human health (Prescott and Logan, 2016).

Importance of Probiotics and Prebiotics

The vast number of current publications, approximately 74,200, focusing on probiotics and prebiotics highlights the significance of these supplements in promoting health and well-being (Reid, 2008). Probiotics have been proven to offer substantial health benefits, particularly those derived from dairy products like milk (Probiotics and prebiotics–progress and challenges) (Mattila-Sandholm et al., 2002). These natural compounds play a crucial role in various aspects of health, including the management of clinical conditions and disease prevention (Dillard and German, 2000).

The term "nutritional benefits of probiotics and prebiotics" underscores the advantages of these microorganisms in preventing and treating specific ailments (Marteau and Boutron-Ruault, 2002; Morais and Jacob, 2006). Probiotics and prebiotics are accepted in the scientific world as having crucial functions in preserving immune homeostasis, intestinal barrier function, and health in general (Sanders et al., 2019). These findings stress on the dietary options containing probiotics as well as prebiotics to improve health effectiveness (Roberfroid et al., 2010).

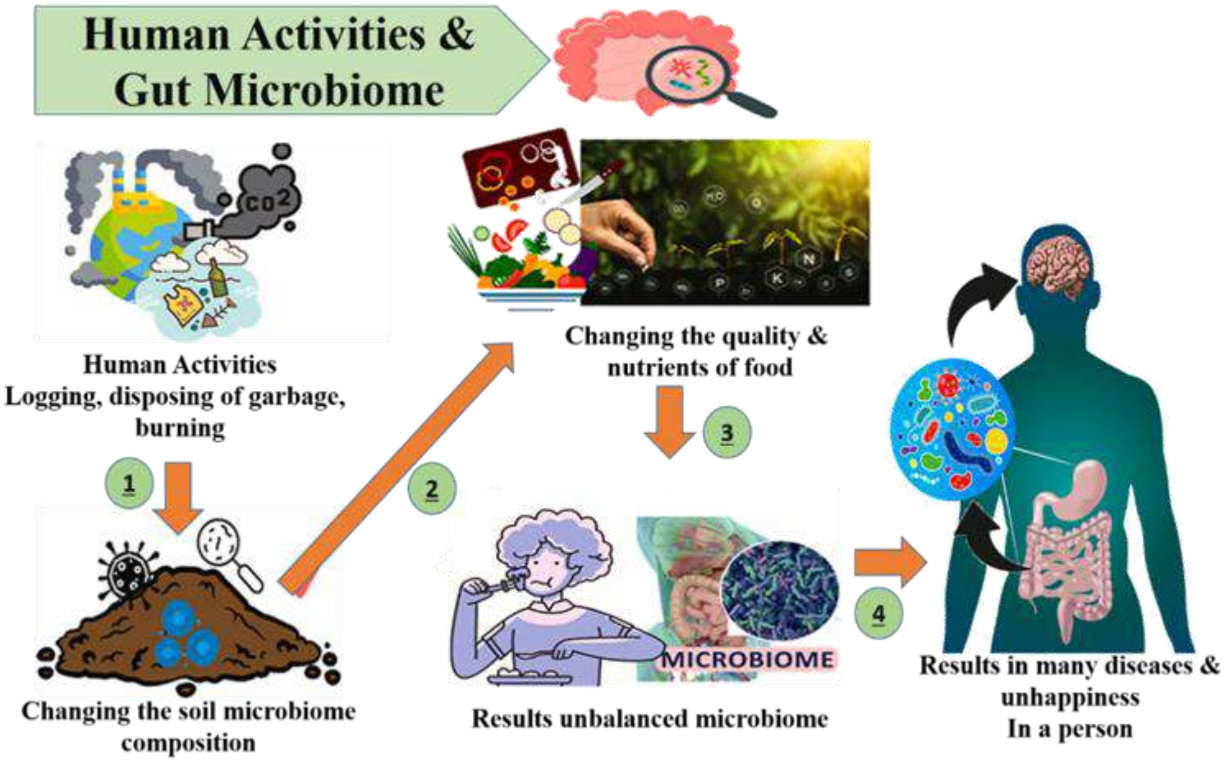

Fig. 6: Shows Importance of gut health in human life

The Future of Digestive Health

Digestive health is set to have a brighter future since researchers and techno-logical developments in the future are ever likely to unveil the workings of the gut-brain axis and the microbiota (Hyland and Stanton, 2023).

Recent Advances in Microbiome Research

These insights will also continue to drive future discovery within the field of microbiome, particularly gut health. Studying the gut-brain connection and the microbiome make it possible to offer more specific treatments (Lau et al., 2021).

Precision Probiotics and Prebiotics

Precision therapies can be seen to have a future in the future as research enters the identification of efficacy of probiotics and prebiotics (Zommiti et al., 2020). Some attempts to improve gut microbiota might include individualised prebiotics, particular probiotics, and dietary interventions (Vandeputte, 2020).

Microbiome Diagnosis for Initial Treatment

Such direct striving for the accurate microbiome testing might dramatically change the treatment of the abnormalities of the gastrointestinal tracts (Malla et al., 2019). When using modern diagnostic techniques, often the anomalies or diseases could be promptly diagnosed in order to treat the specific diseases (Kumari et al., 2023).

Holistic Strategies for Gut Health

Now, the versatility in employing strategic models that integrate the correlation between lifestyle, gut and nutrition is said to contribute towards future growth of the market. Integrative approaches are expected to have a relatively large impact on the value of general and gut health (Sudhakar et al., 2022). In other words, the field of digestive wellness is still growing and availing more potentialized, the most precise, and the most holistic solutions, allowing a person to obtain powerful resources for the betterment of his or her gut (Baty et al., 2014).

Future Trends in Digestive Wellness

As it pertains to the digestive wellness, there has been a significant change of dynamism in the nutritional and health modicum of the current years (Birch, and Bonwick, 2019). The findings of the current and past studies plus the literature indicate that, foods for digestive wellbeing, including fermented foods, natural fibers, and probiotics are increasingly popular (Melini et al., 2019). On this front the industry is pondering ways on how to come up with more than standard digestive products through exploitation of trends (Tudoran et al., 2012). The colon is getting a lot of focus now a day because it plays a primary role in the health and disease prevention and now has taken a paradigm shift in biological medicine (Reid et al., 2003).

In addition, the effect of metabolizable proteins on cardiovascular health is also under investigation, and future developments of nutrient – diets and dietary supplements today and in the future are also considered (Phillips, 2017). Regarding probiotics and prebiotics, they have got much attention in recent years, and now the scientists are focusing on the impact of probiotics on the gut and the connection of particular additives to other overall health categories (Sharifi-Rad et al., 2020). Innovation of products with more plant proteins is a major strategy that food companies follow to meet consumer demands for improving health and a cleaner environment (Batista et al., 2023). Marketing and wellness are related whereby a common focus is made on digestive health products (Bublitz et al., 2013). The consumers in focus are young people to aspire to be sustainably functional foods' generation in 2024 and are always willing to receive health-bearing foods (Frank et al., 2024).

What opportunities and risks functional and medicinal beverages represent are being unveiled as the market emerges, focusing on the opportunities provided by those drinks for the support and optimization of digestion (Nazir et al., 2019). As there are continuous researches on dairy fermentation, Lactobacillus helveticus can be identified as a possible bacterium of future starter cultures (Ayivi and Ibrahim, 2022). More broadly, the nexus of research publications, consumers' choices, and industrial developments will further contribute to the evolution of the digestive health market in the future (Bigliardi and Galati, 2013).

Animal Microbiome Advancements

Modern advances in DNA sequencing have drawn curiosity into the subjective nature of animal associated microbiome in these animals hence enabling new prospect for veterinary medicine and animal health (Gilbert et al., 2016).

Microbiome Therapeutic Potential

It fact, the microbiome has been defined as another therapeutic target in which there is an attempt to find new ways to modify the microbiome for different treatments, especially the gut microbiome (Sorbara and Pamer, 2022).

Inhanced Clinical Investigations Through Next-Gen Sequencing

New-generation sequencing has greatly advanced the knowledge of human microbiome paving way to more medical analysis and further improved human-oriented treatment plans (Gebrayel and Nicco, 2022).

1. Heralded by recombinant technology in microbiome therapeutics, microbiome therapeutics is rapidly developing individualized treatments and processes that unlock the full potential of microbiome (Bober et al., 2018).

Advancements in Microbiome Therapeutics

The use of recombinant technology in microbiome therapeutics is paving the way for targeted treatments and processes that harness the full potential of the microbiome (Bober et al., 2018).

Revolutionizing Gut Microbiome Studies

Recent molecular technologies have characterized the mechanisms and areas of potential interaction between gut microbes and their host organism (Carr et al., 2013).

Technological Innovations in Urobiome Research

Technological development today is offering fresh ideas into the urinary system microbiome and its placement within urology control increasing the academic understanding of the urobiome (Porto et al., 2023).

Microbiome Engineering

Microbiome engineering has been identified as a promising future area of development and further technological development and application of microbial manipulation for several purposes (Cullen et al., 2020).

Culture Based Gut Microbiota Research

This has been very useful especially in developing culture-based approaches towards to gut microbiome as a way of fostering the culture and social aspects of the microbial ecology and the effects on human health (Milani et al., 2017).

Impact on Pediatric Health

Recent developments in the field of DNA sequencing have also provided a better appreciation of the human gut microbiome with practical implications to pediatric care and the understanding of the Microbiome and pediatric diseases (Saulnier et al., 2013).

Conclusion

The future of digestive health seems to be bright because of the growing interest in microbiome technology, tailored treatments and individuals' health management. The complementary interaction that exists between prebiotics and probiotics makes them indispensable in the modulation and regulation of the gut symbionts that are very central to one's wellbeing. From new efficient technologies to unique treatment approaches and dietary interventions, digest health is bound to change dramatically providing novel opportunities for prophylaxis and cure. Thus, as the knowledge increases it will be possible to introduce changes in lifestyle, menu and individualized therapies that will help people to improve their gut function and, consequently, the quality and duration of their life.

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