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GUT HEALTH MICROBIOTA & ANIMAL DISEASES

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Gut Heath, Microbiota and Animal Diseases

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PREFACE

The book Gut Health, Microbes, and Animal Diseases examines gut health, microbes, and animal diseases in livestock and poultry. The book mainly covers the use of probiotics and prebiotics in intestinal diseases, the effect of probiotics on the animal production cycle, the optimization of new feed additives, the effectiveness of probiotics in the control of parasites, the relationship between *Toxoplasma gondii* and intestinal flora, and the pathogenesis of *Escherichia coli* in piglets. In recent years, the scientific community has made impressive progress in gut health and microbial communities and their impact on animal diseases. The gut is not only a site of food digestion but also a complex ecosystem in which the role of microorganisms is crucial. The microbial community's balance and dysbiosis directly affect the host's health and especially play a key role in the development and progression of animal diseases. This book aims to unravel the mysteries of this complex system by synthesizing the latest research findings and providing insights into the structure and function of the gut microbiota and how they affect the health status of animals. The book focuses on the effects of probiotics and other beneficial ingredients on the microbiota and discusses the relationship between microbial imbalances and animal diseases and how gut health can be maintained through feed additions and other interventions.

With the progress of modernization of agriculture, livestock, and poultry farming is becoming more and more prominent as its share in the overall agricultural economy is gradually increasing. However, the intestinal health problems of livestock and poultry are still plaguing the development of the livestock industry. Livestock and poultry intestinal health problems in all months of the year uninterruptedly scattered in the occurrence of a variety of livestock and poultry, its total number of morbidity and mortality than other various diseases, not only to reduce the quality and quantity of livestock and poultry products but also increased feed consumption, which brings a more significant burden on the development of animal husbandry. The application of modern science and technology in livestock and poultry farming has brought great changes to livestock production, which is the specialization and factorization of livestock and poultry farming. The use of prebiotics and probiotics, combined feed additives, and the addition of bioactive ingredients have significantly increased productivity. The intestinal health of animals is also the main issue that we, as veterinarians, are diligently working on to accelerate the modernization of animal husbandry. How to protect the intestinal health of livestock and poultry and the preventive and curative measures to ensure the development of livestock and poultry farming production also deserve universal attention.

Editor

Liu Ping, a member of the Communist Party of China, holds a PhD, is a young professor/associate professor, and a master's supervisor. Executive Director of the Veterinary Surgery Branch of the Chinese Society of Animal Husbandry and Veterinary Medicine, and Director of the Credit Committee for Veterinary Internal Medicine and Clinical Diagnosis and Treatment of the Chinese Society of Animal Husbandry and Veterinary Medicine. Teaching and research work: mainly engaged in teaching veterinary medicine and conducting research and technology promotion and application in the diagnosis, monitoring, and prevention of animal diseases, livestock and poultry nutrition metabolism diseases, disease control and animal reproduction, molecular bioinformatics analysis of diseases, livestock and poultry manure treatment, and environmental control technology research.

Contents

Chapter 01

Recent Trends on Probiotics and Prebiotics in the Gut Health of Animals

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ABSTRACT

In the past two to three decades, efforts to enhance human health have concentrated on developing live microbial supplements, or "probiotics," that can alter the body's natural gut flora. Probiotics constitute a significant and continuously expanding portion of the global functional food market, accounting for around 65% of this enormous industry with a projected value of US$75 billion. Lactic acid bacteria, which include bifidobacteria, enterococci, and lactobacilli, are the most common active ingredients in probiotic products. Probiotics have been linked to a wide range of health benefits, like immune system stimulation, reduction of lactose intolerance, maintenance of normal and healthy intestinal flora, and protection from infections. Probiotics are a group of strains that have been shown to have positive effects and can be found in products in rather large quantities. Furthermore, typically present in the human gastrointestinal tract, bifidobacteria and lactobacilli are thought to be advantageous. They can be promoted by non-digestible food elements such as oligosaccharides, which are referred to as prebiotics. Probiotics and prebiotics can be combined to create a food product known as a synbiotic, which is likely intended to target two "target regions" of GIT.

KEYWORDS
Probiotics, Prebiotics, Synbiotics, Gut disease

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INTRODUCTION

Probiotics were first proposed in 1908 by Nobel Prize winner Eli Metchnikoff, who hypothesized that Bulgarian peasants' long lives were because of the use of fermented milk products. Stillwell and Lilly used the word "probiotic" in 1965 to mention chemicals generated by one organism promoting the growth of another. They were designated as "microbial components of microbial cells that have a beneficial effect on health and well-being" (Gareau et al., 2010).

Numerous microorganisms that are present in the gastrointestinal tract, in the mouth, and on the skin, coincide closely with humans. With a surface area of around 400m^2, the GI tract has the largest concentration of commensal microorganisms. This constitutes the body's second largest surface area, behind the respiratory tract. More than 500 different bacterial species are found in the rich flora of the gastrointestinal tract (GIT), and some of them offer substantial health benefits such as improving the immune system, shielding the host from invasive viruses and bacteria, and facilitating the digestion. The gut microbiota is critical to maintaining human homeostasis, and is acquired quickly after birth, and stays mostly constant throughout life. An individual and distinct intestinal immune system evolves as a result of connections between the developing intestinal microbiota and the host. The task of the host mucosal immune system is to differentiate between benign and infectious species by inducing protective immunity while evading an overabundance of inflammation that could compromise the integrity of the gastrointestinal mucosa. (Tsilingiri and Rescigno 2013).

Among other forms of treatment, the usage of immunosuppressive medication, antibiotics, and radiation may change the composition and impact of the flora. Therefore, recreating the microbial equilibrium and preventing disease may be made easier by the introduction of beneficial bacterial species into the GIT (Marteau et al., 2002).

The Greek word "for life" is where the term "probiotics" originated. The probiotics are "live micro-organisms," which, when given in sufficient proportions, impose health advantage on the host, according to an expert panel that was commissioned by the Food and Agriculture Organization (FAO) and World Health Organization (WHO). The bacterial genera Enterococcus, *Streptococcus, Bifidobacterium, Bacillus, Escherichia*, and *Lactobacillus* are most frequently utilized in the formulations of probiotics. Moreover, a few Saccharomyces fungal strains have also been employed (Di Lena et al., 2015).

The first probiotic, *Lactobacillus rhamnosus* GG (LGG), has gotten the most medicinal interest thus far. Since the *Lactobacillus* strain that dairy companies had previously employed for fermentation could not colonize the gut, *Lactobacillus rhamnosus* strain GG was identified in 1985 through the creation of a list of optimal characteristics for probiotics. It has been demonstrated that *Lactobacillus rhamnosus* strain GG improves gut immunity. Payer's patches experience an increase in antigen uptake due to the increased number of IgA and other immunoglobulin-secreting cells in the intestinal mucosa, localized release of interferons, and facilitated antigen transport to core lymphoid cells (Zendeboodi et al., 2020).

Prebiotics are indigestible food elements that work to improve the health of the host by favourably provoking the development of a specific bacterium or a group of related microbes in the colon. Prebiotics are dietary carbohydrates, such as inulin, gluco-oligosaccharides, and fructo-oligosaccharides circumventing the digestion in the upper gastrointestinal tract and alter the type of substrate that is available to the gut's resident bacteria population. This leads to changes in the bacterial composition of the gut. Probiotics as well as prebiotics are examples of synbiotics. They raise the likelihood that bacteria will survive in the gastrointestinal tract, which increases their beneficial effects (Gupta and Garg 2009).

Selection Criteria of Probiotic Strain

A methodical approach is necessary for the selection of probiotic bacteria, employing a procedure similar to the one illustrated in Fig. 1. Most of the time, a "step-by-step approach" involving a series of tests to gradually narrow down a pool of potential probiotic candidates is necessary due to the high number of isolated strains. The strains exhibiting the greatest number of efficient qualities and, consequently, no unfavorable features are chosen after this process.

- Microorganisms should be capable to communicate or send messages to immune cells linked with the stomach.
- The capacity to endure in the intestine even if the probiotic strain is unable to colonize the stomach
- Should be nonpathogenic
- Resistance to processing

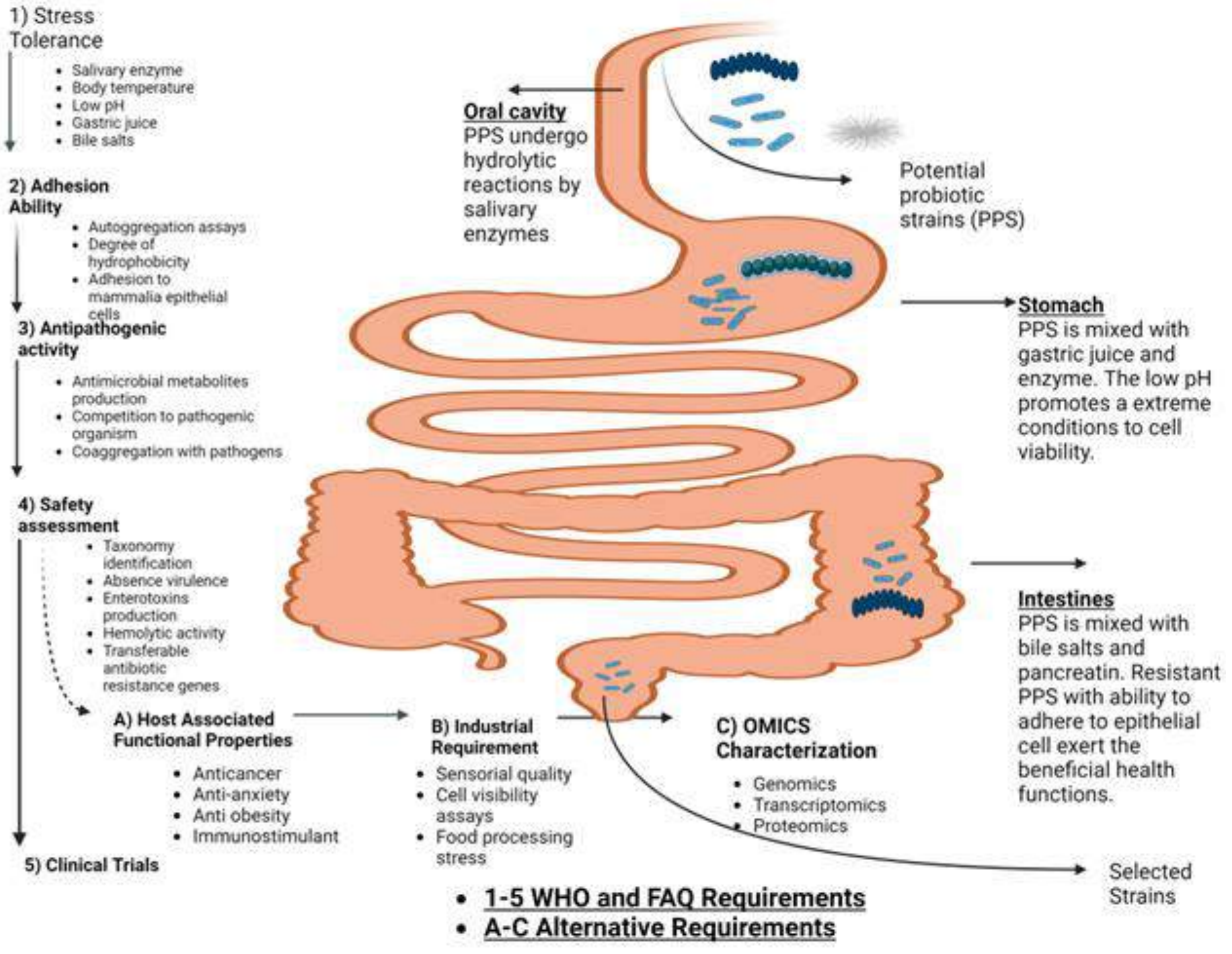

Fig. 1: Depicts the screening methods used for characterizing probiotic strains. According to studies by (Chang et al., 2001; Divya et al., 2012; Fiorda et al., 2016; Luna and Foster, 2015; Maragkoudakis et al., 2006; Ooi and Liong, 2010; Persichetti et al., 2014; WHO/FAO).

Table 1: Micro-organisms used as Probiotics (Jin et al., 2000)

Lactobacillus Spps.	*Bifidobacterium* Spps.	*Streptococcs* Spps.	*Saccharomyces* Spps.	Others
L.paracasei L.plantarum L.reuteri	*B. adolescentis*	*S. thermophiles*	*S. boulardii*	*Bacillus cereus*
L.salivarius L.bulgaricus	*B. infantis*	*S.salivarius subsp thermophilus*		*Escherichia coli*
L. johnsonii	*B. longum*			*Propionibacterium*
	B. lactis			

Mechanism of Action

Probiotics can be categorized according to how the bacteria specifically affect the immune system, allergy disorders, microbial milieu, cancer, intestinal epithelium, and distant mucosal locations.

Antimicrobial Effects on Microflora

Probiotics have long been thought to work by altering the microbiota in the body. A number of studies indicate that eating specific species of lactobacilli can raise endogenous levels of these bacteria and decrease concentrations of E. coli and Bacteroides clostridium in feces. However, the most significant effect is on the flora's metabolic activities, as these bacteria have been shown to reduce the production of substances that cause cancer, like fecal azoreductase, β-glucoronidase and nitroreductase (Wollowski et al., 2001). It's still unclear if colonization is necessary for probiotics to work.

Neonatal reaction to preparations of probiotics depends on gestational age, postnatal age, weight, and previous exposure to antibiotics. A study on infants, found that colonization with Lactobacillus GG happened in 21% of infants weighing less than 1500g compared to 47% of bigger infants. The antibiotic's usage interfered with the probiotic's capability to colonize (Shi and Walker 2004).

Production of Antimicrobial Factors

Short-chain fatty acids, which probiotics produce, can decrease the pH of the colon and promote the growth of less harmful microorganisms. Antimicrobial proteins, called bacteriocins, are produced by probiotic organisms and particularly are potent against Gram-Positive Bacteria. Moreover, lactobacilli generate chemicals that render virus particles inactive (Cadieux et al., 2002). Within minutes, soluble compounds generated by *Lactobacillus fermentum* RC-14 and *Lactobacillus rhamnosus* GR-1 render vesicular stomatitis virus and adenovirus inactive. Lactobacillus GG is a producer of antimicrobial chemicals like lactic acid, hydrogen peroxide, and pyroglutamate, which hinder the growth of several gram-negative and gram-positive bacteria. Furthermore, *Lactobacillus acidophilus* strain LA1 generates an antibacterial agent that is not lactic acid nor bacteriocin and that is effective against a range of gram-negative and gram-positive bacteria (Lievin et al., 2000).

Competition for Nutrients

Probiotics can also compete with pathogenic organisms for the nutrients that they would then absorb. For instance, a probiotic that consumes monosaccharides may inhibit the growth of Clostridium difficile, a bacterium that needs monosaccharides to flourish.

Probiotics as Immune Modification Vehicles

It is possible to genetically modify probiotics like lactobacilli to emit chemicals like IL-10 that have an anti-inflammatory impact. The gastrointestinal tract's inflamed parts may locally release anti-inflammatory cytokines when the host consumes these genetically modified probiotics. (Steidler et al., 2000).

Effect on Humoral Immunity

Numerous studies show that a wide range of probiotics can reliably and powerfully induce a certain type of antibody response. IgA antibody responses specific to the rotavirus are induced by the viable *L. casei* strain GG. Furthermore, by influencing the generation of virus-neutralizing antibodies, the two strains of probiotics—*Lactobacillus acidophilus* CRL431 and *Lactobacillus rhamnosus* GG induced an immunologic reaction against the poliomyelitis vaccine virus. Additionally, in an animal model, eaten *B. bifidum* markedly augmented the amount of immunoglobulin (IgA, IgG, and IgM) secreting cells in the spleen and mesenteric lymph nodes. (Saikali et al., 2004).

Anti-proliferative Effect of Probiotics

Probiotics have the ability to affect several intestinal processes, including immunological status, transit, detoxification, and colonic fermentation. These effects may be linked to the emergence of colon cancer. Probiotic use has been shown to have direct antiproliferative effects on immunological and malignant cells. It has been demonstrated that modulating gut and systemic immunity in rats and specifically reducing carcinogenic bacterial enzymes have the potential to have major antiproliferative effects against colon cancer. The indication is still growing, and additional study is needed, but data point to a favorable role for probiotics in preventing colon cancer (Marotta et al., 2003).

Functional Properties

Growth Promotion in Farm Animals

Probiotic bacteria break down hydrocarbons, which implies food is being broken down into its utmost basic components. This permits nearly complete absorption via the gastrointestinal tract. Probiotics, therefore, significantly improve overall nutrition and promote cellular development and growth at a rapid pace. For example, in young pigs, Bifidobacteria and Lactobacillus boosted weight gain and decreased mortality. Additionally, pigs fed *Bacillus coagulans* performed better than pigs treated with sub-therapeutic antibiotics, with reduced mortality, improved feed conversion, and weight gain compared to un-supplemented piglets.

Defense against Intestinal Infections in the Host

Probiotics help to cleanse the gastrointestinal tract. They penetrate the intestinal walls' layer of filth, attach themselves, and lift the buildup of deterioration. After that, this waste naturally disappears. Probiotic supplements help avoid and occasionally treat fungal and yeast infections. By the production of antimicrobial compounds competing with the pathogens for nutrients, encouraging the intestinal immune system, and adhering to the intestinal mucosa, probiotic bacteria added to feed may defend piglets against pathogens of the intestine through a diversity of potential mechanisms (Ellin, 2001).

Relief of Constipation

Probiotics rapidly alleviate constipation and restore regular bowel motions. You can take lactobacillus both during and after an antibiotic course of therapy. This lessens the symptoms of antibiotic-induced diarrhea, which is brought on by the gastrointestinal tract's "bad" and "good" bacteria dying off randomly.

Anti-carcinogenic Effect

Carcinogenic intestinal beta-glucouronidase and nitroreductase are rendered inactive by Lactobacillus. Research conducted at the University of Nebraska and Sloan Kettering Institute for Cancer Research demonstrated that Lactobacillus inhibits the growth of tumors and has a clear anti-tumor effect. Further research is necessary; however, animal studies have indicated that some lactic acid bacteria may aid prevent colon cancer (Walker and Duffy, 1998; Zabala et al., 2001).

Nutrient Synthesis and Bioavailability

Probiotic strains of Lactobacillus plantarum produce lysine, one of the amino acids that are immediately digested by the body. They generate B vitamins, which function as biocatalysts in the metabolism of food and combat stress. These include pantothenic acid, B6, niacin, folic acid, B12, and riboflavin (Koop-Hoolihan, 2001).

Diseases Treated by Probiotics

- Treating atopy
- Irritable bowel syndrome
- Antibiotic-associated diarrhea
- Traveler’s diarrhea
- Infectious diarrhea
- Necrotizing enterocdiarrheaolitis

Prebiotic Introduction

One may quite readily impact nutrition, which is continuously recognized as one of the most important environmental elements influencing human health, both individually and as a whole in the population. Functional meals have gained popularity recently among lay people who are attempting to live better lives as a result of education and training, as well as among professionals. Food that offers additional health advantages from nutrients whose nutritional value improves the consumer's physical and mental well-being in addition to traditional elements is referred to as functional food. Particularly useful ingredients in nutritious foods are minerals, vitamins, prebiotics, and probiotics. The notion of prebiotics and probiotics is often regarded as the most noteworthy development in the realm of intestinal microbiota support and nutrition. (Hijová et al., 2019)

Prebiotics are beneficial food ingredients, produced artificially by converting carbohydrates through an enzyme process or naturally occurring in foods derived from plants. These substances are often soluble dietary fibers or carbohydrate structures that are preferentially digested by bacteria both within and outside the body. Thus, this activity promotes the growth of particular microorganisms and benefits the host's health. (Gibson et al., 2010). A fresh definition that defies the notion that prebiotic effects must be specific/selective has recently been put out in light of the rapidly expanding field of diet-gut microbiota interactions (Bindels et al., 2015). For generations, people have been consuming foods high in prebiotics; estimates place daily consumption for hunter-gatherer populations as high as 135g (Leach et al., 2010). Banana, sugar beet, beans, human breast milk, artichokes, rye, barley, cow's milk, onion, garlic, tomato, and asparagus are natural sources of prebiotics (Cooper et al., 2022).

Professors Roberfroid and Gibson coined the term "prebiotics" in 1995. Prebiotics were part of the functional food concept that was prepared by both of them. In general, prebiotics are described as "nondigestible food components that pass into the colon in their undamaged state, are resistant to the action of hydrolytic enzymes at the top of the GIT, and beneficially affect the microflora of the host organism by selectively stimulating the growth and/or activity of one or limited number of bacteria in the colon and thus improving the host's health" (Gibson and Roberfroid 1995). The term "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confer benefits upon the host well-being and health" was added to prebiotics definition in 2004 (Gibson et al., 2004). Prebiotics are now defined to include non-carbohydrates and their mode of act is no longer restricted to the gastrointestinal system or to specific foods (Gibson et al., 2017).

Substances used as Prebiotics

According to earlier research, prebiotics are oligosaccharide carbohydrates, primarily xylooligosaccharides (XOS), lactulose, inulin, and the fructose-oligosaccharides (FOS) that are generated from them (Yin et al., 2022; Zhao et al., 2021). Recent research, however, has shown that prebiotics also include other non-carbohydrate substances that satisfy the prebiotic requirements, such as polyphenols that have been extracted from fruits like black raspberries (Gu et al., 2019) and blueberries (Jiao et al., 2019).

Fructans

The most varied class of prebiotics that are commercially accessible are called fructans, and they comprise fructose polymers that are not digested. A review of their impacts on bone mineral density and calcium absorption has been conducted (Roberfroid et al., 2010). The bulk of glycosidic links in every chain of these molecules is beta (b) (2–1) fructosyl–fructose connections, which is one of their defining characteristics. In addition to being naturally occurring in plant-based foods including asparagus, bananas, artichokes, onions, and wheat, these chemicals can also be produced synthetically (Sabater-Molina et al., 2009).

Galactooligosaccharide

Oligosaccharides galacturonic GOS is a novel functional material with inherent qualities that the body finds difficult to absorb and digest. GOS are made up of two to eight sugar units, of which two are galactose and disaccharides (which contain two galactose units) and one is terminal glucose (Delgado-Fernandez et al., 2021).

A combination of galactose-based oligosaccharides with variable DP and connections to various monomers of sugar, such as lactose, and glucose, results in GOS, a well-known prebiotic component. The various health benefits of GOS are explained by their unique oligosaccharide composition. Research has demonstrated that GOS promotes the development of useful gut flora like lactobacillus, and bifidobacteria in early life stages because these substances resemble human milk oligosaccharides which support immunity and gut health in nurturing newborns (Fanaro et al., 2005).

Research on animals has shown that the ingestion of GOS in postmenopausal rat models led to a noteworthy elevation in the calcium content of the skeleton in ovariectomized rats (Chonan et al., 1995) and mineralization of bone in male rats (Weaver et al., 2011). It has also been shown that dietary GOS increases postmenopausal women's absorption of calcium (van den Heuvel et al., 2000).

Lactose Derivatives

The main disaccharide included in dairy products is lactose. It is a combination of galactose and glucose sugar monomers linked by glycosidic bonds. It has been demonstrated that rats fed a diet high in lactose and calcium had stronger and more mineralized bones (Schaafsma et al., 1987). Lactase's enzyme activity diminishes with age in humans, which may permit microbes to break down this sugar when it enters the lower gastrointestinal tract (Misselwitz et al., 2013). Since people with lactose intolerance absorb more calcium from milk containing lactose than people with usual lactase action, this could lead to a larger absorption of minerals (Griessen et al., 1989). Some examples of prebiotics production are given in Fig. 2.

Functional Properties of Prebiotics

When consumed in suitable amounts, probiotic-living microorganisms benefit the health of the host by colonizing the body. Probiotics have the capability to transform the human intestinal microbes' composition and prevent harmful bacteria from colonizing the intestines. Additionally, probiotics have been shown to have the capacity to support the body's development of a robust intestinal mucosa layer, strengthening the function of the intestinal barrier and boosting immunity (Wang et al., 2021). The promotion of prebiotics is necessary for the growth and replication of probiotics. Prebiotics are substances—mostly polysaccharides—that the body is unable to process and absorb. They can aid in the development or propagation of live microorganisms within the host (Li et al., 2021).

Prebiotics influence metabolism, strengthen immune regulation, fend off infections, improve mineral absorption, and generally improve health (Peredo-Lovillo et al., 2020) (Fig. 3). Prebiotics come from a diversity of sources and are typically defined as certain microalgae, oligosaccharides, polysaccharides, and natural plants. The main sources of emerging prebiotics include seeds, peels, fruit juice, algae, polysaccharide- and polyphenol-containing microorganisms, and traditional Chinese medicine (Quigley 2019).

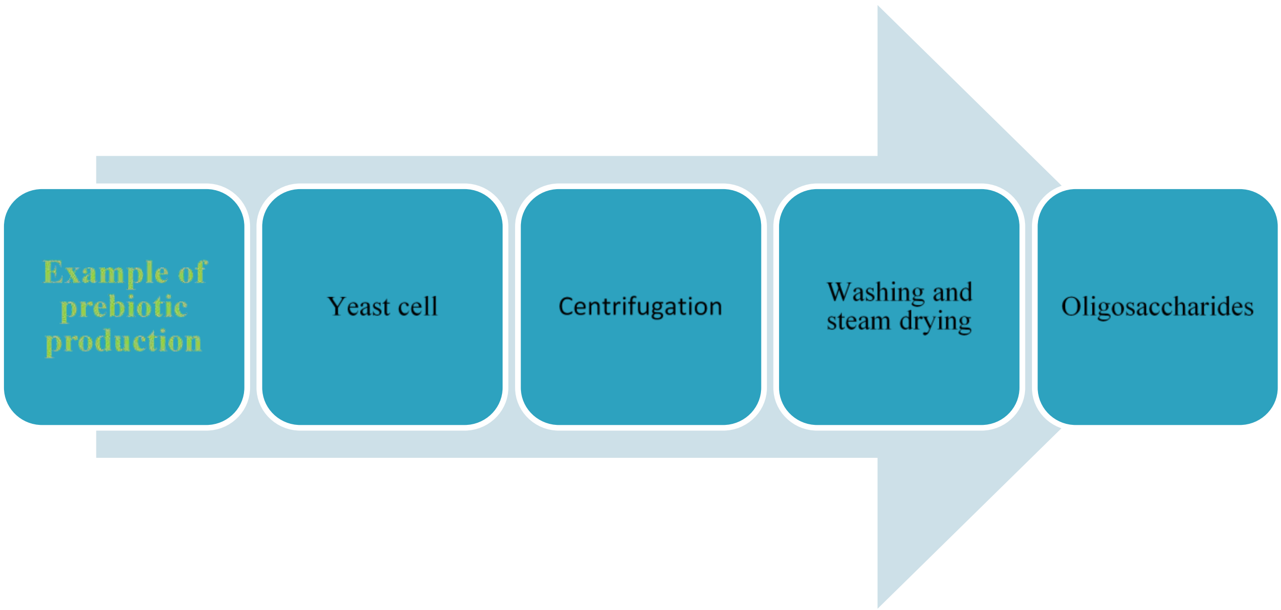

Fig. 2: Example of prebiotic production

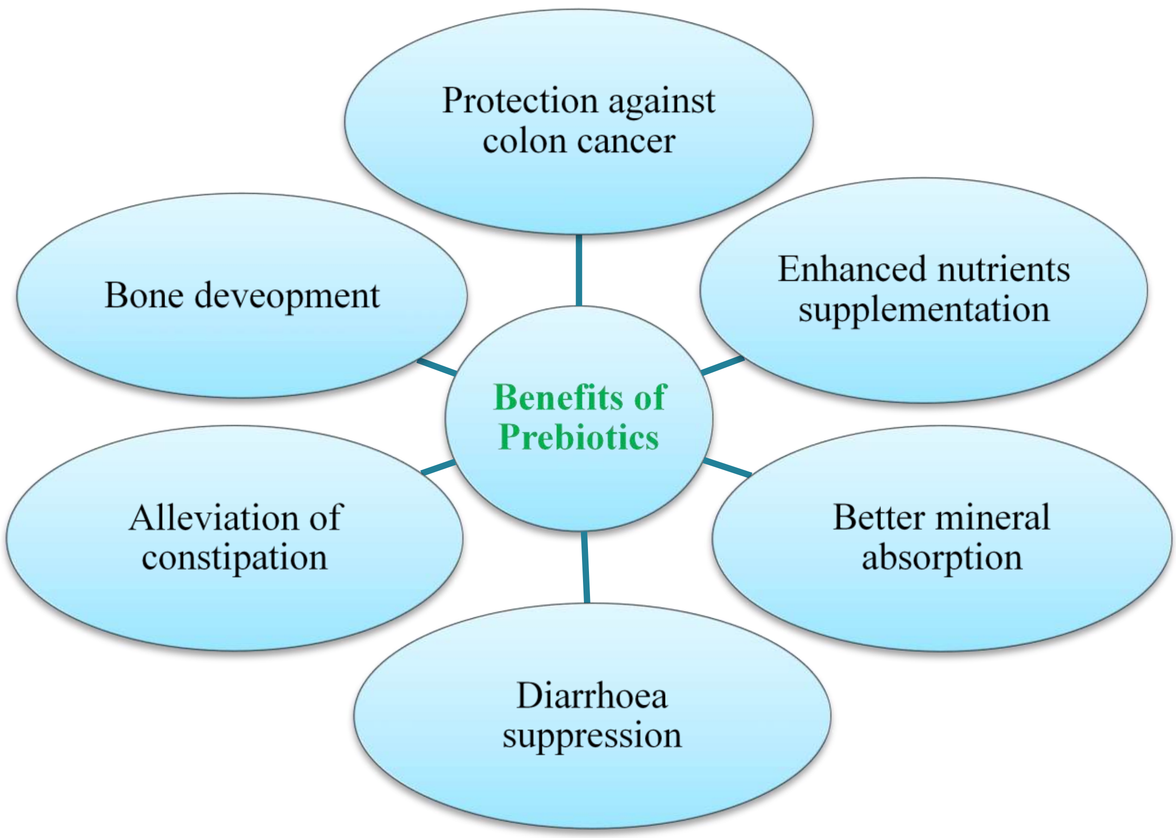

Fig. 3: Benefits of Prebiotics

Mode of Action

Prebiotics can generally withstand digestion in the small intestine by remaining in the gastrointestinal system because of absence of enzymes in the human gut that break down their polymeric bonds. Prebiotics are subsequently supported by the body intact to large intestine, where intestinal flora breaks them down and selectively ferments them to produce definite secondary metabolites. These types of metabolites are then engrossed by intestinal epithelium or go to the liver via the portal vein. These metabolites have positive impacts on the physiological processes of the host, including pathogen resistance, immunity regulation, and increased absorption of minerals, decreased blood lipid, and improved intestinal barrier function (Guarino et al., 2020).

Beneficial bacteria in the gut metabolize the most prevalent SCFAs, such as propionate, butyrate, and acetate, which are good for preserving systemic and intestinal health (Baert et al., 2020) (Fig. 4). Additionally, one particular benefit of prebiotics is that they support the growth of the target bacteria. By safeguarding or encouraging the creation of advantageous fermentation products, they might encourage the proliferation of useful flora to contend with other species following the consumption of particular prebiotics (FOS, inulin, and GOS) (Ashaolu 2020).

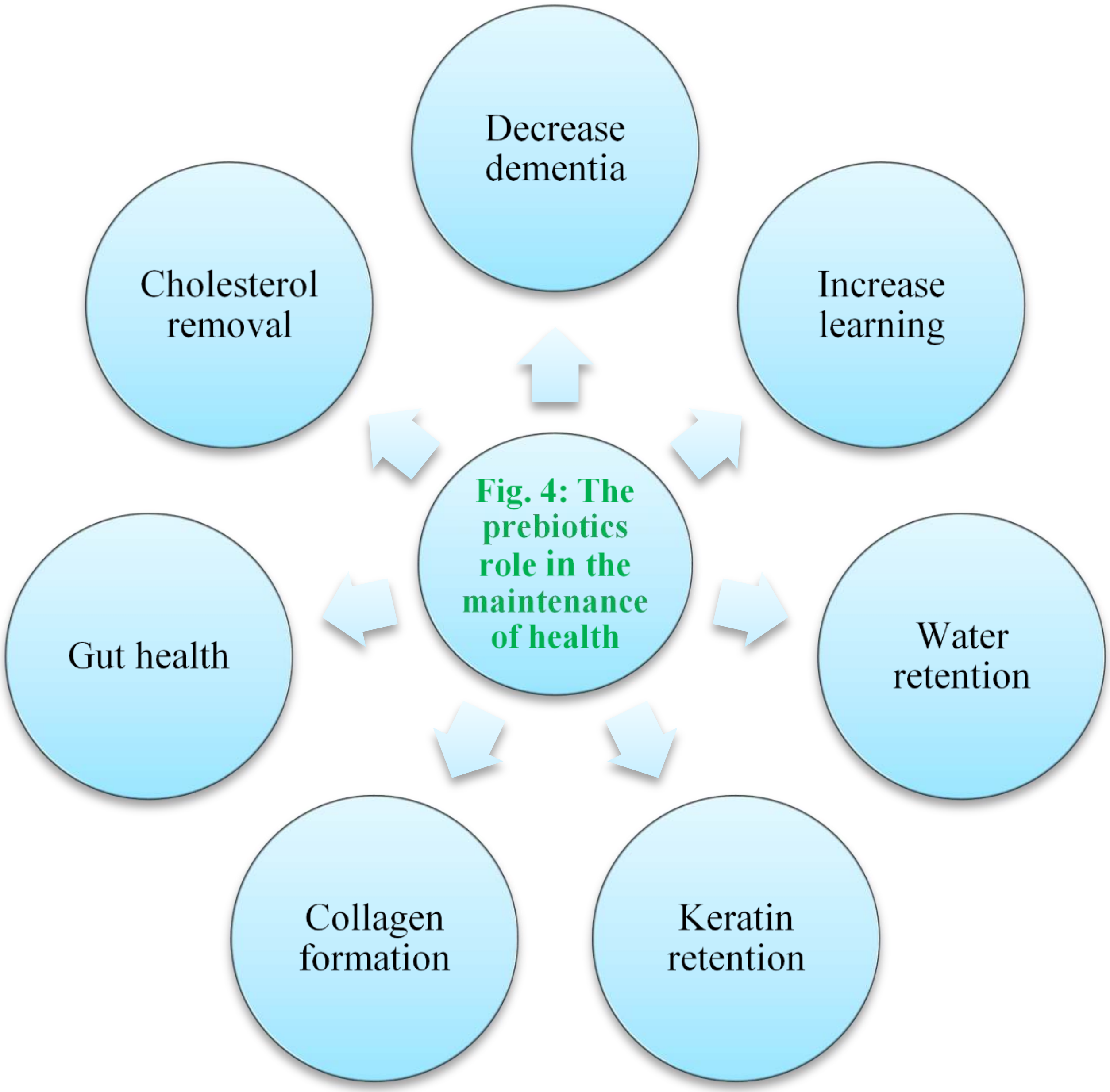

Fig. 4: The prebiotics role in the maintenance of health

Synbiotics and their Importance for Animals

The word "synbiotic" has historically been used to describe a mixture of probiotics and prebiotics increasing the survivability of probiotics that are eaten (Pandey et al., 2015). Combining prebiotics with different chain lengths and monomer connections is one way to use synbiotics; this has been demonstrated to extend the prebiotic impact over a wider GI tract (Coxam 2005). The criterion that each independently delivers a health benefit and that each dose be sufficient to independently accomplish those benefit(s) is shared by definitions of prebiotics and probiotics (Gibson et al., 2017). Long-chain prebiotics such as inulin may be digested more distally or beside a prolonged stretch of the gut, but short-chain prebiotics like oligofructose are thought to be processed in the proximal colon. Often referred to as ITF-mix, this most frequently reported prebiotic mixture has variable chain lengths and is made up of inulin-type fructans. It has been proved that this combination improves bone health outcomes and the absorption of magnesium and calcium in both postmenopausal ladies and adolescents (Abrams et al., 2007).

Prebiotics and plant polyphenols work synergistically, and this has extensive implications for bone health as well (Devareddy et al., 2006). Research on synbiotics—which assesses how well prebiotics work in conjunction with probiotics—is expanding quickly. At the moment, Bifidobacterium species are the most researched in synbiotic applications concerning the health of bones. According to a study, adding GOS to two species of Bifidobacterium—Bifidobacterium bifidum and longum—markedly improved the bioavailability of calcium, magnesium, and phosphorus as well as the mineral content of the hind limbs (Pérez-Conesa et al., 2007).

Up until now, prebiotics and probiotics have had an excellent safety record (Van den Nieuwboer and Claassen 2019), and synbiotics made with them may likewise be considered safe for the same purposes. Nonetheless, new formulations need to be properly evaluated for safety (Ioannidis et al., 2004).

Future Prospects of both Probiotics and Prebiotic

Research on the impact of probiotics on cardiovascular conditions, such as atherosclerosis and myocardial infarction, is being focused on a large scale (Loscalzo et al., 2011). According to neuro-gastroenterologist Dr. Gershon's working hypothesis, there is an enteric nervous system that plays a part in gut physiology and other related gut illnesses (Gershon, 1998). By comprehending the function of BMicrobials endocrinology, the study of the ability of microorganisms to produce and respond to neurochemicals that originate either within the microorganisms themselves or within the host they inhabit, the aforementioned notion can be addressed. Probiotics both produce and react to neuroactive substances (Roshchina 2010).

Conclusion

It is commonly well-known that intestinal flora plays a substantial part in both maintaining health and also preventing disease. Its constant "communication" or interaction with the immune system, endocrine system, central nervous system, and environment reveals the intricate underlying systems. If this delicate equilibrium is upset, it can cause various problems and make it easier for a disease to spread. In particular, it has been proposed that microbiologists ought to be heavily involved in strain isolation, mechanism of action testing, and "packaging these into steadfast products for usage of humans (Potera, 1999). Probiotics and prebiotics most likely have distinct "target" areas. Important information is still required despite the surge in papers on probiotic organisms published in recent years by microbiologists, nutritionists, food scientists, and doctors. More cutting-edge techniques should be created to track modifications in gut flora's makeup and how they interact with the host's metabolism and immune system.

REFERENCES

Abrams, S. A., Griffin, I. J., and Hawthorne, K. M. (2007). Young adolescents who respond to an inulin-type fructan substantially increase total absorbed calcium and daily calcium accretion to the skeleton. *The Journal of Nutrition*, *137*(11), 2524S-2526S.

Ashaolu, T. J. (2020). Immune boosting functional foods and their mechanisms: A critical evaluation of probiotics and prebiotics. *Biomedicine and Pharmacotherapy*, *130*, 110625.

Baert, F., Matthys, C., Mellaerts, R., Lemaître, D., Vlaemynck, G., and Foulon, V. (2020). Dietary intake of Parkinson's disease patients. *Frontiers in Nutrition*, 105.

Bindels, L. B., Delzenne, N. M., Cani, P. D., and Walter, J. (2015). Towards a more comprehensive concept for prebiotics. *Nature Reviews Gastroenterology and Hepatology*, *12*(5), 303-310.

Cadieux, P., Burton, J., Gardiner, G., Braunstein, I., Bruce, A.W., Kang, C.Y., and Reid, G. (2002). Lactobacillus strains and vaginal ecology. *JAMA*, 287:1940-1941.

Chang, Y., Kim, J., Kim, W., Kim, Y., and Park, Y. (2001). Selection of a potential probiotic Lactobacillus strain and subsequent in-vivo studies. Antonne Van Leeuwenhoek. pp. 193-199.

Chonan, O., Matsumoto, K., and Watanuki, M. (1995). Effect of galactooligosaccharides on calcium absorption and preventing bone loss in ovariectomized rats. *Bioscience, Biotechnology, and Biochemistry*, *59*(2), 236-239.

Cooper, T. E., Scholes-Robertson, N., Craig, J. C., Hawley, C. M., Howell, M., Johnson, D. W., and Wong, G. (2022). Synbiotics, prebiotics and probiotics for solid organ transplant recipients. *Cochrane Database of Systematic Reviews*, (9).

Coxam, V. (2005). Inulin-type fructans and bone health: state of the art and perspectives in the management of osteoporosis. *British Journal of Nutrition*, *93*(S1), S111-S123.

Delgado-Fernandez, P., de Las Rivas, B., Munoz, R., Jimeno, M. L., Doyaguez, E. G., Corzo, N., and Moreno, F. J. (2021). Biosynthesis of nondigestible galactose-containing hetero-oligosaccharides by lactobacillus plantarum WCFS1 MelA α-Galactosidase. *Journal of Agricultural and Food Chemistry*, *69*(3), 955-965.

Devareddy, L., Khalil, D. A., Korlagunta, K., Hooshmand, S., Bellmer, D. D., and Arjmandi, B. H. (2006). The effects of fructo-oligosaccharides in combination with soy protein on bone in osteopenic ovariectomized rats. *Menopause*, *13*(4), 692-699.

Di Lena, M., Quero, G.M., Santovito, E., Verran, J., De Angelis, M., and Fusco, V. (2015). A selective medium for isolation and accurate enumeration of Lactobacillus casei-group members in probiotic milks and dairy products. *International Dairy Journal* 2015, 47:27-36.

Divya, J.B., Varsha, K.K., and Nampoothiri, K.M., (2012). Newly isolated lactic acid bacteria with probiotic features for potential application in food industry. *Applied Biochemical Biotechnology,* 167, 1314–1324. https://doi.org/10.1007/s12010-012-9561-7.

Ellin, D.M. (2001). Alternatives to Antibiotic use of growth promotion in animal husbandry. Food Research Institute Briefings.

Fanaro, S., Boehm, G., Garssen, J., Knol, J., Mosca, F., Stahl, B., and Vigi, V. (2005). Galacto-oligosaccharides and long-chain fructo-oligosaccharides as prebiotics in infant formulas: a review. *Acta Paediatrica*, *94*, 22-26.

Fiorda, F.A., de Melo Pereira, G.V., Thomaz-Soccol, V., Medeiros, A.P., Rakshit, S.K., and Soccol, C.R., (2016). Development of kefir-based probiotic beverages with DNA protection and antioxidant activities using soybean hydrolyzed extract colostrum and honey. *LWT Food Science Technology,* 68, 690–697. https://doi.org/10.1016/j.lwt.2016.01

Gareau, M. G., Sherman, P. M., and Walker, W. A. (2010). Probiotics and the gut microbiota in intestinal health and disease. *Nature Reviews Gastroenterology and Hepatology*, *7*(9), 503-514.

Gershon, M.D. (1998). The second brain (pp. 4–7). HarperCollins Publishers.

Gibson, G. R., and Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of Nutrition*, *125*(6), 1401-1412.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., and Reid, G. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology and Hepatology*, *14*(8), 491-502.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., and Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews*, *17*(2), 259-275.

Gibson, G. R., Scott, K. P., Rastall, R. A., Tuohy, K. M., Hotchkiss, A., Dubert-Ferrandon, A., and Buddington, R. (2010). Dietary prebiotics: current status and new definition. *Food Science Technology Bull. Funct. Foods*, *7*(1), 1-19.

Griessen, M., Cochet, B., Infante, F. et al. (1989) Calcium absorption from milk in lactase-deficient subjects. *Am Journal Clinical Nutrition,* 49:377–384.

Gu, J., Thomas-Ahner, J. M., Riedl, K. M., Bailey, M. T., Vodovotz, Y., Schwartz, S. J., and Clinton, S. K. (2019). Dietary black raspberries impact the colonic microbiome and phytochemical metabolites in mice. *Molecular Nutrition and Food Research*, *63*(8), 1800636.

Guarino, M. P. L., Altomare, A., Emerenziani, S., Di Rosa, C., Ribolsi, M., Balestrieri, P., and Cicala, M. (2020). Mechanisms of action of prebiotics and their effects on gastro-intestinal disorders in adults. *Nutrients*, *12*(4), 1037.

Gupta, V., and Garg, R. (2009). Probiotics. *Indian Journal of Medical Microbiology*, *27*(3), 202-209.

Hijová, E., Bertková, I., and Štofilová, J. (2019). Dietary fibre as prebiotics in nutrition. *Central European Journal of Public Health*, *27*(3), 251-255.

Ioannidis, J. P., Evans, S. J., Gøtzsche, P. C., O'neill, R. T., Altman, D. G., Schulz, K., and Consort Group*. (2004). better reporting of harms in randomized trials: an extension of the CONSORT statement. *Annals of Internal Medicine*, *141*(10), 781-788.

Jiao, X., Wang, Y., Lin, Y., Lang, Y., Li, E., Zhang, X., and Li, B. (2019). Blueberry polyphenols extract as a potential prebiotic with anti-obesity effects on C57BL/6 J mice by modulating the gut microbiota. *The Journal of Nutritional Biochemistry*, *64*, 88-100.

Jin, L.Z., Marquardt, R.R., and Zhao, X. (2000). A strain of Enterococcus faecium (18C23) inhibits adhesion of enterotoxigenic Escherichia coli K88 to porcine small intestine mucus. *Applied Environment Microbiology,* 66:4200-4.

Koop-Hoolihan, L. (2001). Prophylactic and therapeutic uses of Probiotics: A Review of Journal of the American Dietetic Association. 147:747-748.

Leach, J. D., and Sobolik, K. D. (2010). High dietary intake of prebiotic inulin-type fructans in the prehistoric Chihuahuan Desert. *British Journal of Nutrition*, *103*(11), 1558-1561.

Li, X., Zhang, Q., Wang, W., and Yang, S. T. (2021). A novel inulin-mediated ethanol precipitation method for separating endo-inulinase from inulinases for inulooligosaccharides production from inulin. *Frontiers in Bioengineering and Biotechnology*, *9*, 679720.

Lievin, V., Peiffer, I., Hudault, S., Rochat, F., Brassart, D., Neeser, J.R., and Servin, A.L. (2000). Bifidobacterium strains from resident infant human gastrointestinal microflora exert antimicrobial activity. *Gut*, 47:646-652.

Loscalzo, J. (2011). Lipid metabolism by gut microbes and atherosclerosis. *Circulation Research*, *109*(2), 127-129.

Luna, R.A., and Foster, J.A., (2015). Gut brain axis: diet microbiota interactions and implications for modulation of anxiety and depression. *Current Opinion Biotechnology,* 32, 35–41. https://doi.org/10.1016/j.copbio.2014.10.007

Maragkoudakis, P.A., Zoumpopoulou, G., Miaris, C., Kalantzopoulos, G., Pot, B., and Tsakalidou, E., (2006). Probiotic potential of Lactobacillus strains isolated from dairy products. *International Dairy Journal,* 16, 189–199.

Marotta, F., Naito, Y., Minelli, E., Tajiri, H., Bertuccelli, J., Wu, C.C., Min, C.H., Hotten, P., and Fesce, E. (2003). Chemopreventive effect of a probiotic preparation on the development of preneoplastic and neoplastic colonic lesions: an experimental study. *Hepatogastroenterology*, 50(54):1914-1918. 72.

Marteau, P., Cuillerier, E., Meance, S., Gerhardt, M.F., Myara, A., Bouvier, M., et al. (2002). Bifidobacterium animalis strain DN- 173 010 shortens the colonic transit time in healthy women: a double-blind, randomized, controlled study. *Aliment Pharmacol Ther,* 16:587-93

Misselwitz, B., Pohl, D., Fru¨hauf, H. et al (2013). Lactose malabsorption and intolerance: pathogenesis, diagnosis and treatment. *United Europe Gastroenterol Journal*, 1:151–159. doi:10.1177/ 2050640613484463.

Olveira, G. and Molero, I.G. (2016). An update on probiotics, prebiotics and synbiotics in clinical nutrition. *Endocrinologia, Diabetese and Nutricion*, 63: 482-494.

Pandey, K. R., Naik, S. R., and Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics-a review. *Journal of Food Science and Technology*, *52*, 7577-7587.

Peredo-Lovillo, A., Romero-Luna, H. E., and Jiménez-Fernández, M. (2020). Health promoting microbial metabolites produced by gut microbiota after prebiotics metabolism. *Food Research International*, *136*, 109473.

Pérez-Conesa, D., Lopez, G., and Ros, G. (2007). Effects of probiotic, prebiotic and synbiotic follow-up infant formulas on large intestine morphology and bone mineralisation in rats. *Journal of the Science of Food and Agriculture*, *87*(6), 1059-1068.

Potera, C. (1999). Probiotics gaining recognition. *ASM News*, 65, 739– 740.

Quigley, E. M. (2019). Prebiotics and probiotics in digestive health. *Clinical Gastroenterology and Hepatology*, *17*(2), 333-344.

Roberfroid, M., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., and Meheust, A. (2010). Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition*, *104*(S2), S1-S63.

Roshchina, V. V. (2010). Evolutionary considerations of neurotransmitters in microbial, plant, and animal cells. *Microbial Endocrinology: Interkingdom Signaling in Infectious Disease and Health*, 17-52.

Sabater-Molina, M., Larque´, E., Torrella, F., and Zamora, S. (2009) Dietary fructooligosaccharides and potential benefits on health. *Journal Physiology Biochemistry,* 65:315–328. doi:10.1007/BF03180584.

Saikali, J., Picard, C., Freitas, M., and Holt, P. (2004). Fermented milks, probiotic cultures, and colon cancer. *Nutrition Cancer*, 49:14-24.

Schaafsma, G., Visser, W. J., Dekker, P. R., and Van Schaik, M. (1987). Effect of dietary calcium supplementation with lactose on bone in vitamin D-deficient rats. *Bone*, *8*(6), 357-362.

Shi, H.N., and Walker, A. (2004). Bacterial colonization and the development of intestinal defenses. *Can Journal Gastroenterology,* 18:493-500.

Steidler, L., Hans, W., Schotte, L., Neirynck, S., Obermeier, F., Falk, W., et al. (2000). Treatment of murine colitis by Lactobacillus lactis secreting interleukin-10. *Science*, 289:1352-1355.

Tsilingiri, K., and Rescigno, M. (2013). Postbiotics: what else?. *Beneficial Microbes*, *4*(1), 101-107.

van den Heuvel, E. G., Schoterman, M. H., and Muijs, T. (2000). Transgalactooligosaccharides stimulate calcium absorption in postmenopausal women. *The Journal of Nutrition*, *130*(12), 2938-2942.

Van den Nieuwboer, M., and Claassen, E. (2019). Dealing with the remaining controversies of probiotic safety. *Beneficial Microbes*, *10*(6), 605-616.

Walker, A.W., and Duffy, L.C. (1998). Diet and bacterial colonization: Role of probiotics and prebiotics: *Review Journal Nutrition Biochemistry,* 9:668-675.

Wang, X., Zhang, P., and Zhang, X. (2021). Probiotics Regulate Gut Microbiota: an effective method to improve immunity. *Molecules,* 26:6076. doi: 10.3390/molecules26196076

Weaver, C. M., Martin, B. R., Nakatsu, C. H., Armstrong, A. P., Clavijo, A., McCabe, L. D., and van den Heuvel, E. G. (2011). Galactooligosaccharides improve mineral absorption and bone properties in growing rats through gut fermentation. *Journal of Agricultural and Food Chemistry*, *59*(12), 6501-6510.

Wollowski, I., Rechkemmer, G., Pool-Zobel, B.L. (2001). Protective role of probiotics and prebiotics in colon cancer. *Am Journal Clinical Nutrition*, 73:451S-455S.

Yin, X., Cai, T., Liu, C., Ma, Y., Hu, J., Jiang, J., and Wang, K. (2022). A novel solvothermal biorefinery for production of lignocellulosic xylooligosaccharides, fermentable sugars and lignin nano-particles in biphasic system. *Carbohydrate Polymers*, *295*, 119901.

Zabala, A., Martin, M.R., Haza, A.I., Fernandez, L., Rodriguez, J.M., and Morales, P. (2001). Anti-proliferative effect of two lactic acid bacteria strains of human origin on the growth of a myeloma cell line. *Letter Applied Microbiology,* 32:287-292.

Zendeboodi, F., Khorshidian, N., Mortazavian, A. M., and da Cruz, A. G. (2020). Probiotic: conceptualization from a new approach. *Current Opinion in Food Science*, *32*, 103-123

Zhao, J., Zhang, X., Zhou, X., and Xu, Y. (2021). Selective production of xylooligosaccharides by xylan hydrolysis using a novel recyclable and separable furoic acid. *Frontiers in Bioengineering and Biotechnology*, *9*, 660266

Chapter 02

Bacteriocin-Producing Lactic Acid Bacteria: Probiotic Approach for the Treatment of Gut's Diseases

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ABSTRACT

Lactic acid bacteria (LAB) are the most common probiotics. It prevents many gastrointestinal (GI) tract diseases such as obesity, inflammatory bowel disease (IBD), dysbiosis, and chronic infection. They produce lactic acid that lowers the pH of the GI tract and inhibits the growth of different pathogenic bacteria. They also provide competition for food and produce different antimicrobial peptides known as bacteriocins, which can effectively kill or inhibit closely related bacterial strains as well as bacterial pathogens such as *E.coli,* and *Salmonella*. Bacteriocins are small cationic peptides that lead to bacterial cell death by forming pores and the release of cytosolic contents. Lactic acid bacteria as probiotics maintain homeostasis and have many beneficial effects on gastrointestinal tract health. They also play an important role in boosting the immune response of the host by stimulating the host immune cells of the intestine, which further activates the other cells by signal complex.

KEYWORDS			
Probiotics, Bacteriocin, Lactic acid bacteria, Gut Disease	Received: 10-May-2024 Revised: 07-July-2024 Accepted: 22-Aug-2024		A Publication of Unique Scientific Publishers

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INTRODUCTION

The gut microbiota plays a crucial role in developing the host's immune system, protecting against harmful microorganisms, and increasing the integrity of the gut. The important gut bacteria include Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria (Zhang et al., 2015) Dysbiosis is a disease, that occurs when there is a disturbance in the normal balance of the gut microbial flora. This can be caused by various factors such as antibiotic use, an unbalanced diet, and infection. Various diseases such as inflammatory bowel disease (IBD), viral infections, and obesity are also linked to the imbalance of intestinal microbiota (Kim et al., 2019). These disturbances can lead to alteration in the gut microbiota, which in turn increases the risk of developing numerous serious diseases (Lange et al., 2016). Dysbiosis also leads to the emergence of diseases associated with immunological dysregulation, such as allergies, autoimmune disorders, and inflammatory conditions (D'Amelio and Sassi, 2018). Probiotics are the living bacteria that are used to treat the imbalance of intestinal microbiota, when consumed in sufficient quantities, they enhance the intestinal beneficial microbiota and give health benefits to humans (Binda et al., 2020).

Bacteriocin-producing probiotics colonize the gut and prevent the adherence of pathogens to intestinal epithelial cells by competing for food and producing inhibitory compounds (Figure 1) (Vieco-Saiz et al., 2019a). Bacteriocins have a distinct method of action compared to antibiotics since they eliminate target cells by creating pores and disrupting the cell membrane (Yang et al., 2014). In addition, bacteriocins possess a more simple biosynthetic process, and having a greater specific activity against microorganisms that are resistant to several drugs provides benefits for their use in medical treatments (Pérez-Ramos et al., 2021a). Bacteriocins are the proteins synthesized by ribosomes and are broken down by proteolytic enzymes. As a result, bacteria are unable to acquire resistance in the gut. Hence, the use of probiotics is an innovative strategy for treating various disorders such as enteric infections, and restoring a beneficial microbial community to promote health (Fong et al., 2020).

Gut Microbiota

The microorganisms that inhabit the digestive tract of humans including viruses, bacteria, and fungi are referred as Gut Microbiota. The gut microflora of mammals contains trillions of bacteria and around 500 different microbial strains and some of the strains are used as probiotics. Microorganisms naturally reside on the skin, mouth cavity, vagina, and gastrointestinal system of humans by birth. Early infancy is a critical phase for the development of the microbiota, which is influenced by factors such as delivery method (vaginal versus cesarean section), gestational age, and usage of antibiotics throughout the perinatal period. Compared to cesarean section deliveries, vaginal births are linked to higher intestine colonization with *Bifidobacteria* but not *lactobacilli*. Conversely, greater colonization by hospital-associated microorganisms such as *Enterobacter*, *Klebsiella*, and *Clostridium* is linked to cesarean section births.

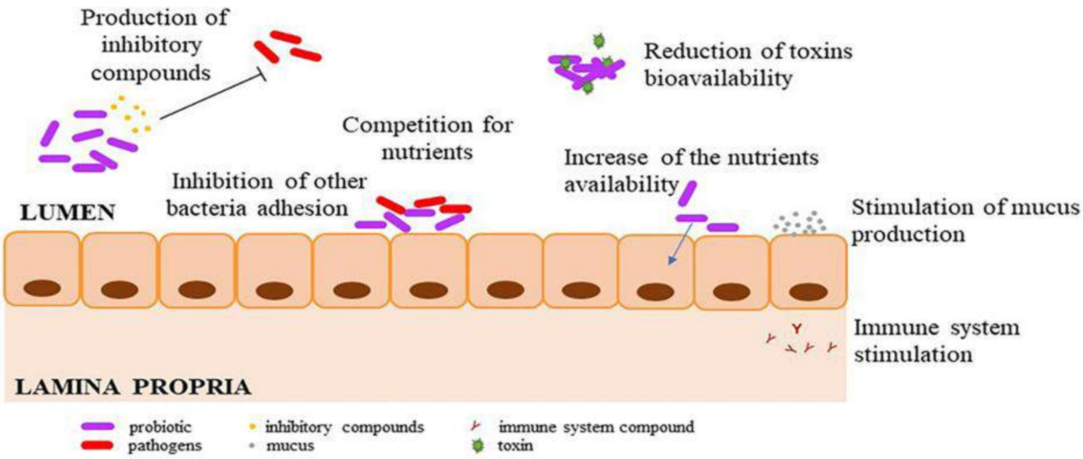

Fig. 1: Pathogen Inhibition mechanism by LAB, (Vieco-Saiz et al., 2019a)

Human microbiota changes during the course of life, from childhood to adulthood and old age. There appears to be a correlation between a lower microbial diversity during infancy and a higher chance of developing disease in later childhood. Reduced diversity of the gut microbiome is a common feature in several disorders that may be associated with dysbioses, necrotizing enterocolitis, chronic diarrhea, and IBD. Early exposure to specific bacteria and variations in the composition of the gut microbiome due to any factor have a lasting impact on an individual's health (Gareau et al., 2010a).

Probiotics

Probiotics are bacteria that have positive health effects on the host when consumed in a specific quantity. Their primary functions in the gut include modulating the immune system and maintaining the balance between beneficial and harmful microbes in the gut (Choi et al., 2010). The important probiotic includes many lactic acid-producing lactobacilli strains and several *Bifidobacteria*. Probiotics are typically regarded as safe for consumption, however, they may produce bacteria-host interactions and unpleasant side effects in rare situations (Gareau et al., 2010a).

Probiotic organisms have many beneficial impacts on intestinal epithelial cells. Certain strains can produce a mucus barrier by releasing mucus from goblet cells or by offering a physical barrier known as colonization resistance that prevents the entry of pathogens into the epithelial cell. They also help in maintaining the permeability of the intestine by more expression of the zona occludens (Gareau et al., 2010a).

Lactic Acid Bacteria

Lactic acid bacteria (LAB) are the important flora of the gut microbiota and have a crucial function in balancing the overall microbial population. Probiotic lactic acid bacteria (LAB) are a diverse collection of non-pathogenic, Gram-positive bacteria that do not produce spores and lack the enzyme catalase. They convert glucose into lactic acid, together with many growth-restricting compounds (Mokoena, 2017). They may be found in different foods such as fermented meats, seafood, drinks, and pickled vegetables, and in the oral and nasal cavities of humans. Significant genera in this context are *Lactococcus, Enterococcus, Streptococcus, Pediococcus, Aerococcus,* and *Lactobacillus*. Lactobacilli are the most abundant probiotics present in humans' gastrointestinal tracts. Sometimes, more than one probiotic is used to treat the disease and regain the host's normal microbiota. They produce different kinds of antimicrobial compounds which help in the prevention of disease (Table 1). Probiotic bacteria play a crucial role in immunoregulation by mediating the signal formation that activates other immune cells through intestinal immune system stimulation. Probiotics also lead to more IgA production which may stimulate interactions between dendritic cells and B and/or lymphocytic T cells (Anjana and Tiwari, 2022). In addition to LAB, *Bifidobacterium* is the predominant microorganism that colonizes the gut of the host and provides health advantages. (O'Callaghan and Van Sinderen, 2016).

LAB and *Bifidobacterium* are used as a cure for several gastrointestinal (GI) diseases. They limit harmful microbes, strengthen the GI barrier, and inhibit the production of proinflammatory cytokines (Xue et al., 2017). Apart from the lactic acid bacteria (LAB), other types of bacteria also prevail in the gut and have essential roles. For instance, the innocuous *E. Coli* Nissle, a probiotic often present in the gut, is mostly used to maintain a healthy balance of intestinal microbiota. It also stimulates the reestablishment of human β-defensin 2 synthesis, which can effectively protect the intestinal barrier against attachment of the pathogenic *E. coli* (Anjana and Tiwari, 2022).

Bacteriocin-producing LAB Bacteria

Lactobacilli bacteria that can produce bacteriocin, are used in treating several diseases of the GI tract. They prevent the colonization of the pathogenic bacteria in the human GI tract. When a bacteriocin-producing bacteria is administered orally, it does not alter the general structure of the gut, but it does cause some positive changes at a lower taxonomic level. However, some of these changes were reversed following the treatment (Umu et al., 2017). Probiotics, such as *Lacticaseibacillus casei*, and *Streptococcus thermophiles* may be taken orally and increase the expression of Ig-A and Ig-G in a way that depends on the dosage. When administered 8 days before infection with vancomycin-resistant *Enterococcus*, the use of nisin Z and pediocin AcH resulted in a decrease in pathogen colonization (Millette et al., 2008).

Table 1: Antimicrobial compounds produce by LAB

Molecule	Examples	Producer	Spectrum	References
Bacteriocins	Nisin	*Lc.lactis* subsp. l*actis*	Broad spectrum	(de Arauz et al., 2009)
	Pediocin PA-1	*Ped.acidilactic*	Broad Spectrum	(Rodríguez et al., 2002)
	Enterolysin AS48	*Ent.faecalis*	Gram-positive and many Enterobacteriaceae	(Karpiński and Szkaradkiewicz, 2013)
Bacteriocin-like inhibitory substances		*Ped.acidikactic Kp10*	*L.monocytogenase*	(Wong et al., 2017)
		Leuc.mesenteroides 406	*L.monocytogenase*	(Arakawa et al., 2016)
Antibiotic	Reutericyclin	*Lb.reuteri*	Gram-positive	(Rattanachaikunsopon and Phumkhachorn, 2010)
	Reuterin	Lb.reuteri DSM 20016	Gram-positive	(Stevens et al., 2011)
Organic acid	Lactic acid, acetic acid	LAB	Broad spectrum	(Vieco-Saiz et al., 2019a)

Bacteriocin, Abp118, which is produced by the *L. Salivarius* UCC118, has anti-listerial action in the gastrointestinal tract (Riboulet-Bisson et al., 2012). The *L. Plantarum* P-8 produces plantaricin, which leads to alterations in the fecal bacteria community in humans (Kwok et al., 2015). Thuricin CD, a bacteriocin, consists of two peptides, Trnα and Trnβ, and can kill several strains of *C. difficile* showing that it is possible to target difficult-to-treat bacteria without impacting the normal bacteria in the distal colon (Anjana and Tiwari, 2022b). Bacteriocin produced by lactic acid bacteria (LAB) has also shown great efficacy against foodborne pathogens such as *Listeria monocytogenes* found in the human gut (Harris et al., 1989; Millette et al., 2008).

Bacteriocins

Bacteriocins are small peptides, effective at low concentrations, and typically target closely related bacterial species, but there is evidence of some bacteriocins having a broader range of activity as well (Chi and Holo, 2018; Goyal et al., 2018). Bacteriocins modulate the levels of anti-inflammatory and pro-inflammatory cytokines via several signaling pathways and play a crucial role in maintaining the host's health via various actions (Sassone-Corsi et al., 2016). Bacteriocin-producing bacteria exhibit resistance to these antimicrobial peptides due to the presence of the immunity proteins on the cell membrane. There are three classes of bacteriocin, Class 1 referred to as lantibiotics, has a molecular weight of less than 5 kDa. They undergo posttranslational modifications, resulting in methyllanthionine formation. On the other hand, Class 2 is categorized as non-lantibiotics. They are heat stable and have a molecular weight of less than 10 kDa (Nishie et al., 2012). Class III comprises bacteriocins that are heat-stable, with a molecular weight of more than 30 kDa. Examples of Class III bacteriocins include enterolysin (Yang et al., 2014).

Bacteriocins have efficiency against several pathogens, making them beneficial in combating numerous human infectious disorders as shown in Table 2. Nisin has great efficacy in treating meningitis, and sepsis resulting from *Streptococcus pneumonia*. The cyclic bacteriocin griselimycin successfully eradicated tuberculosis (Anjana and Tiwari, 2022). Nisin inhibits cancer through ion channel formation on the cell membrane, resulting in the release of lactate dehydrogenase, and mitochondrial respiration disruption in cancer cells. Nisin, when used along with cancer medications, has been shown to have a synergistic effect in eliminating tumors (Preet et al., 2015).

Gut-Brain Axis and Microbial Community

The gut microbiota responds to the pattern of chemical messengers in the central nervous system (CNS) by secretion of different compounds (Anjana and Tiwari, 2022). The vagus nerve, which is tactile to deviated fibers, terminates in the nuclei within the brain stem. The brain stem nuclei may therefore affect a variety of bowel functions and transmit gestures to further CNS zones, including the cerebral cortex and midbrain (Wang and Wang, 2016)**.** Systemic blood flow can potentially facilitate an exchange between the central nervous system and the colon (Gibson and Mehler, 2019). The immunological and endocrine systems participate in duplex transmission halfway and sideways along the brain and microbiota axis (Borre et al., 2014). First, bacteria can replace, combine, and degrade neurotransmitters as well as transmodulators (Anjana and Tiwari, 2022). Additionally, cytokines and activator B cells in the host are produced by the gut microbiota, which operates as a harmful alternative to CNS (Alam et al., 2017). Therefore, through a variety of routes including antibody-mediated neuronal and endocrine systems, microbiota can affect the microbiota-gut-brain axis. Destruction, hypertension, and other coherent disorders may result from these neurological alterations in the brain. Changes in the gut microbiota have been linked to a number of neurological conditions. These include refractory epilepsy and neurodegenerative diseases (Nagpal et al., 2018).

Table 2: LAB involved in different bacterial disease treatment and immune modulation

Lactic acid bacteria	Bacteriocins	Target Pathogen	Animal Model	Reference
L. lactis	NisinZ	Immunomodulatory effect	Murine	(Millette et al., 2008)
L. lactis	NisinF	Respiration effect	Murine	(De Kwaadsteniet et al., 2009)
L. lactis	NisinZ	Enteric pathogen	Mouse	(Millette et al., 2008)
L. lactis	Nisin	Stress reduction	Mouse	(Jia et al., 2018a)
Lactobacillus salivarius	BacteriocinAbp118	Listeriosis	Murine	(Riboulet-Bisson et al., 2012)
L.salivarius NRRLB	BacteriocinOR-7	*Campylobacter jejuni*	Chicken	(Ilinskaya et al., 2017)
Pediococcus acidilactici	PediocinPA1	Listeriosis	Murine	(Dabour et al., 2009)
Enterococcus Mundtii RL35	EnterocinCRL35	Listeriosis	Murine	(Salvucci et al., 2012)

As of right now, no concrete data support the involvement of LAB in the gut-brain axis. On the other hand, some strains have the potential to modify the gut microbiota and hence indirectly impact the gut-brain axis. Some sequencing analyses revealed the correlation between the gut microbiota and the neurochemicals that influence communication between the gut and the brain (Jia et al., 2018a).

Immune Modulation

The immune system defends the gastrointestinal tract against invading microorganisms. The immune system primarily consists of mucosa-associated lymphoid tissue (MALT), and the epithelial layer. Interaction between dendritic cells and *Lactobacilli* in the human gastrointestinal tract activates the adaptive immune system, leading to the production of pro and anti-inflammatory cytokines. Probiotic strains' antigenic fragments have been exposed to M cells in Peyer's patches and intestinal epithelial cells, therefore modulating both the innate and adaptive immune system. Probiotic bacteria also play a crucial role in immunoregulation by mediating the signal formation that activates other immune cells through intestinal immune system stimulation. Probiotics lead to more IgA production which may stimulate interactions between dendritic cells and B and/or lymphocytic T cells (Gareau et al., 2010a)

The *Bifidobacterium breve* boosts the maturation and survival of the dendritic cells. The extended lifespan of dendritic cells (DC) is attributed to heightened amounts of antiapoptotic protein, ultimately enhancing the expression of CD86 and CD83 maturation markers. It plays a role in enhancing the immune response by increasing the ability of the antigen-presenting cells to stimulate the differentiation of the T-cells (Hoarau et al., 2006). Dendritic cells safeguard viable microbiota of the gut and transport germs to "mesenteric lymph nodes," leading to the generation of antibodies that protect against mucosal invasion (Macpherson et al., 2005; Macpherson and Uhr, 2004). The naïve T cells differentiated into several cell lineages, based on the interaction between dendritic cells (DCs) and certain pattern recognition factors. Cytophage-Bacteroides are necessary for the TH17 development in the lamina propria, contributing to maintaining the balance between regulatory T-cell populations and TH-17 cells (Delcenserie et al., 2008; Foligne et al., 2007). Nisin had an immunomodulatory impact, and prolonged treatment of nisin may potentially restore the equilibrium between B and T lymphocyte levels (Shin et al., 2016).

Role of Probiotics in the Treatment of Diseases

The gut microbiota dysbiosis leads to the development of multiple chronic illnesses, such as arthralgia, immune-mediated disorders, metabolic abnormalities, hepatic conditions, and different gastrointestinal ailments (Carding et al., 2015). The potential functions of probiotics in preventing diseases are shown in Figure 2**.** Bacteriocins produced by lactic acid bacteria and probiotics themselves could influence the composition of the host's microbiota, and immune system and contribute to the treatment of many diseases (Anjana and Tiwari, 2022)**.**

Colonic Infections

Bacteriocins-producing probiotics inhibit certain foodborne and clinical pathogens that cause serious illnesses. Bacteriocins function as pore formers, disrupting the potential of the cell and causing ATP to be expelled, ultimately resulting in the death of the cell. Therefore, the bacteriocins produced by LAB have the potential to be used as a substitute for conventional antibiotics in infection treatment (Li et al., 2022; Pérez-Ramos et al., 2021b; Sheoran and Tiwari, 2021). *Clostridium difficile* is the primary pathogenic bacteria responsible for colonic infection. The nisin exhibited a specific ability to deplete *C. difficile*. It is challenging to manipulate the fecal microbial ecology without impacting the existing gut microbiota (Papaconstantinou and Thomas, 2007). The GJ7 Kimchicin is manufactured by *L. Citreum* GJ7 suppressed the growth of *S. typhi* bacteria (Chang and Chang, 2011). The bacteriocin BM1829 is generated by the bacterium *L. Crustorum* MN047 suppressing the growth of *S. typhi* (Yan et al., 2021).

During the current era, viral infections have led to the occurrence of serious health issues. Several antiviral medicines have been developed and tried, and they have lately been shown to be effective in treating these infections. Nevertheless, these treatments exhibited toxicity and failed to achieve full symptom reduction. Bacteriocins have antiviral properties against many viruses by inhibiting the glycoprotein production during the last phase of virus replication (Anjana and Tiwari, 2022). Rotavirus, norovirus, and adenovirus are the primary causes of acute gastroenteritis in children under the age of 5. Rotavirus is a non-enveloped virus with double-stranded RNA that specifically targets and damages the epithelial cell lining in babies, leading to the development of diarrhea (Li et al., 2022).

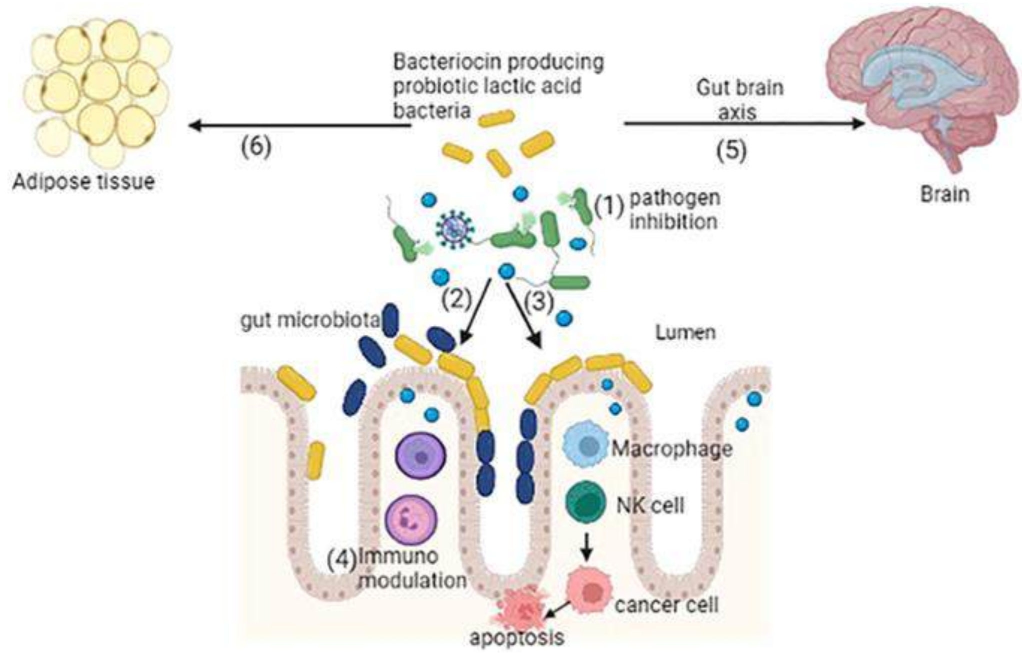

Fig. 1: Representing several potential functions of the Bacteriocin-producing probiotic lactic acid bacteria (1) Inhibition, (2) Colonization, (3) Immune cell activation, (4) Immune modulation, (5) Gut-Brain axis balancing, (6) Antiobesity Activity (Anjana and and Tiwari, 2022)

Bacteriocin prevents the several enzymatic activities that are essential to viral infection (Salman et al., 2020). The *Enterococcus faecium* CRL35 bacteria produces enterocin CRL35, which prevents the herpes simplex virus (Wachsman et al., 1999). Among enteric viruses, the norovirus possesses single-stranded RNA. After infection with the norovirus, the person has less Bacteroidetes and more proteobacteria. Influence on the gut microbiota composition is shown by the direct interaction between norovirus and proteobacteria isolated from feces samples. Furthermore, *L. casei* BL23 may prevent the virus's P-particles from attaching to epithelial cells (Salman et al., 2020).

Inflammatory Bowel Disease (IBD)

Inflammatory Bowel disease includes Crohn's disease (CD) and ulcerative colitis (UC). IBD is a chronic gastrointestinal disorder. It is currently unknown what causes inflammatory bowel disease. Depression, microbiological imbalance, and poor diet often make the problem worse. Still, a limited number of intestinal bacteria, like *Mycobacterium avium*, *C. concisus*, and *E. Coli*, are thought to be involved in the development of IBD (Toumi et al., 2021). The complicated origins of chronic inflammatory diseases, ulcerative colitis (UC), and Crohn's disease (CD) include hereditary genetics, environmental triggers, immune system changes, and aberrant gut microbiome response. A patient with dysbiosis has an imbalance of bacteria associated with certain diseases (Sidhu and van der Poorten, 2017). Although it is a potential treatment for IBD, fecal microbiota transplantation has limited effectiveness (Colman and Rubin, 2014).

Probiotics have been demonstrated to be effective and tolerable for people with IBD; nonetheless, it is unknown what bacteriocin's precise role and mechanism are. Inflammatory bowel disease (IBD) patients have a different microbiome than healthy individuals have (Shadnoush et al., 2015). Proteobacteria and Actinobacteria increased whereas Firmicutes, like *Faecalibacterium prausnitzi*, and Bacteroidetes decreased during IBD. To fight with infections, it is therefore essential to stabilise the gut flora. Probiotics that generate bacteriocins might be rather helpful as they encourage the development of a normal healthy microbiota (Gourbeyre et al., 2011). Probiotic supplementation, however, has been shown to be less successful in treating Crohn's disease than ulcerative colitis. Disturbance in gut microbiota leads to mucus layer changes, which increases the ability of bacteria to travel into the intestines, thereby triggering an immune response (Sicard et al., 2017). Bacteriocin directly kills or inhibits the pathogen, therefore preserving the structural integrity of the gut epithelium. It may also serve as a colonizing normal microbiota, helping *L. Reuteri*, a gut-dwelling bacterium species that generates reuterin, occupy niches in the intestine. Fungi and viruses are among the pathogenic microbes, inhibited by reuterin, and facilitate beneficial bacteria growth. Inflammatory bowel disease, probiotics, and the products of them, including short-chain fatty acids, significantly affect the immune system's reaction and the disturbance of the intestinal microbiological balance. The acidophilus strain isolated from breast milk reduced cholesterol, displayed competitive interactions with intestinal bacteria, and inhibited the growth of human colorectal adenocarcinoma cells (HT-29) (Toumi et al., 2021).

Colorectal Cancer

Colorectal cancer occurs when cells in the colon or rectum grow out of control. It is also known as "colon cancer." The colon is a big gut or bowel. The rectum is a canal that links the colon with the anus. The large intestine sections, the rectum, and the colon are the particular targets of colorectal cancer. The two main symptoms of this disease are a discernible drop in body weight and blood in the stool. The result depends on a number of factors, such as food, way of life, and the aging process itself (Center et al., 2009). *L. acidophilus* strengthens the immune response against colorectal cancer. Among the substances released by probiotic bacteria include toxins, enzymes, and bacteriocins, all of which have been shown to combat cancer. The bacteria *Lactococcus lactis* synthesizes nisin A. In liver hepatocellular carcinoma (HepG2) lactis changes the structural integrity of the cell membrane and inhibits the growth of cancer cells. Nisin opens up the cell membrane and, via an innate process, starts apoptosis. Its ability to slow down the proliferation of melanoma cells also makes it an antimetastatic medication (Norouzi et al., 2018). Moreover, *P. acidolactici* K2a2-3 produces pediocin, which inhibits the development of human colon cancer cells (HT29) (Taherikalani and Ghafourian, 2021). Microcin causes caspase activation, phosphatidylserine release, DNA damage, and depolarization of the cell membrane (Baindara et al., 2018). Moreover, pediocin—produced by the bacterium *Pediococcus acidilactici* K2a2-3—was able to stop cancer cells. The dose of pediocin affect its ability to stop the proliferation of cancer cells. This suggests that colorectal cancer therapy may include bacteriocin, either directly or indirectly (Kaur and Kaur, 2015).

Obesity

It is one of the metabolic disorders closely associated with dysbiosis, an imbalance in the gut flora. Obesity is also influenced by a number of different variables including a poor lifestyle, hormonal imbalances, brain chemistry, and genetic and epigenetic modifications. The complex pathophysiology of obesity may help to explain why clinical obesity therapy is a significant public health policy concern. Although bariatric surgery has been shown to reduce body weight and treat comorbidities associated with obesity, it is a highly intrusive process. Adverse events are highly likely, and pediatric groups have safety concerns. Therefore, it would appear that the most effective way to treat obesity would involve a multimodal strategy that includes medication, behavior therapy, food modifications, and physical activity (Cerdó et al., 2019).

The gut microbiota is an important mediator between the host and the environment, controlling both fat deposition and energy homeostasis and probiotics help to control the flora of the stomach. Gut peptide signaling and neurological system modulation are two ways that gut bacteria affect calorie intake and satiety. The regulatory signaling peptide's balance may be upset if the gut flora changes. Thus, the re-establishing of the intestinal flora could be able to cure obesity. (Turnbaugh et al., 2008). Bacteriocin-producing probiotics reduce adipocyte size, increase the expression of genes linked to fatty acid oxidation, and reduce fatty acid absorption. *L. Plantarum* increases TNFα production and controls leptin hormone release. Probiotics control bacterial composition by generating bacteriocin, which affects obesity. Research is still needed to determine the best way to provide probiotics for the prevention or treatment of obesity, including the dosage, length of treatment, and long-term benefits of the various strains. (Million et al., 2013).

Adverse Effect

Probiotics are usually considered to be safe, however recent research has shown that they might not be the best choice for certain patient populations. For instance, probiotic-using children with short bowel syndrome and central venous catheters have sporadic reports of bacteremia, sepsis, and meningitis (Barton et al., 2001; Land et al., 2005). These people are more likely to experience the translocation of microorganisms, such as probiotic strains of live bacteria and fungi used in clinical settings. A probiotic mixture of six bacteria was administered intraduodenally to patients with severe pancreatitis who were receiving care in an intensive care unit (van Minnen et al., 2007). This human experiment did not lower the incidence of infectious complications of acute pancreatitis, despite preliminary animal research suggesting a possible benefit. Moreover, the population taking the probiotic formulation was found to have a markedly higher chance of poor outcomes. Necrotizing jejunitis was seen in the deceased patients. This discovery suggests that the splanchnic circulation may have already been weakened and that the proximal intestine may have been directly exposed to a large concentration of bacteria. Nonetheless, it is still unclear if using living organisms in high quantities puts some patient populations at an excessive risk of developing severe side effects, such as sepsis (Gareau et al., 2010b).

Conclusion

The bacteriocins are the small cationic peptides, produced by different lactic acid bacteria that has the ability to kill different pathogenic bacteria by pore formation. They had antimicrobial activity against many pathogenic bacteria of the human intestine including *Salmonella*, *Listeria*, *Clostridium*, and *Enterococcus*. They are also effective against diseases caused by viruses like noroviruses, rotavirus, etc. The gut is a very important human body part that helps in food digestion and stabilizing different functions. Most diseases of the gut are caused by an imbalance in the gut microbiota, i.e., IBD and obesity. Probiotics help in regaining the normal microbiota by providing food competition to pathogenic bacteria and the production of bacteriocin which has an antimicrobial affect on the pathogenic bacteria as well as they also boost the immunity of the host against pathogens.

REFERENCES

Alam, R., Abdolmaleky, H. M., and Zhou, J. (2017). Microbiome, inflammation, epigenetic alterations, and mental diseases. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 174*(6), 651–660. https://doi.org/10.1002/ajmg.b.32567

Anjana, and Tiwari, S. K. (2022). Bacteriocin-producing probiotic lactic acid bacteria in controlling dysbiosis of the gut microbiota. *Frontiers in Cellular and Infection Microbiology, 12*, Article 851140. https://doi.org/10.3389/fcimb.2022.851140

Arakawa, K., Yoshida, S., Aikawa, H., Hano, C., Bolormaa, T., Burenjargal, S., and Miyamoto, T. (2016). Production of a bacteriocin-like inhibitory substance by *Leuconostoc mesenteroides* subsp. *Dextranicum 213M0* isolated from Mongolian fermented mare milk, airag. *Animal Science Journal, 87*(3), 449–456. https://doi.org/10.1111/asj.12445

Baindara, P., Korpole, S., and Grover, V. (2018). Bacteriocins: Perspective for the development of novel anticancer drugs. *Applied Microbiology and Biotechnology, 102*, 10393–10408. https://doi.org/10.1007/s00253-018-9420-8

Barton, L. L., Rider, E. D., and Coen, R. W. (2001). Bacteremic infection with *Pediococcus*: Vancomycin-resistant opportunist. *Pediatrics, 107*(4), 775–776. https://doi.org/10.1542/peds.107.4.775

Binda, S., Hill, C., Johansen, E., Obis, D., Pot, B., Sanders, M. E., Tremblay, A., and Ouwehand, A. C. (2020). Criteria to qualify microorganisms as "probiotic" in foods and dietary supplements. *Frontiers in Microbiology, 11*, Article 1662. https://doi.org/10.3389/fmicb.2020.01662

Borre, Y. E., Moloney, R. D., Clarke, G., Dinan, T. G., and Cryan, J. F. (2014). The impact of microbiota on brain and behavior: mechanisms and therapeutic potential *Advance Experiment Medicine Biology*. https://doi.org/10.1007/978-1-4939-0897-4_17

Carding, S., Verbeke, K., Vipond, D. T., Corfe, B. M., and Owen, L. J. (2015). Dysbiosis of the gut microbiota in disease. *Microbial Ecology in Health and Disease, 26*(0). https://doi.org/10.3402/mehd.v26.26191

Center, M. M., Jemal, A., Smith, R. A., and Ward, E. (2009). Worldwide variations in colorectal cancer. *CA: A Cancer Journal for Clinicians, 59*(6), 366–378. https://doi.org/10.3322/caac.20038

Cerdó, T., García-Santos, J. A., Bermúdez, M. G., and Campoy, C. (2019). The role of probiotics and prebiotics in the prevention and treatment of obesity. *Nutrients, 11*(3), Article 3. https://doi.org/10.3390/nu11030635

Chang, J. Y., and Chang, H. C. (2011). Growth inhibition of foodborne pathogens by kimchi prepared with bacteriocin-producing starter culture. *Journal of Food Science, 76*(1). https://doi.org/10.1111/j.1750-3841.2010.01965.x

Chi, H., and Holo, H. (2018). Synergistic antimicrobial activity between the broad spectrum bacteriocin garvicin KS and nisin, farnesol and polymyxin B against gram-positive and gram-negative bacteria. *Current Microbiology, 75*(3), 272–277. https://doi.org/10.1007/s00284-017-1375-y

Choi, K.-O., Nguyen, H.-H., and Kwak, H.-S. (2010). The role of the immune system in the use of probiotic lactic acid bacteria in preventing and treating allergic diseases. *Food Science of Animal Resources, 30*(1), 1–12. https://doi.org/10.5851/kosfa.2010.30.1.1

Colman, R. J., and Rubin, D. T. (2014). Fecal microbiota transplantation as therapy for inflammatory bowel disease: A systematic review and meta-analysis. *Journal of Crohn's and Colitis, 8*(12), 1569–1581. https://doi.org/10.1016/j.crohns.2014.06.008

Dabour, N., Zihler, A., Kheadr, E., Lacroix, C., and Fliss, I. (2009). In vivo study on the effectiveness of pediocin PA-1 and *Pediococcus acidilactici* UL5 at inhibiting *Listeria monocytogenes*. *International Journal of Food Microbiology, 133*(3), 225–233. https://doi.org/10.1016/j.ijfoodmicro.2009.05.005

D'Amelio, P., and Sassi, F. (2018). Gut microbiota, immune system, and bone. *Calcified Tissue International, 102*(4), 415–425. https://doi.org/10.1007/s00223-017-0331-y

de Arauz, L. J., Jozala, A. F., Mazzola, P. G., and Penna, T. C. V. (2009). Nisin biotechnological production and application: A review. *Trends in Food Science and Technology, 20*(3–4), 146–154. https://doi.org/10.1016/j.tifs.2009.01.056

De Kwaadsteniet, M., Doeschate, K. T., and Dicks, L. M. T. (2009). Nisin F in the treatment of respiratory tract infections caused by *Staphylococcus aureus*. *Letters in Applied Microbiology, 48*(1), 65–70. https://doi.org/10.1111/j.1472-765X.2008.02495.x

Delcenserie, V., Martel, D., Lamoureux, M., Amiot, J., Boutin, Y., and Roy, D. (2008). Immunomodulatory effects of probiotics in the intestinal tract. *Current Issues in Molecular Biology, 10*(1–2), 37–54. https://doi.org/10.21775/cimb.010.037

Foligne, B., Zoumpopoulou, G., Dewulf, J., Ben Younes, A., Chareyre, F., Sirard, J.-C., Pot, B., and Grangette, C. (2007). A key role of dendritic cells in probiotic functionality. *PloS One, 2*(3), e313. https://doi.org/10.1371/journal.pone.0000313

Fong, W., Li, Q., and Yu, J. (2020). Gut microbiota modulation: A novel strategy for prevention and treatment of colorectal cancer. *Oncogene, 39*(26), 4925–4943. https://doi.org/10.1038/s41388-020-1341-1

Gareau, M. G., Sherman, P. M., and Walker, W. A. (2010a). Probiotics and the gut microbiota in intestinal health and disease. *Nature Reviews Gastroenterology and Hepatology, 7*(9), 503–514. https://doi.org/10.1038/nrgastro.2010.117

Gareau, M. G., Sherman, P. M., and Walker, W. A. (2010b). Probiotics and the gut microbiota in intestinal health and disease. *Nature Reviews Gastroenterology and Hepatology, 7*(9), 503–514. https://doi.org/10.1038/nrgastro.2010.117

Gibson, D., and Mehler, P. S. (2019). Anorexia nervosa and the immune system—A narrative review. *Journal of Clinical Medicine, 8*(11), 1915. https://doi.org/10.3390/jcm8111915

Gourbeyre, P., Denery, S., and Bodinier, M. (2011). Probiotics, prebiotics, and synbiotics: Impact on the gut immune system and allergic reactions. *Journal of Leukocyte Biology, 89*(5), 685–695. https://doi.org/10.1189/jlb.1109753

Goyal, C., Malik, R. K., and Pradhan, D. (2018). Purification and characterization of a broad spectrum bacteriocin produced by a selected *Lactococcus lactis* strain 63 isolated from Indian dairy products. *Journal of Food Science and Technology, 55*(9), 3683–3692. https://doi.org/10.1007/s13197-018-3298-4

Harris, L. J., Daeschel, M. A., Stiles, M. E., and Klaenhammer, T. R. (1989). Antimicrobial activity of lactic acid bacteria against *Listeria monocytogenes*. *Journal of Food Protection, 52*(6), 384–387. https://doi.org/10.4315/0362-028X-52.6.384

Hoarau, C., Lagaraine, C., Martin, L., Velge-Roussel, F., and Lebranchu, Y. (2006). Supernatant of *Bifidobacterium breve* induces dendritic cell maturation, activation, and survival through a Toll-like receptor 2 pathway. *Journal of Allergy and Clinical Immunology, 117*(3), 696–702. https://doi.org/10.1016/j.jaci.2005.12.1353

Ilinskaya, O. N., Ulyanova, V. V., Yarullina, D. R., and Gataullin, I. G. (2017). Secretome of intestinal Bacilli: A natural guard against pathologies. *Frontiers in Microbiology, 8*, Article 1666. https://doi.org/10.3389/fmicb.2017.01666

Karpiński, T. M., and Szkaradkiewicz, A. K. (2013). Characteristic of bacteriocines and their application. *Polish Journal of Microbiology, 62*(3), 223–235. https://doi.org/10.33073/pjm-2013-030

Kaur, S., and Kaur, S. (2015). Bacteriocins as potential anticancer agents. *Frontiers in Pharmacology, 6*, Article 272. https://doi.org/10.3389/fphar.2015.00272

Kim, S. G., Becattini, S., Moody, T. U., Shliaha, P. V., Littmann, E. R., Seok, R., Gjonbalaj, M., Eaton, V., Fontana, E., and Amoretti, L. (2019). Microbiota-derived lantibiotic restores resistance against vancomycin-resistant enterococcus. *Nature, 572*(7771), 665–669. https://doi.org/10.1038/s41586-019-1501-z

Kwok, L. Y., Guo, Z., Zhang, J., Wang, L., Qiao, J., Hou, Q., Zheng, Y., and Zhang, H. (2015). The impact of oral consumption of *Lactobacillus plantarum* P-8 on faecal bacteria revealed by pyrosequencing. *Beneficial Microbes, 6*(4), 405–414. https://doi.org/10.3920/BM2014.0063

Land, M. H., Rouster-Stevens, K., Woods, C. R., Cannon, M. L., Cnota, J., and Shetty, A. K. (2005). Lactobacillus sepsis associated with probiotic therapy. *Pediatrics, 115*(1), 178–181. https://doi.org/10.1542/peds.2004-2137

Lange, K., Buerger, M., Stallmach, A., and Bruns, T. (2016). Effects of antibiotics on gut microbiota. *Digestive Diseases, 34*(3), 260–268. https://doi.org/10.1159/000443360

Li, Q., Yu, S., Han, J., Wu, J., You, L., Shi, X., and Wang, S. (2022). Synergistic antibacterial activity and mechanism of action of nisin/carvacrol combination against *Staphylococcus aureus* and their application in the infecting pasteurized milk. *Food Chemistry, 380*, Article 132009. https://doi.org/10.1016/j.foodchem.2021.132009

Macpherson, A. J., Geuking, M. B., and McCoy, K. D. (2005). Immune responses that adapt the intestinal mucosa to commensal intestinal bacteria. *Immunology, 115*(2), 153–162. https://doi.org/10.1111/j.1365-2567.2005.02159.x

Macpherson, A. J., and Uhr, T. (2004). Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science, 303*(5664), 1662–1665. https://doi.org/10.1126/science.1091334

Millette, M., Cornut, G., Dupont, C., Shareck, F., Archambault, D., and Lacroix, M. (2008). Capacity of human nisin- and pediocin-producing lactic acid bacteria to reduce intestinal colonization by vancomycin-resistant enterococci. *Applied and Environmental Microbiology, 74*(7), 1997–2003. https://doi.org/10.1128/AEM.02150-07

Million, M., Lagier, J.-C., Yahav, D., and Paul, M. (2013). Gut bacterial microbiota and obesity. *Clinical Microbiology and Infection, 19*(4), 305–313. https://doi.org/10.1111/1469-0691.12172

Mokoena, M. P. (2017). Lactic acid bacteria and their bacteriocins: Classification, biosynthesis and applications against uropathogens: A mini-review. *Molecules, 22*(8), Article 1255. https://doi.org/10.3390/molecules22081255

Nagpal, R., Mainali, R., Ahmadi, S., Wang, S., Singh, R., Kavanagh, K., Kitzman, D. W., Kushugulova, A., Marotta, F., and Yadav, H. (2018). Gut microbiome and aging: Physiological and mechanistic insights. *Nutrition and Healthy Aging, 4*(4), 267–285. https://doi.org/10.3233/NHA-170030

Nishie, M., Nagao, J.-I., and Sonomoto, K. (2012). Antibacterial peptides "bacteriocins": An overview of their diverse characteristics and applications. *Biocontrol Science, 17*(1), 1–16. https://doi.org/10.4265/bio.17.1

Norouzi, Z., Salimi, A., Halabian, R., and Fahimi, H. (2018). Nisin, a potent bacteriocin and anti-bacterial peptide, attenuates expression of metastatic genes in colorectal cancer cell lines. *Microbial Pathogenesis, 123*, 183–189. https://doi.org/10.1016/j.micpath.2018.07.013

O'Callaghan, A., and Van Sinderen, D. (2016). Bifidobacteria and their role as members of the human gut microbiota. *Frontiers in Microbiology, 7*, Article 206360. https://doi.org/10.3389/fmicb.2016.00925

Papaconstantinou, H., and Thomas, J. (2007). Bacterial colitis. *Clinics in Colon and Rectal Surgery, 20*(1), 18–27. https://doi.org/10.1055/s-2007-970196

Pérez-Ramos, A., Madi-Moussa, D., Coucheney, F., and Drider, D. (2021a). Current knowledge of the mode of action and immunity mechanisms of LAB-bacteriocins. *Microorganisms, 9*(10), Article 2107. https://doi.org/10.3390/microorganisms9102107

Pérez-Ramos, A., Madi-Moussa, D., Coucheney, F., and Drider, D. (2021b). Current knowledge of the mode of action and immunity mechanisms of LAB-bacteriocins. *Microorganisms, 9*(10), Article 2107. https://doi.org/10.3390/microorganisms9102107

Preet, S., Bharati, S., Panjeta, A., Tewari, R., and Rishi, P. (2015). Effect of nisin and doxorubicin on DMBA-induced skin carcinogenesis—A possible adjunct therapy. *Tumor Biology, 36*(11), 8301–8308. https://doi.org/10.1007/s13277-015-3571-3

Rattanachaikunsopon, P., and Phumkhachorn, P. (2010). Lactic acid bacteria: Their antimicrobial compounds and their uses in food production. https://www.cabidigitallibrary.org/doi/full/10.5555/20113034130

Riboulet-Bisson, E., Sturme, M. H., Jeffery, I. B., O'Donnell, M. M., Neville, B. A., Forde, B. M., Claesson, M. J., Harris, H., Gardiner, G. E., and Casey, P. G. (2012). Effect of *Lactobacillus salivarius* bacteriocin Abp118 on the mouse and pig intestinal microbiota. *PloS One, 7*(2), e31113. https://doi.org/10.1371/journal.pone.0031113

Rodríguez, J. M., Martínez, M. I., and Kok, J. (2002). Pediocin PA-1, a wide-spectrum bacteriocin from lactic acid bacteria. *Critical Reviews in Food Science and Nutrition, 42*(2), 91–121. https://doi.org/10.1080/10408690290825475

Salman, J. A.-S., Mahmood, N. N., Abdulsattar, B. O., and Abid, H. A. (2020). The effectiveness of probiotics against viral infections: A rapid review with focus on SARS-CoV-2 infection. *Open Access Macedonian Journal of Medical Sciences, 8*(T1), 496–508. https://doi.org/10.3889/oamjms.2020.5483

Salvucci, E., Saavedra, L., Hebert, E. M., Haro, C., and Sesma, F. (2012). Enterocin CRL35 inhibits *Listeria monocytogenes* in a murine model. *Foodborne Pathogens and Disease, 9*(1), 68–74. https://doi.org/10.1089/fpd.2011.0972

Sassone-Corsi, M., Nuccio, S.-P., Liu, H., Hernandez, D., Vu, C. T., Takahashi, A. A., Edwards, R. A., and Raffatellu, M. (2016). Microcins mediate competition among *Enterobacteriaceae* in the inflamed gut. *Nature, 540*(7632), 280–283. https://doi.org/10.1038/nature20557

Shadnoush, M., Hosseini, R. S., Khalilnezhad, A., Navai, L., Goudarzi, H., and Vaezjalali, M. (2015). Effects of probiotics on gut microbiota in patients with inflammatory bowel disease: A double-blind, placebo-controlled clinical trial. *Korean Journal of Gastroenterology, 65*(4), 215–221. https://doi.org/10.4166/kjg.2015.65.4.215

Sheoran, P., and Tiwari, S. K. (2021). Synergistically-acting enterocin LD3 and plantaricin LD4 against gram-positive and gram-negative pathogenic bacteria. *Probiotics and Antimicrobial Proteins, 13*(2), 542–554. https://doi.org/10.1007/s12602-020-09708-w

Shin, J. M., Gwak, J. W., Kamarajan, P., Fenno, J. C., Rickard, A. H., and Kapila, Y. L. (2016). Biomedical applications of nisin. *Journal of Applied Microbiology, 120*(6), 1449–1465. https://doi.org/10.1111/jam.13033

Sicard, J.-F., Le Bihan, G., Vogeleer, P., Jacques, M., and Harel, J. (2017). Interactions of intestinal bacteria with components of the intestinal mucus. *Frontiers in Cellular and Infection Microbiology, 7*, Article 387. https://doi.org/10.3389/fcimb.2017.00387

Sidhu, M., and van der Poorten, D. (2017). The gut microbiome. *Australian Family Physician, 46*(4), 206–211. https://doi.org/10.3316/informit.741870194405168

Stevens, M., Vollenweider, S., Lacroix, C., and Zurich, E. T. H. (2011). The potential of reuterin produced by *Lactobacillus reuteri* as a broad spectrum preservative in food. In *Protective Cultures, Antimicrobial Metabolites and Bacteriophages for Food and Beverage Biopreservation* (pp. 129–160). Elsevier. https://doi.org/10.1533/9780857090522.1.129

Taherikalani, M., and Ghafourian, S. (2021). Anticancer properties of colicin E7 against colon cancer. *Gastroenterology Review/Przegląd Gastroenterologiczny, 16*(4), 364–368. https://doi.org/10.5114/pg.2021.110458

Toumi, R., Samer, A., Soufli, I., Rafa, H., and Touil-Boukoffa, C. (2021). Role of probiotics and their metabolites in inflammatory bowel diseases (IBDs). *Gastroenterology Insights, 12*(1), 56–66. https://doi.org/10.3390/gastroent12010006

Turnbaugh, P. J., Bäckhed, F., Fulton, L., and Gordon, J. I. (2008). Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host and Microbe, 3*(4), 213–223. https://doi.org/10.1016/j.chom.2008.02.015

Umu, Ö. C. O., Rudi, K., and Diep, D. B. (2017). Modulation of the gut microbiota by prebiotic fibres and bacteriocins. *Microbial Ecology in Health and Disease, 28*(1), Article 1348886. https://doi.org/10.1080/16512235.2017.1348886

van Minnen, L. P., Timmerman, H. M., Lutgendorff, F., Verheem, A., Harmsen, W., Konstantinov, S. R., Smidt, H., Visser, M. R., Rijkers, G. T., and Gooszen, H. G. (2007). Modification of intestinal flora with multispecies probiotics reduces bacterial translocation and improves clinical course in a rat model of acute pancreatitis. *Surgery, 141*(4), 470–480. https://doi.org/10.1016/j.surg.2006.12.013

Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Gancel, F., Kempf, I., and Drider, D. (2019). Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Frontiers in Microbiology, 10*, Article 57. https://doi.org/10.3389/fmicb.2019.00057

Wachsman, M. B., Farías, M. E., Takeda, E., Sesma, F., de Ruiz Holgado, A. P., de Torres, R. A., and Coto, C. E. (1999). Antiviral activity of enterocin CRL35 against herpesviruses. *International Journal of Antimicrobial Agents, 12*(4), 293–299. https://doi.org/10.1016/S0924-8579(99)00078-3

Wang, H.-X., and Wang, Y.-P. (2016). Gut microbiota-brain axis. *Chinese Medical Journal, 129*(19), 2373–2380. https://doi.org/10.4103/0366-6999.190667

Wong, F. W. F., Ariff, A. B., Abbasiliasi, S., and Stuckey, D. C. (2017). Recovery of a bacteriocin-like inhibitory substance from *Pediococcus acidilactici* Kp10 using surfactant precipitation. *Food Chemistry, 232*, 245–252. https://doi.org/10.1016/j.foodchem.2017.04.015

Xue, L., He, J., Gao, N., Lu, X., Li, M., Wu, X., Liu, Z., Jin, Y., Liu, J., and Xu, J. (2017). Probiotics may delay the progression of nonalcoholic fatty liver disease by restoring the gut microbiota structure and improving intestinal endotoxemia. *Scientific Reports, 7*(1), Article 45176. https://doi.org/10.1038/srep45176

Yan, H., Lu, Y., Li, X., Yi, Y., Wang, X., Shan, Y., Liu, B., Zhou, Y., and Lü, X. (2021). Action mode of bacteriocin BM1829 against *Escherichia coli* and *Staphylococcus aureus*. *Food Bioscience, 39*, Article 100794. https://doi.org/10.1016/j.fbio.2020.100794

Yang, S.-C., Lin, C.-H., Sung, C. T., and Fang, J.-Y. (2014). Antibacterial activities of bacteriocins: Application in foods and pharmaceuticals. *Frontiers in Microbiology, 5*, Article 241. https://doi.org/10.3389/fmicb.2014.00241

Zhang, Y.-J., Li, S., Gan, R.-Y., Zhou, T., Xu, D.-P., and Li, H.-B. (2015). Impacts of gut bacteria on human health and diseases. *International Journal of Molecular Sciences, 16*(4), 7493–7519. https://doi.org/10.3390/ijms16047493

Chapter 03

Probiotics and Innovative Feed Additives for Optimal Gut Health

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ABSTRACT

The livestock industry relies heavily on maintaining optimal intestinal health for maximizing productivity. However, concerns regarding antibiotic resistance and environmental impact have prompted the search for alternative feed additives. This comprehensive review explores the latest advancements in feed additives and their impact on animal health and sustainability. Various categories of feed additives are examined, including natural additives such as herbs and phytochemicals, technological additives like organic acids, and alternatives to antibiotic growth promoters such as tannins, Dicarboxylic acids, and ionophores. Additionally, the potential of phytogenic feed additives, Phytases, and nonstructural polysaccharides in enhancing nutrient utilization and animal health is discussed. Probiotics have emerged as a promising alternative to antibiotics, promoting gut microbiota balance and enhancing immune function. Recent research highlights the efficacy of probiotics in improving animal performance while reducing reliance on antibiotics. Furthermore, advancements in feed additives aim to mitigate environmental impact, with additives like zeolites and phyllosilicates showing excellent results in reducing enteric methane emissions and improving feed conversion efficiency.

KEYWORDS		
Gut health, Feed additives, Antibiotics, AGPs, Phytases, Tannins, Dicarboxylic acids	Received: 18-May-2024 Revised: 12-June-2024 Accepted: 02-August-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Production of animals is the most important aspect of the economy, and maintaining intestinal health is crucial to their peak performance. The intestine is composed of a single layer of cells known as the intestinal epithelium (IEC). Maintaining an optimal state ensures proper nutrient absorption and digestion, intestinal barrier integrity, and gut bacteria balance (Ji et al., 2019). The GIT functions include defense against pathogens and non-pathogens, transportation of digested and ingested feed along the GIT, energy, and nutrient absorption, secretion of endogenous materials, hosting of intestinal microbiota, and excretion of digested feed and metabolic waste (Yegani and Korver, 2008). The gastrointestinal tract's homeostasis is developed and maintained by a variety of physiological and functional elements that make up gut health. These include the mucus layer's growth and maintenance, the control of barrier function, the synchronization of energy generation and host metabolism, appropriate nutritional uptake and digestion, and a variety of mucosal immunological responses (Kogut and Arsenault, 2016). It takes much more than simply probiotics and prebiotics to modify the gut microflora to maintain or improve "gut health”. In contrast to commensal bacteria, which are essential to host health and metabolism, which might have detrimental consequences directly or indirectly, pathogenic bacteria interact with their host, with themselves, and with the host's nutrition (Yadav and Jha, 2019). Therefore, anything that impacts the condition of the gut will surely have an effect on the animal as a whole, changing its requirements for and ability to absorb nutrients. As a result, "gut health" is a very complicated terminology that includes the immune system's state, the micro- and macro-structural integrity of the gut, and the balance of micro flora (Kelly and Conway, 2001). To ensure optimal gut function feed additives must be used which include antibiotics, prebiotics, probiotics, enzymes, organic compounds etc. Antibiotics have been used extensively in livestock management to reduce disease, enhance performance, and boost output. Furthermore, probiotic, prebiotic, postbiotic, and symbiotic supplements have become the most widely utilized antibiotic substitutes (Hamasalim, 2016). Throughout the world, animal feed additives are used to improve feed utilization, support growth performance, and give livestock especially poultry vital nutrients. Industry norms and customer awareness are driving an increase in demand for natural and non-residual substitutes. Additives to herbal feed, such as ascorbic acid, prebiotics, probiotics, and herbal extracts, have therapeutic properties that improve immune-stimulant activity, digestibility, antibacterial, anti-inflammatory, and antioxidant traits. The key to sustained cow production is standardizing dose schedules (Pallauf and Müller, 2006).

Feed Additives

Feed additives are the products used in animal nutrition to improve the quality of feed and food obtained from animals or to improve the animals' overall health and performance. Increasing the digestibility of the feed ingredients is one of the examples. Sensory additives are a type of additive used to make a diet more appealing, thus encouraging voluntary consumption. They usually work by changing the diet's flavor or color. For instance, additives like vanilla extract can stimulate piglets to consume their feed. To improve the appeal of feed and zootechnical performance, sensory feed additives often include aromatic herbs, spices, and essential oils. These are commonly used in pig farming to stimulate consumption and promote growth. This approach leverages the natural appeal of these substances to enhance the effectiveness of animal feed (Clouard and Val-Laillet, 2014). Nutritional additives are additives that give animals the precise nutrition they need for healthy growth. A vitamin, amino acid, or trace minerals are a few examples. Vitamins have catalytic properties that aid in the synthesis of nutrients, regulating metabolism and impacting the well-being and productivity of poultry. Vitamin-supplemented diets are essential for the treatment and prevention of disease because they enable an animal to use proteins and energy for growth, reproduction, FCR, and overall health (Whitehead, 2002). Zoo technical additives are the additives that supply nutrients and facilitate the more effective utilization of the nutrients already present in the feed. These additions enhance the nutrient status and productivity of the livestock. An example of such an additive would be a direct-fed microbial product or an enzyme, both of which improve digestive tract conditions, making it possible to take nutrients from food more efficiently. Coccidiostats and histomonostats are the chemicals that have a direct influence on poultry health management. These substances are not categorized as antibiotics and are used to regulate the intestinal health of chickens. They work directly on the parasite organisms that live in the intestines (Amit Kumar Pandey, 2019). Technological additives are A set of additives that affect the feed's technological features are included in this classification. These additions may have an indirect impact on the feed's nutritional value by enhancing its handling or hygienic qualities, but they have no direct effect on it. An organic acid for feed preservation is an example of such an addition (Amit Kumar Pandey, 2019).

Importance of Feed Additives in Animal Health

Natural Feed Additives

Natural feed additives are important for nutrition and overall wellness. The emergence of microbial resistance to antibiotic medications and the ensuing health effects on humans, as well as consumer demands that animal diets be devoid of all non-plant xenobiotic substances, have led to an increased interest in the use of natural feed additives in livestock production(Muneendra Kumar, 2014). Feed additives enhance the flavor of farm animal's feed, and as a result, they can affect the feeding habits, digestive fluid output, and overall amount of feed consumed. Through their antimicrobial properties or by favorably stimulating the eubiosis of the micro biota, herbs or phytochemicals can specifically affect microorganisms. Most natural feed additives work by denaturing and coagulating proteins in the bacterial cell wall, which is how they have their antibacterial action(Muneendra Kumar, 2014).

Antibiotics as Feed Additives

Antibiotics are a class of natural, semi-synthetic, or chemical compounds that have anti-microbial activity. They are widely used to treat and prevent infectious diseases in humans and animals, and they are also added to animal feed as growth promoters to aid in the animals' development (Apata, 2009). Antibiotics are frequently used as a treatment, preventative measure, and growth stimulant. Because antibiotics have generally enhanced chicken performance both economically and effectively, farmers and the economy as a whole view the use of antibiotics in poultry and cattle production as beneficial (Apata, 2009). Animal welfare, quality and growth efficiency, feed efficiency boosters, economic output, carcass quality, and public health were the primary reasons why antibiotics were used in livestock production (Van Boeckel et al., 2015). Antibiotic growth promoters reduced the populations of potentially harmful bacteria such as Salmonella, E. coli, and Clostridium perfringens and enhanced their growth performance (ME, 2011). Feed additives enhance animals' energy balance by focusing on four key objectives: (i) shifting methane production towards propionate; (ii) minimizing protein degradation in feed to boost amino acid availability in the small intestine; (iii) slowing down the breakdown of rapidly fermentable carbohydrates like starch and sucrose, while managing lactic acid levels; and (iv) optimizing fiber digestion. Ionophore antibiotics in the rumen have been effective in achieving these goals, particularly in reducing acid production and preventing lactic acidosis when consistently given at low concentrations (20–40 p.p.m.) in meals (Osborne et al., 2004).They decreased the deamination of amino acids, which increased the passage of peptides from the rumen into the small intestine and decreased foamy bloat in cattle feeding on legume pastures; these actions alleviated methane generation by redirecting metabolic H use towards propionate production. There have also been reports of ionophores' post-ruminal effects. They have demonstrated efficacy against coccidiosis, for instance (Gallardo et al., 2005; Wallace, 1990).

Negative Effect of Antibiotics as Growth Promoters

The development of "antibiotic alternatives" has been spurred by concern about the growing number of bacteria that are resistant to antibiotics as a result of the overuse of antibiotics and a decline in the number of innovative antibiotics (Cheng et al., 2014). The reduced activity of bile salt hydrolyase, an enzyme generated by gut bacteria that negatively affects host fat digestion and utilization, was linked to the growth-promoting action of antibiotics (Lin, 2014). Concerns about the emergence of resistant bacteria and the potential for these bacteria to spread from animals to people have been provoked by the overuse of antibiotics. Resistance to drugs that were never used on farms is among the multidrug-resistant (MDR) infections that are associated with non-therapeutic antimicrobial usage (Cheng et al., 2014). The health and feed efficiency of farm animals has improved due to the use of antibiotics as growth promoters in commercial animal production, which has increased overall growth performance by about 18% (Duckett and Pratt, 2014).

Alternatives to Antibiotic Feed Additives

Concerns about the use of antibiotics as feed additives in animal agriculture are growing among the scientific community and the public. Many human pathogenic microorganisms are becoming resistant to antibiotics, which is a cause for concern (Manero et al., 2006; Parveen et al., 2006). The possibility that meals derived from animals may include antibiotic residues that promote growth. Due to all these factors, the European Union (EU) decided, on September 22, 2003, by EU regulation no. 1831/2003 of the European Parliament and the Council, that the use of antibiotics in cattle as production enhancers would be prohibited as of January 1, 2006. This prohibition essentially puts an end to the non-therapeutic usage of antibiotics for almost 50 years. It includes all types of antibiotics, including ionophores, a class of compounds widely employed in chicken production as coccidiostats and in ruminant agriculture as growth promoters or productivity enhancers (Gallardo et al., 2005; McDougall et al., 2004; Melendez et al., 2006).To lessen the risk of drug resistance in human health, and experts have searched for natural substitutes for feed additives.

Tannins

Plants rich in tannins have been explored as potential ruminant feed additives. These plants, high in protein and available during hot, dry seasons when other feeds are scarce, play a crucial role in animal nutrition (Yang et al., 2015). Many forage species contain high levels of tannins, a type of plant-based phenolic compound (Han-Chung Lee, 2005; Makkar, 2003). Tannins are naturally occurring secondary plant chemicals that have varying molecular weights. They are found in nearly all vascular plants and are typically given to ruminants(Wang et al., 2015). There are two types of tannins: condensed tannins (CT) and hydrolyzable tannins (HT).

Particularly, tannins have strong anti-bloat properties that prevent proteins from being broken down by the rumen and lower intestinal parasites, urine nitrogen excretion, and enteric methane emission (greenhouse gas). These benefits can then be transferred to increased milk production, wool growth, immunological responses, and reproductive efficiency (Attia et al., 2016; Aufrère et al., 2012; Min and Hart, 2003; Ramírez-Restrepo and Barry, 2005; Waghorn, 2008).

Dicarboxylic Acid

Additives to acidifier feed are thought to be essential for promoting rumen fermentation, which enhances animal health, productivity, and product quality. Acidifiers are often used as alternatives to antibiotic development marketers because of their ability to create an ideal digestive environment for beneficial microbes that may lead to increased nutritional digestibility, increased growth performance, and decreased diarrhea (Vassilis Papatsiros and Billinis, 2012). Since they function as "hydrogen sinks" during the conversion of Dicarboxylic acids e.g. aspartate, malate, and fumarate to propionate, some of the significant acidifying chemicals that dairy producers now utilize have been studied for use as feed additives (Newbold and Rode, 2006).

Ionophore

The most researched and utilized substances in cow diets are ionophores, which are primarily used to optimize fermentation pathways, change the ruminal microbiota, and lower the incidence of digestive problems (Duffield et al., 2012; Nagaraja and Lechtenberg, 2007; Tedeschi et al., 2003). Ionophores are naturally occurring antibiotics that are carboxylic polyethers, generated by a strain of Streptomyces spp. Utilizing ionophores to alter the ruminal environment and fermentation dynamics also enhances the absorption of protein and dietary energy (McGuffey et al., 2001; Russell and Strobel, 1989; Weimer et al., 2008). As a result of the diet's inclusion of rapidly fermentable carbohydrates, ionophores also help to prevent bloat and the buildup of short-chain fatty acids (SCFA), which includes lactic acid (acidosis) (Nagaraja and Lechtenberg, 2007; Tedeschi et al., 2003). As a result, ionophores have been utilized to enhance beef cattle's health, ruminal fermentation characteristics, and performance. Ionophores in diets improve feed efficiency and performance in ruminants by modulating the rumen microbiome and fermentation routes, increasing energy and nitrogen efficiency metabolism. However, their effects may vary depending on dosage, animal, and diet. In feedlot diets, ionophores improve body weight gain and reduce feed intake, while in forage-based diets, they increase body weight gain but increase feed intake (Tedeschi et al., 2003).

Phytogenic Feed Additives

An increasing amount of research has demonstrated that adding plant-based feed additives or phytogenic feed additives (PFAs) to diets improves zoo technical and animal health indicators. This suggests the potential of PFAs in animal nutrition. Herbs, spices, essential oils, and non-volatile extracts from plants like clove, anise, thyme, fennel, or Melissa, among many others, are examples of phytogenic compounds used in PFAs (Steiner and Shah, 2015). A primary benefit of PFAs is thought to be enhanced digestibility and feed conversion. PFAs affect several parameters, such as the release of digestive juices and enzymes, immune system modulation, alterations in intestinal morphology, and enhanced nutrition utilization, all of which lead to increased performance. Reduced amounts of microbial metabolites in the digestive tract as a result of intestinal microbiota stabilization soothe the immune system and increase the amount of energy available for muscle accretion (Steiner and Syed, 2015).

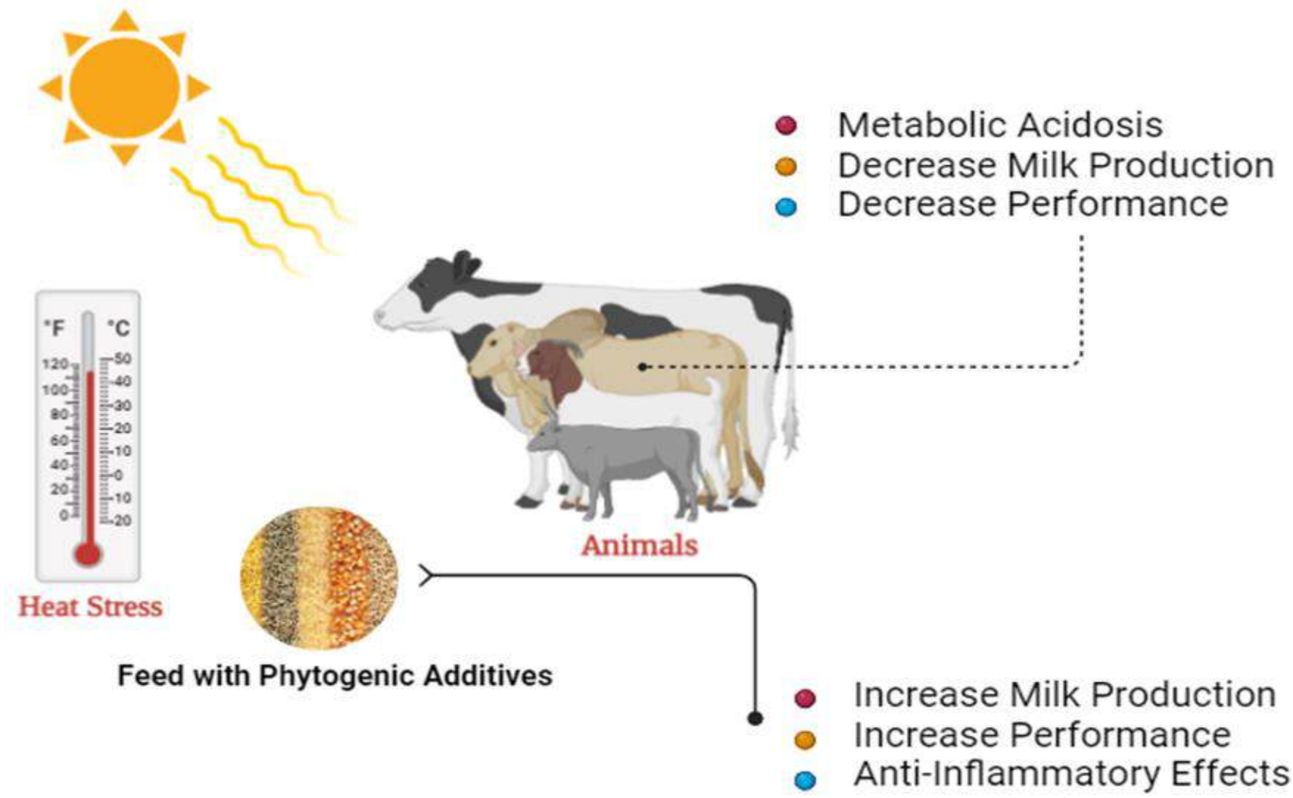

Fig. 1: Effect of phytogenic feed additives on the heat stressed animals (Swelum et al., 2021)

Phytases

Enzymes may cut feed costs by enhancing feed use, which will result in less feed being consumed. Enzymes are also necessary to enhance the sustainability of meat and egg production due to the higher feed utilization efficiency(Bundgaard et al., 2014). Phytase is added to animal feed to counteract the antinutritional effect of phytate, increase the availability of myoinositol, decrease phosphate emissions to the environment, and make use of an existing phosphorus source in the feed (Selle and Ravindran, 2007). The usage of phytate has increased after the use of animal protein sources, such as meat and bone meal, was prohibited. In poultry, it raises the digestion of phytate from about 25% to 50–70% (Papadopoulos and Lioliopoulou, 2023).

Nonstructural Polysaccharides (NSPs)

Nonstructural polysaccharide includes all plant polysaccharide except starch. NSPs can make up as much as 90% of a plant's cell wall (Selvendran and Robertson, 2005), are primarily found in plant cell walls as structural polysaccharides where they are associated with and/or substituted for other polysaccharides, proteins, and phenolic compounds like lignin (Kumar et al., 2012). Typically, they make up less than 10% of the grain's weight. The three NSPs that are most prevalent in plant cell walls are cellulose, hemicelluloses, and pectin; fructans, glucomannans, and galactomannans are the storage polysaccharides that are part of the less prevalent NSP category. When combined with water, soluble NSPs can create dispersions and increase the viscosity of digesta; insoluble NSPs, on the other hand, cannot do this but are distinguished by their capacity for fecal-bulking(Davidson and McDonald, 1998; Habte-Michael Habte-Tsion, 2018). Cereal-based diets have high concentrations of soluble NSPs, which have a negative impact on animal performance and the effectiveness of the digestive system. Animal diets are frequently supplemented with marketed exogenous enzyme combinations, which include NSP enzymes, to lessen the adverse effects of dietary NSPs. These enzymes can partially hydrolyze NSPs, lower the viscosity of the gut's contents, and enhance the digestion and absorption of nutrients (Almirall et al., 1995; Bedford and Apajalahti, 2001; Sternemalm et al., 2008). By decreasing undigested substrates and antinutritive factors and maybe by generating oligosaccharides from dietary NSPs with possible prebiotic effects, dietary added enzymes can benefit the ecology of the digestive microbial population (Habte-Tsion and Kumar, 2018).

Probiotics for Optimal Animal Health

The use of growth promotants and antibiotics, however, has raised concerns about the emergence of food-borne allergies, an increase of bacteria resistant to these drugs, and the harm that these substances due to the environment, including runoff from agriculture. Moreover, growing concern among consumers regarding the impact of growth promotants and antibiotics on human health is a factor that is still being discussed. Researchers employed Probiotics supplementation as a substitute, either as a single strain or multiple strains in the diet of animals to solve this issue (Lipsitch et al., 2002).

Probiotics are defined as mono or mixed strains of living microorganisms that, when used appropriately, confer a desirable health benefit on the host (*FAO/WHO. Guidelines for the Evaluation of Probiotics in Food.*, 2002). A microorganism is considered probiotic if it is nonpathogenic, able to produce a viable cell count, beneficial to the host's health, and improves intestinal tract functioning. Probiotics must meet certain requirements to be used and stored: (i) Probiotic bacteria must be prepared in a viable way and on a large scale; (ii) they must be able to survive in the digestive tract; (iv) the probiotics must have both direct and indirect positive effects on the host (better intestinal microflora); and (v) their safety must be obvious (Vanbelle et al., 1990). Probiotics can be made as capsules, paste, powder, granules, fermented feed, pelleted feed, and more. It has recently been suggested that inactivated bacteria should be broadly classified as probiotics since they too have probiotic effects, especially immunological ones (Tsukahara T, 2005).

Common Probiotics

Lactobacillus acidophilus, Lactobacillus lactis, Lactobacillus plantarum, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus helveticus, Lactobacillus salivarius, Bifido bacteria, Enterococcus faecium, Enterococcus faecalis, Streptococcus thermophilus, Escherichia coli bacteria, and other probiotic fungi like *Saccharomyces cerevisiae* and *Saccharomyces boulardii* are the most widely used probiotics(McFarland, 2006; Naseem et., 2023). The assertion is that microbial products enhance performance and feed conversion for the targeted species, lower morbidity or mortality, and benefit consumers by improving the quality of the product. Genetically modified organisms (GMOs) are being used for biomedical purposes in a novel way, with recombinant probiotics and alternative gene therapy as their basis. There are no clinical adverse effects from probiotic therapy.

Mechanism of Action of Probiotics

Since Lactobacillus and Bifidobacterium are not pathogenetic, they are typically regarded as beneficial bacteria for health. As a result, fostering these beneficial bacteria may enhance host health. The use of live bacterial supplements improves the intestinal microbial equilibrium of the host animal, which has a positive impact on the animal's health(Ohashi and Ushida, 2009).

Pathogens struggle to survive in the gut because probiotics compete with them for nutrition and receptor-binding sites. Short-chain fatty acids (SCFA), organic acids, hydrogen peroxide, and bacteriocins are produced by probiotics and serve as anti-microbial agents, reducing the number of harmful bacteria in the gut. Probiotics also help the intestinal barrier.

Function by promoting the synthesis of mucin proteins, controlling the expression of tight junction proteins like claudin 1 and occluding, and controlling the immunological response within the gut (Latif et al., 2023).

Probiotics have several important methods of action, including modifying the gut microbiota by feeding some helpful bacteria and preventing harmful bacteria from colonizing the gut, which preserves the integrity of the gut mucosa. Probiotics provide a food supply for host-beneficial bacteria like Lactobacillus (LAB) and Bifidobacteria in the lower GIT rather than being digested or absorbed in the upper GIT(Adhikari and Kim, 2017).In the end, this prevents infections, such as Salmonella, from attaching and fosters gut flora. Certain sugars can prevent infections from adhering to the mucosa. For instance, MOS can attach to the mannose-specific lectin of gram-negative bacteria, including E. coli that produces Type-1 fimbriae, causing the bacteria to be expelled from the intestine. Yeast and the outer cell of yeast are common sources of MOS. It has been discovered that MOS alters the immune system and gets rid of infections in the digestive tract. (Adhikari and Kim, 2017)

Recent Advancements in the Feed Additives

As grazers, livestock production also contributes significantly to the restoration of carbon (C) to grassland ecosystems, as well as to the improvement of biodiversity and wildlife habitat. Therefore, strategies for reducing enteric CH4 emissions must be developed without compromising cattle output. In addition to adding to the greenhouse gas emissions from the production of livestock, enteric CH4 emissions also represent an energy loss of up to 11% of the gross energy intake from food (Moraes et al., 2014). Enteric CH4 emissions can be effectively reduced by changing the diet and adding feed additives. Since feed additives may be more economical, they might end up being an effective approach (Roque et al., 2019). Feed additives can reduce enteric CH4 emissions from ruminants by directly interfering with the methanogenesis process, which prevents the production of CH4. Chemical inhibitors are the term for these additives (Kelly and Kebreab, 2023). Numerous studies have demonstrated that adding zeolites to the diet increases feed conversion and/or average daily gain in pigs, sheep, and broiler chickens. Zeolites also improve sows' ability to reproduce, raise dairy cows' milk yields and laying hens' egg output, and have positive impacts on egg weight and internal egg features(Filippidis et al., 1996; Mumpton and Fishman, 1977). Zeolites supplementation appears to be an effective, supplementary, supportive strategy in the prevention of certain diseases and the improvement of animals' health condition, aside from the good impacts on animals' performance(Placinta et al., 1999). Because they have layered crystalline structures and comparable physicochemical properties to zeolites, phyllosilicates like bentonite and hydrated sodium calcium aluminosilicates (HSCAS) have been successfully applied to poultry, pigs, sheep, cattle, and lab animals for this reason (Papaioannou et al., 2005) represented in table 1.

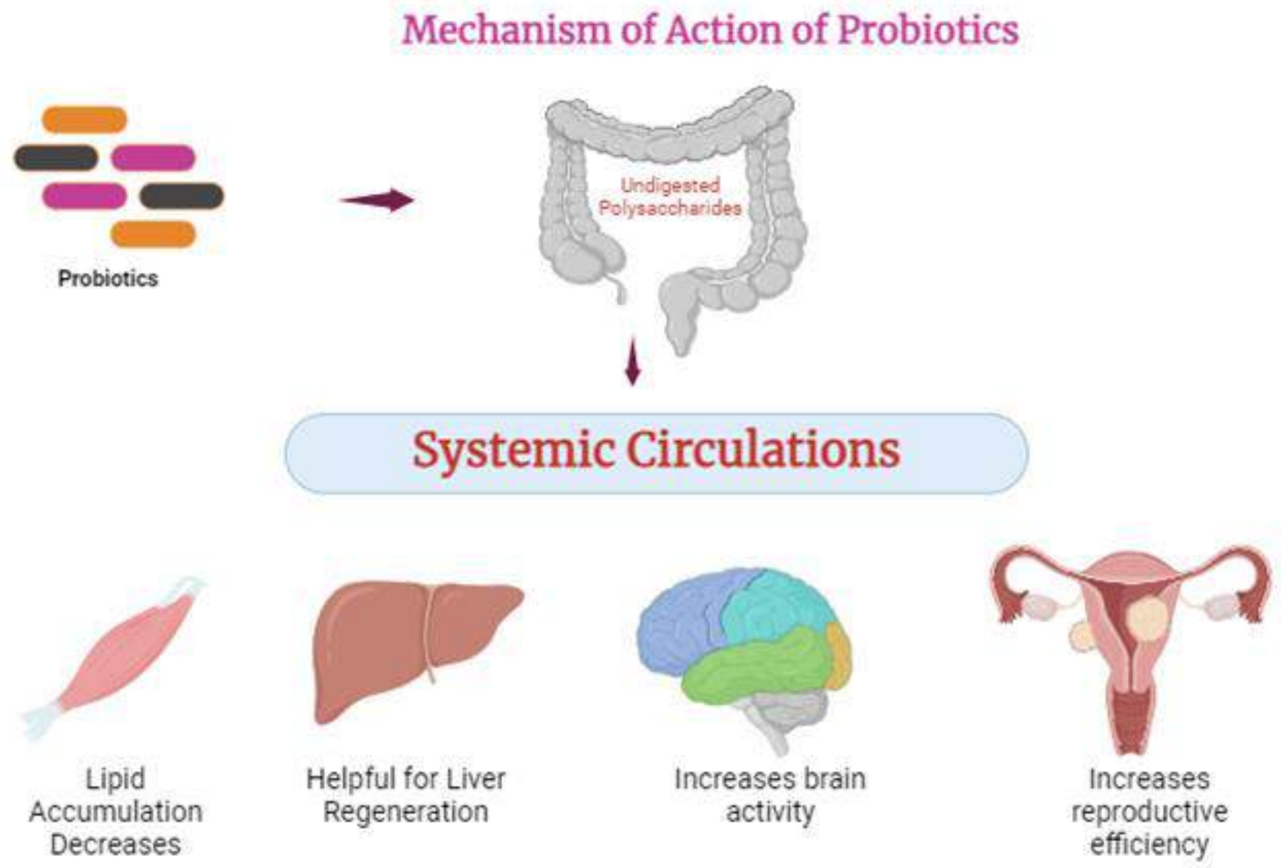

Fig. 2: Mechanism of action of probiotics.

Table 1: Effects of Various Feed Additives on Ruminant Health and Performance

Feed additive	Source	Effects	References
Essential oils	Anise, thymol, eugenol, cinnamon	Decrease protozoa Increase *S.ruminatum, R.albus, B.fibrisolvens* fungi	(Cardozo et al., 2006; Fraser et al., 2007)
Condensed tannins	*Calliandra calothyrsus*, waterdock roots, persimmon fruit	Decreased cellulolytic and proteolytic bacteria, strong antiparasitic properties	(McSweeney et al., 2001; Mueller-Harvey et al., 2019)
Saponins	Chinese chives, tea, yam tubers	Decreased protozoa, decreased CH4, improved feed digestion and ruminal fermentation	(Ramdani et al., 2023)
Ionophores	Monensin, lasalocid, narasin, salinomycin	Improve ruminal fermentation, decreased acidosis and bloat, reduced methane production	(de Sales Silva et al.)
Probiotics	*Aspergillus oryzea, S. cerevisea,B.cereus, E.facieum*	Increase weight gain and feed conversion, decrease incidence of diarrhea	(Al-Jaf and Del, 2019)

Conclusion

In the commercial livestock industry, ensuring sustainable animal production requires addressing several important issues, including environmental protection, public acceptance, consumer safety, animal welfare, and sustainability. Protein, phosphate, and water resources can be maintained, contaminants can be decreased, and performance can be improved by raising feed conversion. So, further advancements in Supplements containing probiotics, prebiotics, and probiotics lead to the stimulation of animal development, boosting immunological function and improving health in animals. Probiotics such as Lactobacillus and Bifidobacterium help to maintain gut microbiota. The development of animals' digestive tracts depends on pro- and prebiotics, metabolic modifiers, and antibacterial agents. Metabolic modifiers affect the metabolism of antimicrobial drugs, but pro- and prebiotics have distinct effects on digestive processes. Enzymes enhance general health, decrease digestive problems, and facilitate digestion. They can be applied to elderly animals to improve on-site feedstuffs with high dietary fiber content or nutrients that are poorly digested, while also reducing nutrient release. Additive feeding appears to have a promising future. To boost meat production, conserve feed, and resist disease, nutritionists are always creating new and improved additives.

REFERENCES

Adhikari, P. A., and Kim, W. K. (2017). Overview of prebiotics and probiotics: focus on performance, gut health and immunity-a review. *Annals of Animal Science*, *17*(4), 949.

Al-Jaf, K. A. H., and Del, Y. K. (2019). Effect of different feed additives on growth performance and production in livestock. *International Journal Agriculture Forestory*, *9*(1), 16-31.

Almirall, M., Francesch, M., Perez-Vendrell, A. M., Brufau, J., and Esteve-Garcia, E. (1995). The Differences in Intestinal Viscosity Produced by Barley and β-Glucanase Alter Digesta Enzyme Activities and Ileal Nutrient Digestibilities More in Broiler Chicks than in Cocks123. *The Journal of Nutrition*, *125*(4), 947-955. https://doi.org/https://doi.org/10.1093/jn/125.4.947

Amit Kumar Pandey, P. K., and Saxena, M. J. (2019). Feed Additives in Animal Health. *Nutraceuticals in Veterinary Medicine*, 345-362. https://doi.org/https://doi.org/10.1007/978-3-030-04624-8_23 (Springer International Publishing)

Apata, D. (2009). Antibiotic resistance in poultry. *International Journal of Poultry Science*, *8*(4), 404-408.

Attia, M., Nour El-Din, A., Elzarkouny, S., El-Zaiat, H., Zeitoun, M., Sobhy, M., and Sallam, S. (2016). Impact of Quebracho Tannins Supplementation on Productive and Reproductive Efficiency of Dairy Cows. *Open Journal of Animal Sciences*, *6*, 269-288. https://doi.org/10.4236/ojas.2016.64032

Aufrère, J., Dudilieu, M., Andueza, D., Poncet, C., and Baumont, R. (2012). Mixing sainfoin and lucerne to improve the feed value of legumes fed to sheep by the effect of condensed tannins. *Animal : an International Journal of Animal Bioscience*, *7*, 1-11. https://doi.org/10.1017/S1751731112001097

Bedford, M., and Apajalahti, J. (2001). Microbial interactions in the response to exogenous enzyme utilization. *CABI*, 299–314. https://doi.org/10.1079/9780851993935.0299

Bundgaard, A. M., Dalgaard, R., Gilbert, C., and Thrane, M. (2014). Assessment of the potential of digestibility-improving enzymes to reduce greenhouse gas emissions from broiler production. *Journal of Cleaner Production*, *73*, 218-226. https://doi.org/https://doi.org/10.1016/j.jclepro.2013.12.055

Cardozo, P. W., Calsamiglia, S., Ferret, A., and Kamel, C. (2006). Effects of alfalfa extract, anise, capsicum, and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high-concentrate diet. *Journal Animal Science*, *84*(10), 2801-2808. https://doi.org/10.2527/jas.2005-593

Cheng, G., Hao, H., Xie, S., Wang, X., Dai, M., Huang, L., and Yuan, Z. (2014). Antibiotic alternatives: the substitution of antibiotics in animal husbandry? *Frontiers in Microbiology*, *5*, 217.

Clouard, C., and Val-Laillet, D. (2014). Impact of sensory feed additives on feed intake, feed preferences, and growth of female piglets during the early postweaning period. *Journal of Animal Science*, *92*(5), 2133-2140.

Davidson, M. H., and McDonald, A. (1998). Fiber: Forms and functions. *Nutrition Research*, *18*(4), 617-624. https://doi.org/https://doi.org/10.1016/S0271-5317(98)00048-7

de Sales Silva, F. A., de Castro Silva, B., Lopes, S. A., Millen, D. D., Berchielli, T. T., Borges, A. L. D. C. C., Prados, L. F., Chizzotti, M. L., Pacheco, M. V. C., and e Silva, F. F. Feed aditives for beef cattle.

Duckett, S. K., and Pratt, S. L. (2014). MEAT SCIENCE AND MUSCLE BIOLOGY SYMPOSIUM--anabolic implants and meat quality. *Journal Animal Science*, *92*(1), 3-9. https://doi.org/10.2527/jas.2013-7088

Duffield, T. F., Merrill, J. K., and Bagg, R. N. (2012). Meta-analysis of the effects of monensin in beef cattle on feed efficiency, body weight gain, and dry matter intake1. *Journal of Animal Science*, *90*(12), 4583-4592. https://doi.org/10.2527/jas.2011-5018

FAO/WHO (2002). Guidelines for the Evaluation of Probiotics in Food.

Filippidis, A., Godelitsas, A., Charistos, D., Misaelides, P., and Kassoli-Fournaraki, A. (1996). The chemical behavior of natural zeolites in aqueous environments: Interactions between low-silica zeolites and 1 M NaCl solutions of different initial pH-values. *Applied Clay Science*, *11*(2-4), 199-209.

Fraser, G. R., Chaves, A. V., Wang, Y., McAllister, T. A., Beauchemin, K. A., and Benchaar, C. (2007). Assessment of the effects of cinnamon leaf oil on rumen microbial fermentation using two continuous culture systems. *Journal Dairy Science*, *90*(5), 2315-2328. https://doi.org/10.3168/jds.2006-688

Gallardo, M. R., Castillo, A. R., Bargo, F., Abdala, A. A., Maciel, M. G., Perez-Monti, H., Castro, H. C., and Castelli, M. E. (2005). Monensin for Lactating Dairy Cows Grazing Mixed-Alfalfa Pasture and Supplemented with Partial Mixed Ration. *Journal of Dairy Science*, *88*(2), 644-652. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(05)72728-4

Habte-Michael Habte-Tsion, V. K., and Ross, W. (2018). *Perspectives of nonstarch polysaccharide enzymes in nutrition*. acedemic press. https://doi.org/doi.org/10.1016/B978-0-12-805419-2.00011-3

Habte-Tsion, H.-M., and Kumar, V. (2018). Chapter 9 - Nonstarch polysaccharide enzymes—general aspects. In C. S. Nunes and V. Kumar (Eds.), *Enzymes in Human and Animal Nutrition* (pp. 183-209). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-805419-2.00009-5

Hamasalim, H. J. (2016). Synbiotic as Feed Additives Relating to Animal Health and Performance. *Advances in Microbiology*, *6*(4), 288-302. https://doi.org/http://dx.doi.org/10.4236/aim.2016.64028

Han-Chung Lee, S.-S. C., and Shang-Tzen Chang, (2005). Antifungal property of the essential oils and their constituents from Cinnamomum osmophloeum leaf against tree pathogenic fungi. *Science of the Food and Agriculture*, *85*(12), 2047-2053. https://doi.org/https://doi.org/10.1002/jsfa.2216

Hossam, H., and Azzaz, H. (2015). Utility of Ionophores for Ruminant Animals: A Review. *Asian Journal of Animal Sciences*, *9*(6), 254-265. https://doi.org/ 10.3923/ajas.2015.254.265

Ji, F. J., Wang, L. X., Yang, H. S., Hu, A., and Yin, Y. L. (2019). Review: The roles and functions of glutamine on intestinal health and performance of weaning pigs. *Animal*, *13*(11), 2727-2735. https://doi.org/https://doi.org/10.1017/S1751731119001800

Kelly, D., and Conway, S. (2001). Genomics at work: the global gene response to enteric bacteria. *Gut*, *49*(5), 612-613. https://doi.org/10.1136/gut.49.5.612

Kelly, L., and Kebreab, E. (2023). Recent advances in feed additives with the potential to mitigate enteric methane emissions from ruminant livestock. *Journal of Soil and Water Conservation*, *78*(2), 111-123.

Kogut, M. H., and Arsenault, R. J. (2016). Editorial: Gut Health: The New Paradigm in Food Animal Production [Editorial]. *Frontiers in Veterinary Science*, *3*. https://doi.org/10.3389/fvets.2016.00071

Kumar, V., Sinha, A. K., Makkar, H. P. S., de Boeck, G., and Becker, K. (2012). Dietary Roles of Non-Starch Polysachharides in Human Nutrition: A Review. *Critical Reviews in Food Science and Nutrition*, *52*(10), 899-935. https://doi.org/10.1080/10408398.2010.512671

Latif, A., Shehzad, A., Niazi, S., Zahid, A., Ashraf, W., Iqbal, M. W., Rehman, A., Riaz, T., Aadil, R. M., Khan, I. M., Özogul, F., Rocha, J. M., Esatbeyoglu, T., and Korma, S. A. (2023). Probiotics: mechanism of action, health benefits and their application in food industries. *Front Microbiol*, *14*, 1216674. https://doi.org/10.3389/fmicb.2023.1216674

Lin, J. (2014). Antibiotic growth promoters enhance animal production by targeting intestinal bile salt hydrolase and its producers. *Frontier Microbiology*, *5*, 33. https://doi.org/10.3389/fmicb.2014.00033

Lipsitch, M., Singer, R. S., and Levin, B. R. (2002). Antibiotics in agriculture: When is it time to close the barn door? *Proceedings of the National Academy of Sciences*, *99*(9), 5752-5754. https://doi.org/doi:10.1073/pnas.092142499

Makkar, H. P. S. (2003). Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research*, *49*(3), 241-256. https://doi.org/https://doi.org/10.1016/S0921-4488(03)00142-1

Manero, A., Vilanova, X., Cerdà-Cuéllar, M., and Blanch, A. R. (2006). Vancomycin- and erythromycin-resistant enterococci in a pig farm and its environment. *Environmental Microbiology*, *8*(4), 667-674. https://doi.org/https://doi.org/10.1111/j.1462-2920.2005.00945.x

McDougall, S., Young, L., and Anniss, F. M. (2004). Production and Health of Pasture-Fed Dairy Cattle Following Oral Treatment with the Ionophore Lasalocid. *Journal of Dairy Science*, *87*(9), 2967-2976. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(04)73428-1

McFarland, L. V. (2006). Meta-Analysis of Probiotics for the Prevention of Antibiotic Associated Diarrhea and the Treatment of Clostridium difficile Disease. *Official Journal of the American College of Gastroenterology | ACG*, *101*(4), 812-822. https://journals.lww.com/ajg/fulltext/2006/04000/meta_analysis_of_probiotics_for_the_prevention_of.24.aspx

McGuffey, R. K., Richardson, L. F., and Wilkinson, J. I. D. (2001). Ionophores for Dairy Cattle: Current Status and Future Outlook. *Journal of Dairy Science*, *84*, E194-E203. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(01)70218-4

McSweeney, C. S., Palmer, B., Bunch, R., and Krause, D. O. (2001). Effect of the tropical forage calliandra on microbial protein synthesis and ecology in the rumen. *Journal Appliedd Microbiology*, *90*(1), 78-88. https://doi.org/10.1046/j.1365-2672.2001.01220.x

Me, H. (2011). Histiric perspective: prebiotics, probiotics, and other altenatives to antibiotics. *Poultry Science*, *90*, 2663-2669.

Melendez, P., Goff, J. P., Risco, C. A., Archbald, L. F., Littell, R. C., and Donovan, G. A. (2006). Effect of administration of a controlled-release monensin capsule on incidence of calving-related disorders, fertility, and milk yield in dairy cows. *America Journal Veterinary Research*, *67*(3), 537-543. https://doi.org/10.2460/ajvr.67.3.537

Min, B., and Hart, S. (2003). Tannins for suppression of internal parasites. *81*.

Moraes, L. E., Strathe, A. B., Fadel, J. G., Casper, D. P., and Kebreab, E. (2014). Prediction of enteric methane emissions from cattle. *Glob Chang Biology*, *20*(7), 2140-2148. https://doi.org/10.1111/gcb.12471

Mueller-Harvey, I., Bee, G., Dohme-Meier, F., Hoste, H., Karonen, M., Kölliker, R., Lüscher, A., Niderkorn, V., Pellikaan, W. F., and Salminen, J.-P. (2019). Benefits of condensed tannins in forage legumes fed to ruminants: Importance of structure, concentration, and diet composition. *Crop Science*, *59*(3), 861-885.

Mumpton, F., and Fishman, P. (1977). The application of natural zeolites in animal science and aquaculture. *Journal of Animal Science*, *45*(5), 1188-1203.

Muneendra Kumar, V. K., Debashis Roy, Raju Kushwaha, Shalini Vaiswani. (2014). appilcation of herbal feed additives in animal nutrition. *International Journal of Livestock Research*.

Naseem, C., Bano, M., Hussain, A., Mujahid, U., Maqsood, H., Shams, R., Ahmad, M., and Fazilani, S. A. (2023). Combination of probiotics and phytase supplementation positively affects health of Japanese quails. *International Journal of Agriculture and Biology,* 30, 312-316.

Nagaraja, T. G., and Lechtenberg, K. F. (2007). Acidosis in feedlot cattle. *Veterinary Clinical North America Food Animal Pract*, *23*(2), 333-350, viii-ix. https://doi.org/10.1016/j.cvfa.2007.04.002

Newbold, C. J., and Rode, L. M. (2006). Dietary additives to control methanogenesis in the rumen. *International Congress Series*, *1293*, 138-147. https://doi.org/https://doi.org/10.1016/j.ics.2006.03.047

Ohashi, Y., and Ushida, K. (2009). Health-beneficial effects of probiotics: Its mode of action. *Animal Science Journal*, *80*(4), 361-371.

Osborne, J. K., Mutsvangwa, T., Alzahal, O., Duffield, T. F., Bagg, R., Dick, P., Vessie, G., and McBride, B. W. (2004). Effects of monensin on ruminal forage degradability and total tract diet digestibility in lactating dairy cows during grain-induced subacute ruminal acidosis. *Journal Dairy Science*, *87*(6), 1840-1847. https://doi.org/10.3168/jds.S0022-0302(04)73341-X

Pallauf, J., and Müller, A. S. (2006). Chapter 6 Inorganic feed additives. In R. Mosenthin, J. Zentek, and T. Żebrowska (Eds.), *Biology of Growing Animals* (Vol. 4, pp. 179-249). Elsevier. https://doi.org/https://doi.org/10.1016/S1877-1823(09)70093-1

Papadopoulos, G. A., and Lioliopoulou, S. (2023). Enzymes as Feed Additives. In *Sustainable Use of Feed Additives in Livestock: Novel Ways for Animal Production* (pp. 101-116). Springer.

Papaioannou, D., Katsoulos, P.-D., Panousis, N., and Karatzias, H. (2005). The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases: A review. *Microporous and Mesoporous Materials*, *84*(1-3), 161-170.

Parveen, S., Lukasik, J., Scott, T. M., Tamplin, M. L., Portier, K. M., Sheperd, S., Braun, K., and Farrah, S. R. (2006). Geographical variation in antibiotic resistance profiles of Escherichia coli isolated from swine, poultry, beef and dairy cattle farm water retention ponds in Florida. *Journal Applied Microbiology*, *100*(1), 50-57. https://doi.org/10.1111/j.1365-2672.2005.02773.x

Placinta, C., D'Mello, J. F., and Macdonald, A. (1999). A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. *Animal Feed Science and Technology*, *78*(1-2), 21-37.

Ramdani, D., Yuniarti, E., Jayanegara, A., and Chaudhry, A. S. (2023). Roles of Essential Oils, Polyphenols, and Saponins of Medicinal Plants as Natural Additives and Anthelmintics in Ruminant Diets: A Systematic Review. *Animals*, *13*(4), 767. https://www.mdpi.com/2076-2615/13/4/767

Ramírez-Restrepo, C., and Barry, T. (2005). Alternative temperate forages containing secondary compounds for improving sustainable productivity in grazing ruminants. *Animal Feed Science and Technology - ANIM FEED SCI TECH*, *120*, 179-201. https://doi.org/10.1016/j.anifeedsci.2005.01.015

Roque, B. M., Van Lingen, H. J., Vrancken, H., and Kebreab, E. (2019). Effect of Mootral-a garlic- and citrus-extract-based feed additive-on enteric methane emissions in feedlot cattle. *Transl Animal Science*, *3*(4), 1383-1388. https://doi.org/10.1093/tas/txz133

Russell, J. B., and Strobel, H. J. (1989). Effect of ionophores on ruminal fermentation. *Applied Environment Microbiology*, *55*(1), 1-6. https://doi.org/10.1128/aem.55.1.1-6.1989

Selle, P. H., and Ravindran, V. (2007). Microbial phytase in poultry nutrition. *Animal Feed Science and Technology*, *135*(1), 1-41. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2006.06.010

Selvendran, R. R., and Robertson, J. A. (2005). THE CHEMISTRY OF DIETARY FIBRE - AN HOLISTIC VIEW OF THE CELL WALL MATRIX. In (pp. 27-43). https://doi.org/10.1533/9781845698195.2.27

Steiner, T., and Shah, S. B. A. (2015). Phytogenic Feed Additives in Animal Nutrition. In (Vol. 1, pp. 403-423). https://doi.org/10.1007/978-94-017-9810-5_20

Steiner, T., and Syed, B. (2015). Phytogenic feed additives in animal nutrition. *Medicinal and aromatic plants of the world: Scientific, Production, Commercial and Utilization Aspects*, 403-423.

Sternemalm, E., Höije, A., and Gatenholm, P. (2008). Effect of arabinose substitution on the material properties of arabinoxylan films. *Carbohydrate Research*, *343*(4), 753-757. https://doi.org/https://doi.org/10.1016/j.carres.2007.11.027

Swelum, A., Hashem, N., Abdelnour, S., Taha, A., Ohran, H., Khafaga, A., El-Tarabily, K., and Elgammal, M. (2021). Effects of Phytogenic Feed Additives on the Reproductive Performance of Animals. *Saudi Journal of Biological Sciences*, *28*. https://doi.org/10.1016/j.sjbs.2021.06.045

Tedeschi, L. O., Fox, D. G., and Tylutki, T. P. (2003). Potential environmental benefits of ionophores in ruminant diets. *Journal Environment Qual*, *32*(5), 1591-1602. https://doi.org/10.2134/jeq2003.1591

Tsukahara, T.B.W., Kan, T., and Ushida, K. (2005). Effect of a cell prepration of Enterococcus feacalis strain EC-12 on digesta flow and recovery from constipation in a pig model and human subjects. *Microbial Ecology in Health and Disease*, *17*, 107-113.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A., and Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*, *112*(18), 5649-5654. https://doi.org/doi:10.1073/pnas.1503141112

Vanbelle, M., Teller, E., and Focant, M. (1990). Probiotics in animal nutrition: A review. *Archiv für Tierernaehrung*, *40*(7), 543-

567. https://doi.org/10.1080/17450399009428406

Vassilis Papatsiros, V. G. P., and Billinis, C. (2012). The Prophylactic Use of Acidifiers as Antibacterial Agents in Swine. In B. Varaprasad (Ed.), *Antimicrobial Agents* (pp. Ch. 14). IntechOpen. https://doi.org/10.5772/32278

Waghorn, G. (2008). Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. *Animal Feed Science and Technology*, *147*(1), 116-139. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2007.09.013

Wallace, R. J., Newbold, J., and McKain, N. (1990). Influence of ionophores and energy inhibitors on peptide metabolism by rumen bacteria. *Journal of Agricultural Science*, *115(2)*, 285-290 https://doi.org/https://doi.org/10.1017/S0021859600075250

Wang, Y., McAllister, T., and Acharya, S. (2015). Condensed Tannins in Sainfoin: Composition, Concentration, and Effects on Nutritive and Feeding Value of Sainfoin Forage. *Crop Science*, *55*, 13-22. https://doi.org/10.2135/cropsci2014.07.0489

Weimer, P. J., Stevenson, D. M., Mertens, D. R., and Thomas, E. E. (2008). Effect of monensin feeding and withdrawal on populations of individual bacterial species in the rumen of lactating dairy cows fed high-starch rations. *Applied Microbiology Biotechnology*, *80*(1), 135-145. https://doi.org/10.1007/s00253-008-1528-9

Whitehead, C. (2002). Vitamins in feedstuffs. *CABI*, 181–190. https://doi.org/10.1079/9780851994642.0181

Yadav, S., and Jha, R. (2019). Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. *Journal Animal Science Biotechnology*, *10*, 2. https://doi.org/10.1186/s40104-018-0310-9

Yang, L., Sado, T., Hirt, M., Pasco-Viel, E., Arunachalam, M., Li, J., Wang, X., Freyhof, J., Saitoh, K., Simons, A., He, S., and Mayden, R. (2015). *Yang et al. 2015 Supplementary materials*.

Yegani, M., and Korver, D. R. (2008). Factors Affecting Intestinal Health in Poultry. *Poultry Science*, *87*(10), 2052-2063. https://doi.org/https://doi.org/10.3382/ps.2008-00091

Chapter 04

The Potential use of Probiotics as Medicine

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ABSTRACT

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host. Currently, seven different bacterial genera are used as probiotics. The extensive investigation of probiotic microbes is mostly because of their promising health benefits. They have a broad spectrum of health benefits that range from gut restoration to disease treatment and from the eradication of pathogens to increasing the shelf life of food. The production of antimicrobial substances like bacteriocins can also enhance their usage potential. Probiotics are used in medical practices, clinical settings, agriculture, aquaculture, disease treatment, enhancing host functionality, improving mental health, the food industry, healthcare industries, and beautification. Due to the overwhelming effects, the medicinal aspects of probiotics are also explored, and surprisingly, they were found to be astounding. Strains from different sources, both in single and multiple forms, with different formulations and a vast route of administration, are used for the treatment of digestive, respiratory, and other diseases. Unlike medicine, there are no strict criteria, but different guidelines are proposed that must be followed while administering probiotic products. The most commonly used probiotics for medicinal purposes are from *Lactobacillus*, while strains from other sources are also used. Some often, i.e., blotting, mild gas production, and headaches, and others, like sepsis and infections, are the rare, documented shortcomings in the medicinal potential of probiotics. The medicinal potential of probiotics can be advanced by using state-of-the-art technologies that focus on accurate strain identification, deep genomic analysis, and the design of new probiotic strains with the desired properties. The application of artificial intelligence can also help in their advancement. This chapter will explain the potential of probiotics as medicine, shed light on their therapeutic potential, the advantages and disadvantages of using probiotics as therapeutic agents, and explain the guidelines that help consumers while taking probiotics as medicine. Moreover, the Islamic perspective of probiotics as medicine is also elucidated.

KEYWORDS		
Bacterial therapy, Disease, *Lactobacillus,* Medicine	Received: 27-Jun-2024 Revised: 12-Jul-2024 Accepted: 19-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

The overwhelming and potential spectrum of probiotics confirms a stronghold in almost all aspects of life and the scientific field. The field is growing exponentially, and currently, thousands of scientific publications are dedicated to exploring new areas and potential applications. For instance, in the first decade of the 21st century, over 5000 publications were dedicated to their medical aspects (Rijkers et al., 2011; Verna and Lucak, 2010). It is anticipated that the commercial market for probiotics will touch 77 billion USD in 2025, indicating their huge application in a vast area, including pharmaceuticals (Baral et al., 2021). The concept, which was developed a century ago, now has roots in biotechnology, medicine, pharmaceuticals, and industries. Different potential applications have been identified and are still ongoing. Nobel laureate Elie Metchnikoff in 1907 proposed the idea of enhancing lifespan by changing gut-healthy bacteria. He proposed that if the gut microbiota is changed with healthy bacteria (now called probiotics), it will help to increase the life of an individual. The idea became more prompting when, in the 1950s, it was described by Vergin that these are active substances that help in healthy development. In 1965, Lilly and Stillwell coined the term probiotics and described them as an immune-modulatory substance that has the potential to alter host immunity and enhance intestinal functions (Hussain, 2023a; Butel, 2014). In the 21st century, the World Health Organization in 2002, and the International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2013 defined probiotics as "live microorganisms that, when administered in adequate amounts, confer a benefit to the host" (Hussain et al., 2023; Maftei et al., 2024; Hill et al., 2014; Nueno-Palop and Narbad, 2011; Damodharan et al., 2020). Thus, new, and emerging applications are identified in many areas and have become one of the most researched topics.

The human and other animal intestine is a complex and dynamic population of 1000 species, constituting approximately 10^{14} microorganisms (Piqué et al., 2019; George Kerry et al., 2018; Heshmati, 2021; Sharma et al., 2013). The bulk of these bacteria in the human body are thought to reside in the gastrointestinal tract (GIT), with an estimated ten times more bacteria than body cells (Fijan, 2014). Normally, there is an eubiosis status of all gut microbiota (the presence of all types of microorganisms in the gut), which can lead to dysbiosis once the balance is changed. Different systems, i.e., quorum sensing, are involved in inter- and intra-bacterial communication, which is facilitated by small peptides called auto-inducers (Khoso et al., 2024). Likewise, the gut microbiota can also affect the physiology, endocrinology, and psychological aspects of the individual, and thus, any dysbiosis in the gut microbes can lead to vast detrimental effects. Collectively, all these microbes create micro-ecological niches in the gut. Similarly, probiotics are also considering the common residents of the gut with potential positive attributes. The word probiotics has its roots in Greek, which means "for life" (Maftei et al., 2024; George Kerry et al., 2018).

The potential positive attributes of probiotics give them unique properties, including strain safety, safe origin, production of antimicrobial substances, etc., and hence limit the number of probiotic microorganisms. Currently, seven genera are proposed to have probiotic strains dominated by the lactic acid bacteria (*Lactobacillus*, *Bifidobacterium*, and *Enterococcus*), followed by some yeast species (Hussain et al., 2023; Maftei et al., 2024). This strain constraint in probiotics is due to its strain-dependent nature and selection criteria. The strain-dependent phenomena explains that we cannot generalize a statement about a genus, and even the presence of one or two virulence traits (negative characters) did not exclude the species from probiotic selections. There are selection criteria that must be followed to propose or claim a strain for its probiotic potential. These criteria comprise non-pathogenic nature, no antibiotic and virulence characteristics, capacity of bacteriocin production, killing of pathogens, immunomodulatory potential, tolerance properties, aggregation formation capacity, long shelf life, short generation time, and viability and survivability during and after processes. Some criteria are proposed for their specific applications (Piqué et al., 2019; Hussain, 2023a).

The clear mechanism of action is not completely understood, but the proposed mechanisms are largely dependent on their viability and effectiveness in the host. Their effectiveness can be affected by the secretion of metabolites and proteins, the expression of surface molecules, and their direct interaction with the host cells, similarly, their survivability is related to their potential to endure harsh gut conditions and their adherence potential to mucosal surfaces (Hussain, 2023a).

Probiotics covered a wide range of health benefits in animals and humans through direct and indirect usage. Particularly, their treatment potential for different diseases has increased very rapidly and is still ongoing, though there is no pure dose-dependent profile like drugs (Tachibana et al., 2020; Naseem et al., 2023). Their health benefits include strengthening of the immune system; improvement in intestinal function (Hill et al., 2014); reduction in allergic reactions (Pandey et al., 2015; Araújo and Ferreira, 2013); and metabolic illnesses; alteration in pain perceptions; and advancement in food consumption (Pandey et al., 2015). Diseases caused by T-cell imbalance (asthma, rhinitis, dermatitis, eczema, etc.) are also treated with probiotics (Fijan, 2014; Benyacoub et al., 2003; Piqué et al., 2019; Islam, 2016; Hussain, 2023b).

However, the development of probiotics, particularly their commercialization, is not an easy task but rather a dangerous, expensive, and complex process in terms of their selection, processing, safety assessment, and authorization. Commercially, probiotics come in many forms, including powder, gel, capsules, tablets, granules, etc., and all have the exact information of their respective genus, species, strains, shelf life, number of colonies per ml, storage conditions, serving size, and associated health claims and consumer information (Sharma et al., 2013; Sanders et al., 2019; Pandey et al., 2015; Anadón et al., 2006). The production of different probiotic metabolites, including short-chain fatty acids, nitrous oxide, hydrogen peroxide, etc., is also used in different applications.

Besides the well-established, validated, and authentic applications, there are still some areas in which ambiguities are found. The scarce area is due to no or fewer clinical trials, small, tested populations, limited efficacy, poor genomic analysis, and post-experiment operational analysis. There is also a controversial debate about whether a probiotic strain can be used as medicine, although there are examples indicating the medicinal properties and therapeutic potential of probiotics. The regulatory authorities have strict guidelines that must be followed by probiotic strains that are proposed to be used as medicine. To the best of our knowledge, the available data on this aspect is not sufficient and well documented. Hence, keeping in mind the literature gap, this article aims to provide recent, updated, and conclusive literature about the medicinal properties, potential, and recommendations of probiotics. We also enlist some basic guidelines and proposed properties that are followed during this probiotic potential.

The Health Profile of Probiotics

Probiotics have an excellent health profile, indicating their intrinsic potential to treat or reduce disease prevalence. These huge benefits are due to their intrinsic potential, and recently, some have been developed due to their genome editing capabilities. The benefits of probiotics are equally applicable to humans and other animals, besides their biotechnological and industrial aspects (Hussain, 2023b). The beneficial spectrum of probiotics imparts some widespread applications, some with frequent benefits, and others are specific, as summarized in Fig 1. Additional benefits include regulation, stimulation, and modulation of immunity; improving intestinal barrier function (Hill et al., 2014); helping in the treatment of necrotizing enterocolitis (George Kerry et al., 2018; Nueno-Palop and Narbad, 2011); being used in the treatment of enteric infection (Shanahan, 2003; Damodharan et al., 2020); and increasing cell survivability by preventing apoptosis (O'Hara and Shanahan, 2007). The metabolites produced by probiotics, commonly called postbiotics, also have potential health effects. For instance, the production of hydrogen peroxide (H_2O_2), organic acids, and short-chain fatty acids (SCFAs) enables them to survive in the gut and is greatly involved in psychological disorders (Hussain et al., 2023). Probiotics also have the potential to restore gut dysbiosis and help in the treatment of nonalcoholic fatty liver disease (NAFLD) (Heshmati, 2021; Plaz-Diaz et al., 2019). Probiotics are also used as an alternative to antibiotics; this area was recently summarized (Rabetafika et al., 2023).

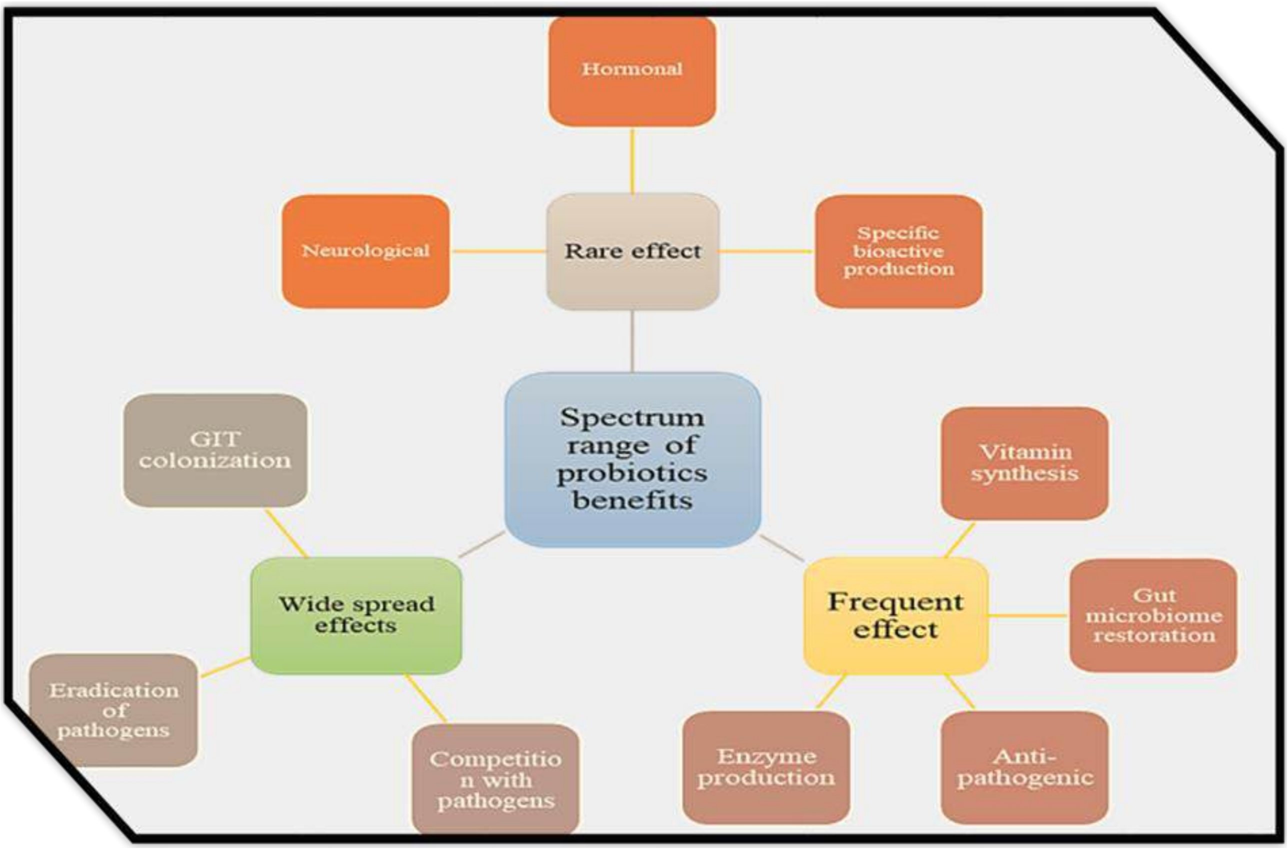

Fig. 1: The different potentials of probiotics in their health profiles—gut restoration and antimicrobial—are the exceptional benefits of probiotics (Hussain A. 2023b)

The concept of the gut-brain axis revolutionized the potential use of gut microbiota, particularly probiotics, in the field of psychological disorders. It's now well established that probiotics can greatly affect brain and mental function, helping in the treatment of neurodegenerative disorders. The term psychobiotics (coined in 2013) is dedicated to potentially describing those probiotics that have a role in cognition. Other physiological properties, like sleep, mood, personality, etc., are greatly influenced by probiotics (Fuochi and Furneri, 2023). A pretty well-known body of literature is available describing the association, role, mechanisms, and pathways that are involved in the gut-brain axis. This potential confirmed the probiotic role in clinical settings and medicine (Hussain, 2023a; Hussain, and Ali, 2024a).

Aging, which is considered the natural and progressive loss of physical and physiological aspects of body cells, is creating a new horizon in the area of probiotics research. The advancement in geroscience entails the process of reducing cell age and enhancing life span. The concept of gerobiotics (probiotics with anti-aging potential) attracts researchers to determine the exact role and mechanisms of how probiotics help in this regard. Although this area is of limited research, different strains have been identified that show promising anti-aging properties in animal models (Abrar and Arisha, 2023). Some of the currently available probiotics products include Florastor (*Saccharomyces boulardii)*, Florajen, RisaQuad, Bacid (LAC), Risa-Bid, Novaflor, Dofus, Flora-Q, (*L. acidophilus*) Intestinex (*L. acidophilus),* Florajen3, Zelac, Prodigen, Provella (*Bifidobacterium* and *Lactobacillus*), Floranex (*L. acidophilus* and *bulgaricus*), etc. (Drug.com). The currently used probiotics in human applications are summarized in Table 1.

Table 1: The currently used probiotic strains in humans for medicinal purposes (B; *Bifidobacterium*, L; *Lactobacillus*, S; *Streptococcus*, E; *Enterococcus*, AAD; antibiotic associated diarrhea (AAD), IBS; Irritable bowel syndrome)

Probiotics	Description	References
*E. faecium SF68 (*NCIMB 10415*)*	It is used in the treatment of enteritis and diarrhea, to prevent cell death, and to enhance immune responses.	(Holzapfel et al., 2018; Fu et al., 2022; Lodemann et al., 2015).
E. faecalis (Symbioflor 1)	Regulate immune diseases like chronic sinusitis or bronchitis.	(Cebrián et al., 2012)
E. faecium EK 13	It causes a reduction in fecal *E. coli* counts.	(Franz et al., 2011; Suvorov et al., 2019).
L. rhamnosus GG	It affects IBS.	(Chapman et al., 2011)
E. faecium L3	It shows antimicrobial, antiviral, anti-pathogenic, and anti-fungal activities.	(Aziz et al., 2019)
B. breve M-16-V	It suppresses the pro-inflammatory cytokine production.	(Piqué et al., 2019)
E. faecium CRL 183	It helps with colon tumors and enhances IL-4, IFN-γ, and TNF-α.	(Hanchi et al., 2021)
L. johnsonii	It inhibits the growth of H. pylori.	(Piqué et al., 2019)
*E. faecium (*PR88*)*	Relief symptoms in IBS	(Ferreira et al., 2013)
S. thermophilus CRL1190	It enhanced protection against H. pylori.	(Piqué et al., 2019)
L. reuteri 17938	It is widely studied for the treatment of colic in infants.	(Sanders et al., 2018)
VSL#3 (multi-strain probiotic)	It is used in the treatment of IBS and the prevention of endotoxin passage.	(Chapman et al., 2011; Piqué et al., 2019; Weichselbaum, 2009).
B. breve C50 and *S. thermophilus* 065	Reduce atopy in children	(Piqué et al., 2019)
L. reuteri, L. rhamnosus, and *P. freudenreichii*	This combination was found to enhance IBS symptoms and reduce mucin degradation.	(Chapman et al., 2011)
*B. bifidum (*MG731)*, L. reuteri (*MG5346)*,* and *L. rhamnosus (*MG5200)	The mixture significantly induces apoptosis in human gastric cancer	(Fuochi and Furneri, 2023)
L. rhamnosus 19070-2 and *L. reuteri* DSM 122460	It was found to improve the symptoms of atopic dermatitis (AD).	(Chapman et al., 2011)
B. lactis and *L. rhamnosus GG*	These can reduce the severity of eczema.	(Kechagia et al., 2013)
E. coli Nissle 1917	It can relapse in Crohn's disease patients.	(Santosa et al., 2006)
L. fermentum CECT5716	It can decrease the incidence of GIT and respiratory infections in infants.	(Butel, 2014)
L. reuteri and *B. breve*	It regulates the intestinal microbiota and improves the metabolism of tryptophan. Also used in the treatment of diarrhea.	(Fuochi and Furneri, 2023; Piqué et al., 2019).
Saccharomyces cerevisiae	Help in the regulation of antibiotic-associated pseudomembranous colitis	(Piqué et al., 2019)
L. acidophilus HA122	It is commercialized for the treatment of infantile colic.	(Piqué et al., 2019)
S. thermophilus	Help in the production of IgA	(Piqué et al., 2019; Maftei et al., 2024)
Saccharomyces boulardii	Have the potential to treat AAD	(Santosa et al., 2006)
L. paracasei subsp. paracasei CNCM I-1518	It has preventive effects on upper respiratory tract infections.	(Maftei et al., 2024)

Safety and Guidelines for Human Consumption of Probiotics

The safety of probiotics in animals' usage can be determined at different levels. Probiotics are widely used in husbandry science and pet foods, while their administration in humans is limited. (Sanders et al., 2018). The quality, amount, storage, reliability, accuracy, and proper labeling of probiotic products for animal use must be clearly stated. To ensure the safe use of probiotics in animals, certain autonomous regulatory bodies provide an unbiased opinion on probiotic products Likewise, before a probiotic product is commercialized, its excellent safety profile must be maintained and properly stated to the target consumers (Sanders et al., 2018). Data suggests that before taking a probiotic product, it is important to study its mode of action, preventive or treatment properties, and clinical trials (Maftei et al., 2024). These properties can be studied from the available literature, consumers' information, the country's guidelines, etc., for probiotic products. For instance, the American Gastroenterological Association (AGA) documented the use of probiotic products for the treatment of gastrointestinal disorders (Maftei et al., 2024). According to the literature, the widely available data about probiotic's potentials are based on skepticism. Earlier, Reid (2005) analyzed 25 probiotic products and reported that less than 1% of the claimed viability is present instead of the billions of bacteria mentioned (Reid, 2005). Thus, it is suggested that many probiotic products have been mislabeled and don't have the labeled cfu/mL in the products (Reid, 2005). The use of probiotics in humans has some criteria and guidelines proposed for safe usage and precautions that are followed. These guidelines and precautions are represented in Fig. 2. (data collected from (Sanders et al., 2018; Tegegne and Kebede, 2022; Rijkers et al., 2011; Maftei et al., 2024; Reid et al., 2003; Gupta and Garg, 2009; Quijano, 2011)).

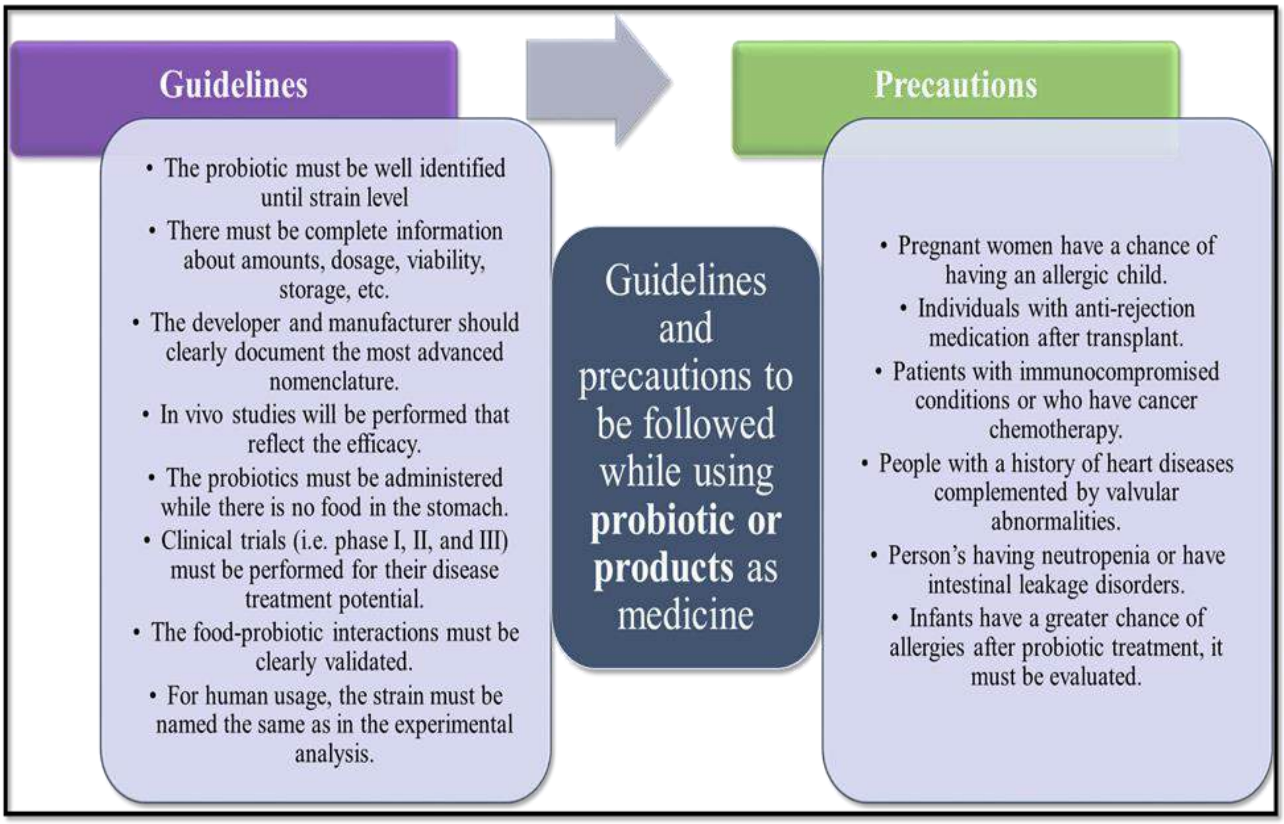

Fig. 2: The schematic illustration shows the guidelines and precautionary measurements while taking probiotics or their products for animal and human usage, particularly as medicine.

Routes of Administration of Probiotics in Humans

Probiotics are developed in different forms, each with their pros and cons. These forms include capsules, sprays, granules, powders, etc. The administration of probiotics in humans depends on the type of formulation, purpose of usage, and strains of probiotics. Probiotics may be administered via mouth, vagina, injection, or in spray form, depending on the objectives. The route of intake of probiotics also has its advantages and disadvantages (Verna and Lucak, 2010). Recently, Baral et al. (2021) summarized the formulation, dosage, and route of administration of probiotics (Baral et al., 2021).

Oral administration of probiotics is considered an easy and potential route of intake as different formulations are taken via mouth. More versatile probiotic species are taken via rectal therapy, but they fail if the probiotics don't have strong pH resistance (Mombelli and Gismondo, 2000). Vaginal intake of probiotics is good for lactobacilli repopulation and is used during bacterial vaginosis (Mombelli and Gismondo, 2000). Besides the routes of intake and form of probiotics, how they will be taken is also important. Food additives and the yogurt vs. milk delivery system have their properties (Verna and Lucak, 2010). Besides the administration, probiotic therapy also has the advantages of low cost and fewer negative reactions (Sarkar, 2013). A new combinatorial approach of probiotics with other substances (for instance, plant oils) is used to achieve maximum benefits (Hussain, 2023c). The medicinal aspects of probiotics, their pros and cons, challenges, advancements, future perspectives, etc. are summarized in Fig 3.

Medicinal Applications of Probiotics and their Safety Concerns

The health profile of probiotics is vast and has proven usage in animals and humans. Nasreen et al. (2024) documented that 76% of physicians are confident that probiotics could help in patient management (Nasreen et al., 2024). Their potential in these subjects enhanced the value of probiotics, particularly their medicinal aspects. As described, commercialization of probiotic strains is not an easy task, and it has become more tedious and requires more investigation when it is used in animal settings. The use of probiotics in humans even required more clarification, investigations, experimental validations, animal studies, and clinical trials (Sanders et al., 2018). However, the application of probiotics in humans is much less than that which is claimed and submitted for approval. These regulations become more severe when the selected strains are from doubtful sources; for instance, the genus *Enterococcus* has a doubtful nature but is still used as a probiotic and hence requires more careful evaluation when used in humans (Hussain et al., 2023; Butel, 2014). The bacterial therapy or probiotic medicinal domains are illustrated in Fig 4.

The dependency on probiotics for medicinal value also depends on the age and gender of an individual and also on the probiotic's formula, amounts, forms, and duration (Sarkar, 2013; Santosa et al., 2006). Recently, Poindexter et al. (2021) documented the role of probiotics in preterm infants and concluded that a good number of clinical trials were dedicated to this aspect (Poindexter et al., 2021). Different studies were conducted which show that single and multiple-strain probiotic combinations have a role in the treatment of necrotizing enterocolitis (NEC) in preterm infants (Poindexter et al., 2021). Likewise, it is more important to carefully select a probiotic for elderly people, as they have weak immunity and multiple disease statuses (Baker et al., 2009). *Lactobacillus rhamnosus* GG (LGG) and *B. lactis* BB-12 are the most studied probiotics for disease prevention, and *L. reuteri* SD2222 is the most investigated probiotic for disease treatment (Gupta and Garg, 2009; Reid et al., 2003). The different human diseases that are treated with single or multiple probiotics are compiled in Table 2.

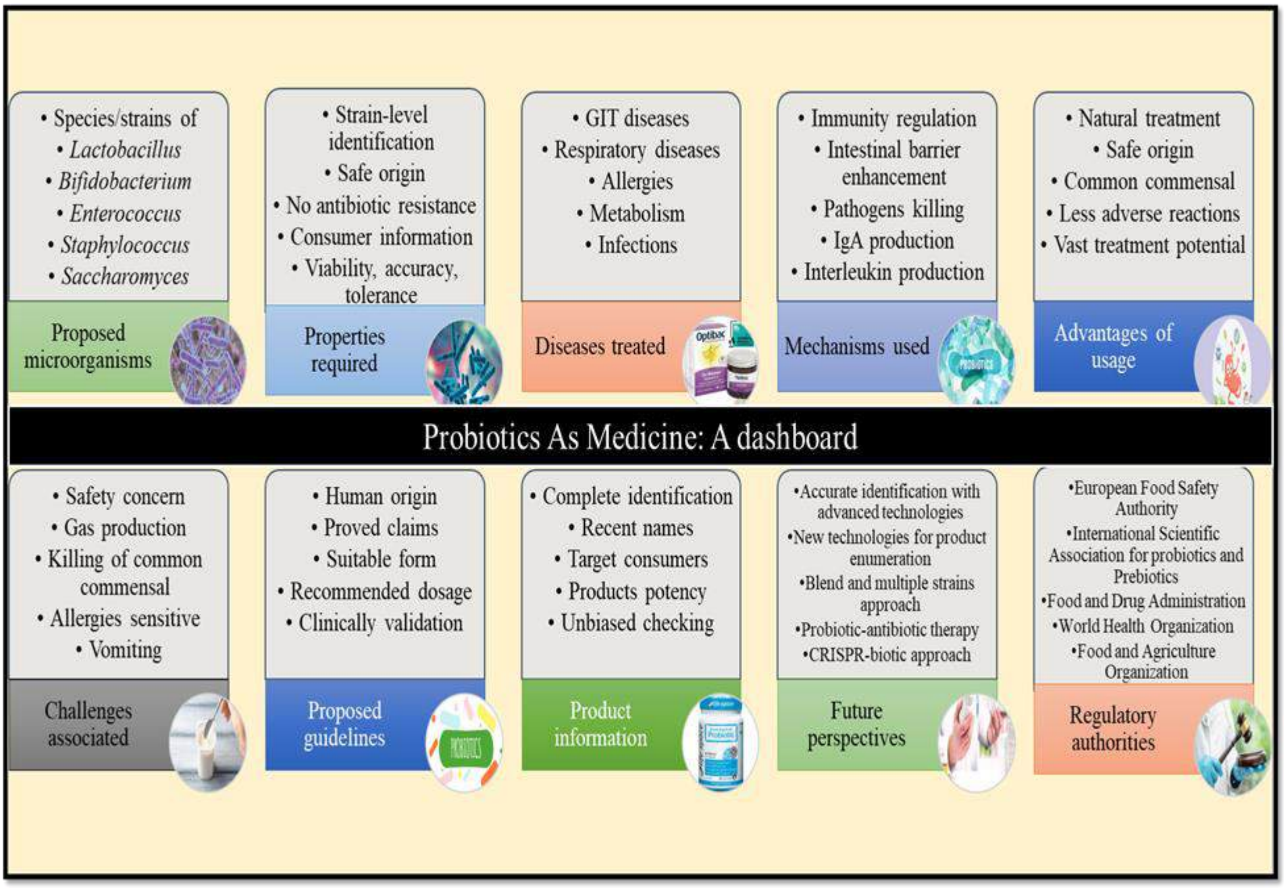

Fig. 3: A dashboard exploring the medicinal aspects of probiotics, reflecting the potential usage of probiotics as medicine and their allies.

The Harmful Nature of Probiotics in Human Usage

Probiotics, although having a broad spectrum of health benefits, also have negative aspects. The harmful nature of probiotics may be due to the strain's intrinsic properties, the potential of acquired traits, or whether they are developed after usage. Blotting, mild gas production, vomiting, headaches, etc. are the well-known negative consequences of probiotics (Islam, 2016; Maftei et al., 2024). Allergy to probiotic usage is also one of the key harmful aspects. The production of postbiotics with toxic effects in animals can enhance their harmful aspects. For instance, postbiotic D-lactate in children with short bowel syndrome may create an acidosis condition that leads to hyperventilation or encephalopathy (Butel, 2014). Some probiotics produce thirst in the body when taken for the first time. Some probiotics have less capacity to colonize in the host, thus creating problems. Likewise, poor viability during storage, single and multiple strain effects, and some strain's intrinsic drawbacks makes them of less use in humans (Sarkar, 2013).

Different studies were conducted to evaluate the various aspects of probiotics in animals and humans. It is also known that not only the probiotic microbes but also their products (metabolites/ postbiotics) have disease treatment potential and have good health benefits (Fuochi and Furneri, 2023; Piqué et al., 2019). The safety concerns with probiotics in animal and human usage are timely compiled by the European Food Safety Authority (EFSA) (Piqué et al., 2019). It was also established that single strains and multiple strains have different effects on the health profile (Poindexter et al., 2021). Chapman et al. (2011) documented the health benefits of probiotics in terms of single and multiple-strain approaches, both in animals and humans (Chapman et al., 2011).

The acquisition of foreign genetic materials creates a great risk of probiotic usage in humans. For example, the transfer of antibiotic resistance genes from the host to the probiotic strains and then to the common commensal of gut produce creates antibiotic resistance, and thus, the potential of antibiotics vanishes (Butel, 2014). Sepsis, endocarditis, liver abscess, etc. are the rare side effects of probiotics (Islam, 2016; Snydman, 2008).

Table 2: The different types of human diseases that are prevented or treated with probiotics (L; *Lactobacillus*, B; *Bifidobacterium*)

Diseases	Probiotics	References
Gastrointestinal tract		
Inflammatory bowel disease (IBD)	*S. boulardii* and *L. rhamnosus GG*	(Weichselbaum, 2009; Santosa et al., 2006; Maftei et al., 2024).
Irritable bowel syndrome (IBS)	*L. plantarum* 299v, *L. plantarum,* and *B. breve*	(Santosa et al., 2006; Islam, 2016).
Ulcerative colitis (UC)	*Escherichia coli Nissle (EcN)*, VSL#3	(Weichselbaum, 2009; Sanders et al., 2018; Islam, 2016).
Crohn's disease (CD)	L. *rhamnosus GG (LGG), B. breve, B. longum,* and *L. casei*	(Weichselbaum, 2009; Santosa et al., 2006; George Kerry et al., 2018; Verna and Lucak, 2010).
Pouchitis	*L. paracasei subsp. paracasei, L. plantarum, L. acidophilus, L. delbrueckii. bulgaricus, B. longum, B. breve,, and S. salivarius*	(Maftei et al., 2024; Verna and Lucak, 2010).
Constipation	*L. casei Shirota (LcS)*	(Weichselbaum, 2009).
Infantile colic	*L. reuteri* 17938	(Sanders et al., 2018).
Necrotizing enterocolitis (NEC)	*B. breve* BBG-001, *L. acidophilus, B. infantis*	(Sanders et al., 2018; Poindexter et al., 2021; Gupta and Garg, 2009).
Gastroenteritis	*L. rhamnosus GG, L. rhamnosus* GR-1, and *L. fermentum* RC-14	(Brown and Valiere, 2004; Reid et al., 2003).
Antibiotic-associated diarrhea (AAD)	*L. casei* DN-114 001, *L. bulgaricus, S. thermophilus, L. acidophilus, L. rhamnosus, L. reuteri, L. bulgaricus, L. rhamnosus GG,* and *S. boulardii*	(Weichselbaum, 2009; Santosa et al., 2006; Sanders et al., 2018; Maftei et al., 2024; Brown and Valiere, 2004).
Acute diarrhea	*S. boulardii, LGG, B. lactis* BB-12, and *L. reuteri* SD 2222	(Weichselbaum, 2009; Islam, 2016; Brown and Valiere, 2004; Gupta and Garg, 2009).
Traveler's diarrhea	*S. boulardii, LGG, L. acidophilus, L. bulgaricus, B. bifidum,* and *S. thermophilus*	(Weichselbaum, 2009; Santosa et al., 2006; Islam, 2016; Brown and Valiere, 2004).
Immune system diseases		
Common cold	*L. gasseri* PA 16/8, *B. longum* SP 07/3, and *B. bifidum* MF 20/5	(Weichselbaum, 2009).
Type 1 diabetes	*B. (longum, infantis, breve); L. (acidophilus, delbrueckii. Bulgaricus, plantarum*	(Tegegne and Kebede, 2022).
Japanese cedar pollen (JCP)	*L. casei Shirota (LcS), L. rhamnosus* ATCC 53103, and *L. acidophilus* L-92	(Weichselbaum, 2009).
Eczema/dermatitis	*L. rhamnosus* HN001, *B. animalis subsp. lactis* HN019	(Weichselbaum, 2009; Islam, 2016).
Infections		
Helicobacter pylori infections	*L. gasseri OLL 2716(LG21), L. casei, L. gasseri, L. johnsonii, and L. reuteri* DSM 17648	(Brown and Valiere, 2004; Gupta and Garg, 2009; Kimura, 2004; Sarkar, 2013; Reid et al., 2003; Rabetafika et al., 2023).
Chronic kidney disease (CKD)	*L. casei HY2743* and *L. casei HY7201*	(Ranjha et al., 2021).
Urinary tract infections (UTI)	*L. (fermentum, brevis, casei, vaginalis, delbrueckii, salivarius, reuteri,* and *rhamnosus).*	(Mombelli and Gismondo, 2000; George Kerry et al., 2018).
Surgical Infections	*L. fermentum* RC-14, *L. plantarum* 299, *L. acidophilus, L. lactis, L. casei. B. longum, B. bifidum, and B. infantis*	(Gupta and Garg, 2009; Rabetafika et al., 2023).
Vaginosis	*L. johnsonii, L. acidophilus, L. rhamnosus* GR-1, *L. fermentum* RC-14, and *L. crispatus* CTV-05	(Mombelli and Gismondo, 2000; Cheng et al., 2019; Reid et al., 2003; Rabetafika et al., 2023).
Uro-genital infections	*L. rhamnosus* GR-1 *and L. fermentum* RC-14	(Gupta and Garg, 2009).
Clostridium difficile colitis (CDC)	*L. rhamnosus GG, S. boulardii,* and *L. casei*	(Reid et al., 2003; Verna and Lucak, 2010; Rabetafika et al., 2023).
Genitourinary tract infections	*L. GR-1 and B-54* or *RC-14*	(Brown and Valiere, 2004).
Dermatological diseases	*L. salivarius* LS03*; Lactococcus and Streptococcus salivary; B. adolescentis* SPM0308	(Maftei et al., 2024).
Virus related disorders		
SARS-CoV-2	*Bacillus (coagulans, subtilis, clausii), L. plantarum,* KABP022, KABP023, and KAPB033	(Maftei et al., 2024).

Respiratory tract infections	*L. paracasei subsp. paracasei* CNCM I-1518*; L. plantarum* HEAL9*; L. paracasei* 8700	(Maftei et al., 2024; Rabetafika et al., 2023).
Influenza	*L. paracasei* CNCM I-1518	(Maftei et al., 2024).
HIV	*L. plantarum, Pediococcus acidilactici, Lactobacilli*	(Maftei et al., 2024; Gupta and Garg, 2009).
HPV	*L. rhamnosus* GR-1, *Limosilactobacillus reuteri* RC-14	(Maftei et al., 2024).
Cancer		
Breast cancer	*B. infantis* and *L. acidophilus*	(Brown and Valiere, 2004; Gupta and Garg, 2009).
Bladder cancer	*L. casei, B. longum*	(Mombelli and Gismondo, 2000).
Colon cancer	*L. johnsonii, L. reuteri, L. bulgaricus, B. longum, L. rhamnosus GG, B. lactis* Bb12*, and L. fermentum* NCIMB-5221 and -8829	(Santosa et al., 2006; Tegegne and Kebede, 2022; George Kerry et al., 2018).
Metabolic disorders		
Hypercholesterolemia	*L. johnsonii and L. reuteri*	(Mombelli and Gismondo, 2000; Quijano, 2011).
Bloating	*L. reuteri and B. breve*	(Piqué et al., 2019).
Hepatic diseases	VSL#3	(Brown and Valiere, 2004).
Lactose digestion	*Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus*	(Brown and Valiere, 2004).
Hyperlipidemia	*L. reuteri, L. gasseri*	(Brown and Valiere, 2004).
Lactose intolerance	*Lactobacilli, L. bulgaricus B. animalis, L. paracasei, B. animalis. lactis* BB12, *L. acidophilus* NFCM, *S. thermophilus,* and *L. johnsonii* La1	(Mombelli and Gismondo, 2000; Singh et al., 2011; Quijano, 2011; Sarkar, 2013).
Anti-obesity	*L. gasseri* BNR17, *L. casei, L. acidophilus,* and *B. longum*	(George Kerry et al., 2018).
non-alcoholic fatty liver disease	*B. infantis, L. acidopilus,* and *Bacillus cereus*	(Cheng et al., 2019).
Others		
Oral candidiasis	*B. animalis, Lactococcus lactis, L. helveticus, L. rhamnosus GG* ATCC53103, *L. rhamnosus* LC705	(Gupta and Garg, 2009; Singh et al., 2011; Allaker and Stephen, 2017).
Halitosis	*Streptococcus salivarius* K12, *L. salivarus* WB21	(Ranjha et al., 2021; Allaker and Stephen, 2017).
Mental health	*L. rhamnosus, L. helveticus, L. brevis* DPC6108, *L. plantarum, L. fermentum, B. longum spp. Infantis, L. acidophilus,* and *L. casei*	(Cheng et al., 2019; Roobab et al., 2020; George Kerry et al., 2018).
Anti-sclerosis	*B. subtilis* and *B. coagulans*	(Roobab et al., 2020).
Osteoporosis	*L. reuteri* and *B. longum.*	(Ranjha et al., 2021).
Antiparasitic	*L. acidophilus* NCFM	(Nasreen et al., 2024).
Obesity	*B. pseudocatenulatum* SPM 1204*, B. longum* SPM 1205*, and B. longum* SPM 1207	(Ranjha et al., 2021).

Probiotics as Medicine: The Islamic Perspective

Probiotics, as described, have a crucial role in the prevention and treatment of different diseases. Humans are facing plenty of diseases that need to be treated with different substances. Disease occurring is natural, and it was created by ALLAH Almighty. Islam, which is a comprehensive religion that covers all aspects of someone's life, is hence called the complete code of life. Islam is the second-largest religion in the world, with approximately 1.8 billion followers, and this number is increasing rapidly (Hussain, 2024). Islam has a complete set of rules, commands, and guidelines that compel Muslims to follow them in every situation except in emergencies. Emergency, from an Islamic perspective, has its criteria and is known as Durrha (Badiuzzamani and Gunardi, 2021). Halal and haram are the two opposite terms in Islam, in which the former is allowed or permissible for use or doing, while the latter is non-permissible or not allowed to do or use. The effect of halal and haram is not only because of religious commands but in a real sense, these have a bad effect on humans if the harm is used. As the ALLAH almighty, create the human, and ALLAH knows what is good for us and what is not good to use, even if it seems the other way around, i.e., the haram seems good or beneficial for usage. Currently, the halal food industry is growing fast and has become the leading industry, particularly among the Muslim population (Yap and Al-Mutairi, 2023) (Hussain A, & Ali, S.A, 2024b).

The concept of halal and haram is vast and similarly applicable to food substances. Halal food means that is free from any haram or najas source, does not contain haram ingredients, and is not processed in haram or najas instruments. In the Quran, it is mentioned that “eat halal and tayybha,” which means the item must be halal and should be clean for usage (Mohd et al., 2018). Haram is the opposite of halal and is not allowed to be used except in Durrah situations. Almost all things must be halal until they are not declared haram in Islam. Halal pharmaceutical substances must be taken using the described halal criteria (Mohd et al., 2018). The Islamic approach to medicine and its aspects are shown in Fig 5.

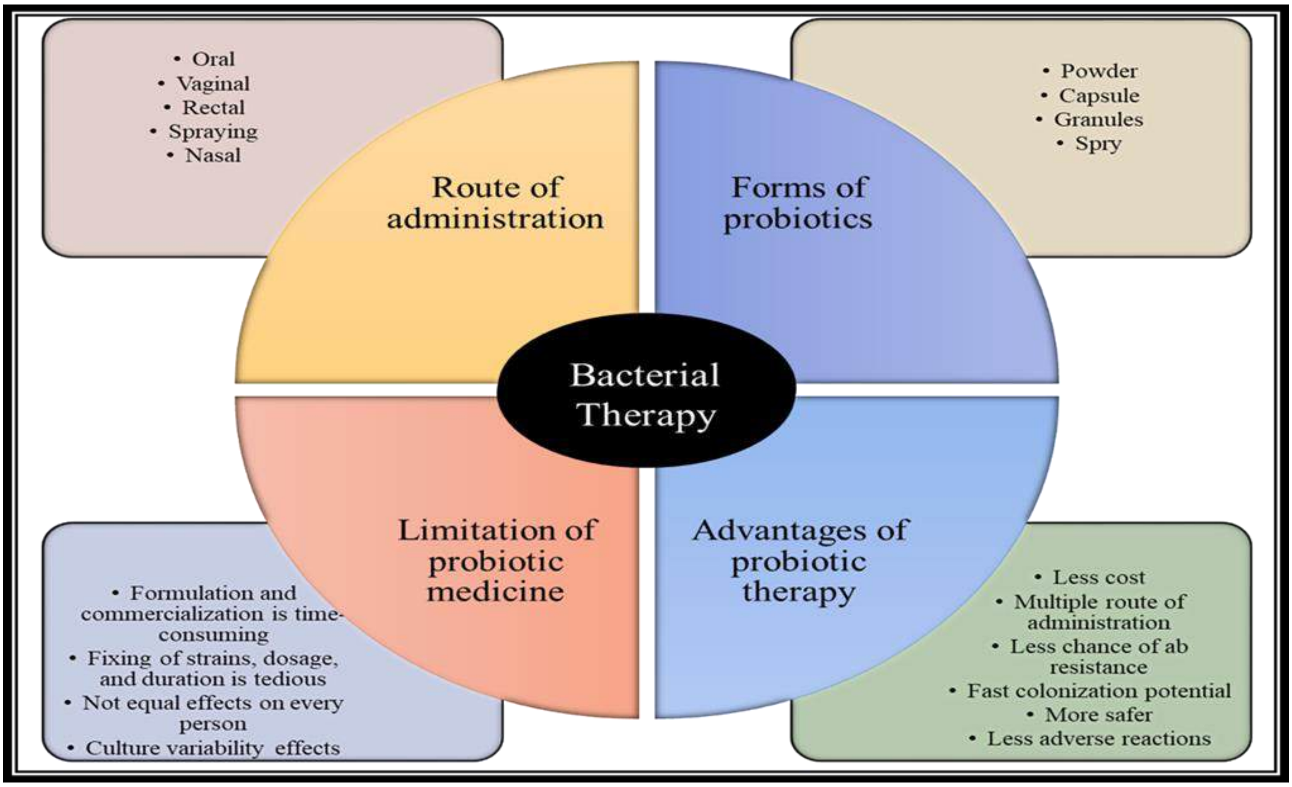

Fig. 4: The potential domains of bacterial therapy (probiotic as medicine)

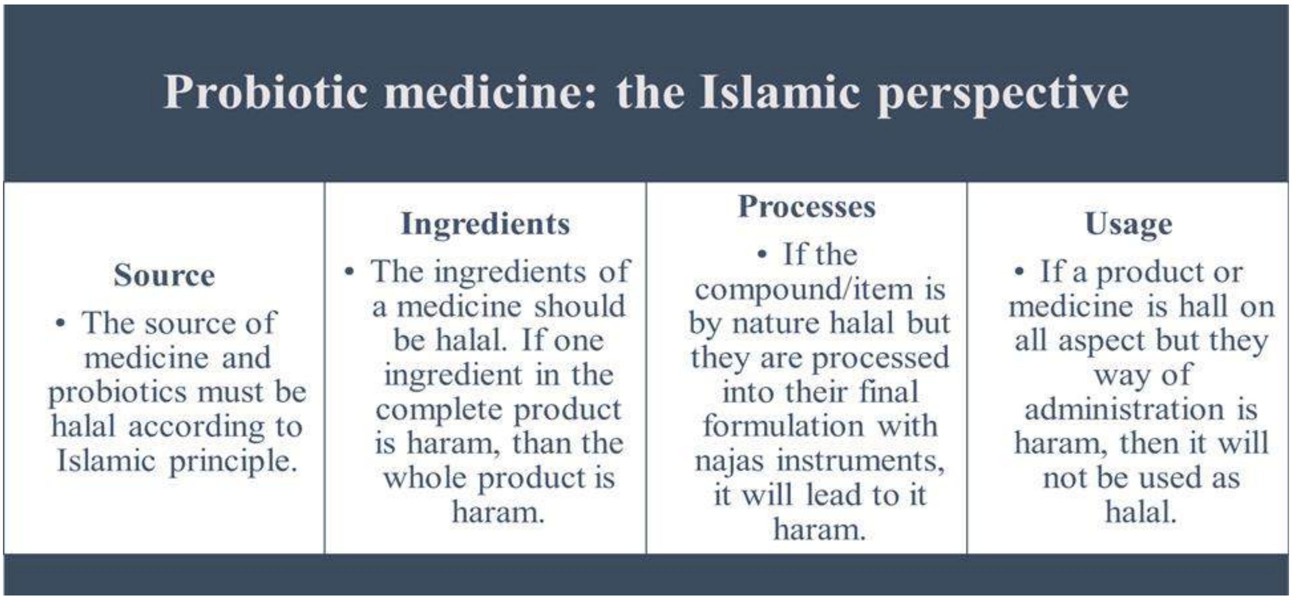

Fig. 5: The Islamic perspective of probiotic medicine

In medicine, the same halal and haram concepts are applied, as medicine is something that is ingested or taken inside the body. The source, process, ingredients, usage, etc. must be halal for any medicine to be taken. In the case of probiotics, most strains are isolated from human organs, and according to Islamic guidelines, the use of any human organ is not allowed, thus creating doubt about the use of probiotics. With the exception of two-year-old babies, which are fed with breastfeeding, they have advantages as their derived strains are used for human consumption, as the urine of a two-year-old baby is considered clean, as described in a hadith (Badiuzzamani and Gunardi, 2021; Yap and Al-Mutairi, 2023). The pivotal points that determine the halal perception of microbial products are the source, nature of microbes, growth media compositions, metabolites, production process, and the additives that help them be used for specific functions (Kurniati and Hafsan, 2022). Hence, it is important to check the halal and haram nature of probiotic medicine before administration.

Challenges

The literature showed the potential of probiotics or their products to be used as medicine or as a therapeutic agent to reduce and treat animals' diseases. The key mechanisms for this are their gut restoration and immunity modulation abilities, which help to repopulate the normal flora and, thus, aid in disease treatment. Currently, there is strong evidence that shows their medicinal potential, but it still needs to be complemented with more experimental data and animal studies (Stavropoulou and Bezirtzoglou, 2020). The antibiotic resistance potential is considered a positive attribute for probiotics but it creates problems when the person is infected so the antibiotic will not be working, thus creating a greater risk in disease treatment. The potential challenges associated include (i) the authorization and regulation of probiotic strains; (ii) the safety profile in terms of genetic stability, i.e., no acquisition of foreign substances; (iii) the creation of a dose-dependent profile; (iv) due to the strain-dependent nature, one strain may show different effects; and (v) the individual genetic profile, which showed different reactions against probiotics (Mejía-Caballer et al., 2021; Wolfe et al., 2023; Nami et al., 2015).

Recent Advancement and Future Perspectives

Modern technologies enable researchers to play with the genetics of microorganisms. New methods, procedures, and protocols are constantly developed for the better usage of microorganisms. Biotechnology, in this regard, contributes significantly. Genetic tools like the CRISPR-Cas system and genomic analysis deeply reflect the potential of microorganisms for any possible application. In the same area, the field of probiotics has advanced with different technologies. The development of CRISPR-biotics, next-generation probiotics, psychobiotics, gerobiotics, immunobiotics, and engineered probiotics are a few glimpses of using advanced technologies (Tegegne and Kebede, 2022).

Likewise, the competitions are still ongoing and are anticipated to be more precise, advanced, and accurate in the future. These advancements are supposed to help in strain identification, enhance reliability, and improve reproducibility (Maftei et al., 2024).

The future perspectives in the field of probiotic medicine include, but are not limited to, the following developments:

- The development of designer probiotics with the required properties is currently getting attention. These probiotics have advanced properties and have greater potential.
- Synthetic biology and probiotics are a new approach that has the potential to aid more probiotic products and elucidate new applications.
- The recent trend of fecal bacteriotherapy or fecal microbiota transplantation (FMT) (the transferring of good or healthy bacteria to the patient for gut restoration) can be more productive when probiotic strains are used (Tegegne and Kebede, 2022).
- The role of probiotics in gnotobiotics can be experimentally validated.
- The development of emerging technologies like 3D bioprinting can also be applied to enhance the field with more potential and accuracy (Hussain et al., 2024).
- Next-generation probiotics development is increasing, identifying different novel strains with probiotic potential using genetic analysis methods.
- The development of CRISPR-biotics (using the CRISPR-Cas system for probiotic development) is also a new trend in the probiotic field, which enhances the properties of probiotics (Hussain, A. & Ali, SA. 2023d).
- Gerobiotics, which are anti-aging probiotics, also received greater attention and showed their potential in combating aging processes.
- Psychobiotics, which are probiotics with the potential to treat cognitive function impairments, are currently under consideration.
- The potential role of artificial intelligence (AI) and allied technologies can be used to identify more advanced applications.

Conclusion

The spectrum of different applications of probiotics confirms their roots in multiple domains of life. Ranging from food and biotechnological applications to human disease treatment, this is just a glimpse of their strength. Due to the overwhelming effects, the medicinal aspects of probiotics are also explored, and surprisingly, they were found to be astounding. Strains from different sources, both in single and multiple forms, were screened for different diseases, and they showed promising results. Although, unlike medicine, there are no strict criteria, different guidelines are proposed that must be followed while administering probiotics. The formulation of probiotics, route of administration, delivery system, etc. are the contributing factors to the therapeutic potential of probiotics. Some often, i.e., blotting, mild gas, and allergies, and others, like sepsis and infections, are the documented shortcomings in the medicinal potential of probiotics. This study is limited to providing the theory and guidelines about probiotics medicine, although there is no case study or particular population studied, were added. Advancement in the field can be elaborated by fast and accurate methods of identification, genetic manipulation for profound properties, and the development of new aspects of applications using advanced technologies of artificial intelligence, machine learning, and deep learning.

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Ethical Declaration
Not applicable

Conflict of Interest
The authors have no conflict of interest

Data Access Statement
Not analyzed new data

REFERENCES

Allaker, R. P., and Stephen, A. S. (2017). Use of Probiotics and Oral Health. *Current Oral Health Reports*, *4*(4), 309–318. https://doi.org/10.1007/s40496-017-0159-6

Anadón, A., Rosa Martínez-Larrañaga, M., and Aranzazu Martínez, M. (2006). Probiotics for animal nutrition in the European Union. Regulation and safety assessment. *Regulatory Toxicology and Pharmacology*, *45*(1), 91–95. https://doi.org/10.1016/j.yrtph.2006.02.004

Araújo, T. F., and Ferreira, C. L. de L. F. (2013). The genus Enterococcus as probiotic: Safety concerns. *Brazilian Archives of Biology and Technology*, *56*(3), 457–466. https://doi.org/10.1590/S1516-89132013000300014

Aziz, F., Khan, M. N., Ahmed, S., and Andrews, S. C. (2019). Draft genome sequence of *Enterococcus faecium* SP15, a potential probiotic strain isolated from spring water. *BMC Research Notes*, *12*(1), 10–12. https://doi.org/10.1186/s13104-019-4136-0

Badiuzzamani, A. A., and Gunardi, S. (2021). The Use of Human-Strain Probiotics as a Dietary Supplement from Islamic Perspective. *Journal of Halal Industry and Services*, *4*(1), 1–15. https://doi.org/10.36877/jhis.a0000255

Baker, H. C., Tran, D. N., and Thomas, L. V. (2009). Health benefits of probiotics for the elderly: a review. *Journal of Foodservice*, *20*(5), 250–262. https://doi.org/10.1111/j.1748-0159.2009.00147.x

Baral, K. C., Bajracharya, R., Lee, S. H., and Han, H. K. (2021). Advancements in the pharmaceutical applications of probiotics: Dosage forms and formulation technology. *International Journal of Nanomedicine*, *16*, 7535–7556. https://doi.org/10.2147/IJN.S337427

Benyacoub, J., Czarnecki-Maulden, G. L. von der Weid, T., Czarnecki-Maulden, G. L., and Anderson, R. E (2003). Supplementation of food with *Enterococcus faecium* (SF68) stimulates immune functions in young dogs. *Journal of Nutrition*, *133*(4), 1158–1162. https://doi.org/10.1093/jn/133.4.1158

Brown, A. C., and Valiere, A. (2004). Probiotics and medical nutrition therapy. *Nutrition in Clinical Care: An Official Publication of Tufts University*, *7*(2), 56–68.

Butel, M. J. (2014). Probiotics, gut microbiota and health. *Medecine et Maladies Infectieuses*, *44*(1), 1–8. https://doi.org/10.1016/j.medmal.2013.10.002

Cebrián, R., Baños, A., Valdivia, E., Pérez-Pulido, R., Martínez-Bueno, M., and Maqueda, M. (2012). Characterization of functional, safety, and probiotic properties of *Enterococcus faecalis* UGRA10, a new AS-48-producer strain. *Food Microbiology*, *30*(1), 59–67. https://doi.org/10.1016/j.fm.2011.12.002

Chapman, C. M. C., Gibson, G. R., and Rowland, I. (2011). Health benefits of probiotics: Are mixtures more effective than single strains? *European Journal of Nutrition*, *50*(1), 1–17. https://doi.org/10.1007/s00394-010-0166-z

Cheng, D., Song, J., Xie, M., and Song, D. (2019). The bidirectional relationship between host physiology and microbiota and health benefits of probiotics: A review. *Trends in Food Science and Technology*, *91*(March), 426–435. https://doi.org/10.1016/j.tifs.2019.07.044

Damodharan, K., Palaniyandi, S. A., Suh, J. W., and Yang, S. H. (2020). Probiotic Characterization of *Lactobacillus* paracasei subsp. paracasei KNI9 Inhibiting Adherence of Yersinia enterocolitica on Caco-2 Cells In Vitro. *Probiotics and Antimicrobial Proteins*, *12*(2), 600–607. https://doi.org/10.1007/s12602-019-09535-8

Drug.com. (Accessed,April, 2024). https://www.drugs.com/drug-class/probiotics.html

Fijan, S. (2014). Microorganisms with claimed probiotic properties: An overview of recent literature. *International Journal of Environmental Research and Public Health*, *11*(5), 4745–4767. https://doi.org/10.3390/ijerph110504745

Franz, C. M. A. P., Huch, M., Abriouel, H., Holzapfel, W., and Gálvez, A. (2011). Enterococci as probiotics and their implications in food safety. *International Journal of Food Microbiology*, *151*(2), 125–140. https://doi.org/10.1016/j.ijfoodmicro.2011.08.014

Fu, X., Lyu, L., Wang, Y., Zhang, Y., Guo, X., Chen, Q., and Liu, C. (2022). Safety assessment and probiotic characteristics of *Enterococcus lactis* JDM1. *Microbial Pathogenesis*, *163*, 105380. https://doi.org/10.1016/j.micpath.2021.105380

Fuochi, V., and Furneri, P. M. (2023). Applications of Probiotics and Their Potential Health Benefits. *International Journal of Molecular Sciences*, *24*(21). https://doi.org/10.3390/ijms242115915

George Kerry, R., Patra, J. K., Gouda, S., Park, Y., Shin, H. S., and Das, G. (2018). Benefaction of probiotics for human health: A review. *Journal of Food and Drug Analysis*, *26*(3), 927–939. https://doi.org/10.1016/j.jfda.2018.01.002

Gupta, V., and Garg, R. (2009). Probiotics. *Indian Journal of Medical Microbiology*, *27*(3), 202–209.

https://doi.org/10.4103/0255-0857.53201

Hanchi, H., Mottawea, W., Sebei, K., and Hammami, R. (2018). The genus *Enterococcus*: Between probiotic potential and safety concerns-an update. Frontiers in Microbiology, 9, 1–16. https://doi.org/10.3389/fmicb.2018.01791

Heshmati, H. (2021). Gut Microbiome Modulation in the Management of Nonalcoholic Fatty Liver Disease. *Academia Letters*, *September 2021*, 1–8. https://doi.org/10.20935/al3470

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., and Sanders, M. E. (2014). Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology*, *11*(8), 506–514. https://doi.org/10.1038/nrgastro.2014.66

Holzapfel, W., Arini, A., Aeschbacher, M., Coppolecchia, R., and Pot, B. (2018). *Enterococcus faecium* SF68 as a model for efficacy and safety evaluation of pharmaceutical probiotics. *Beneficial Microbes*, *9*(3), 375–388. https://doi.org/10.3920/BM2017.0148

Hussain, A & Ali SA. (2024a). The Role of Probiotics in the Prevention and Treatment of Psychological Disorders. Conferene Proceedings of International Psychology Conference on Mental Health and Resilience (IPC- MHR), 22-February, 1–24. https://doi.org/10.13140/RG.2.2.19473.20325

Hussain, A. (2023b). Health benefits of probiotics. Council for Scientific approaches, Pakistan. https://www.csapakistan.com/2023/06/06/health-benefits-of-probiotics/

Hussain, A. (2023a). Molecular assessment and validation of the selected enterococcal strains as probiotics. *Dissertation Submitted to University of Karachi*, pakistan, 1–130.

Hussain, A., Rajpoot, T., and Rehman, M. (2024). The Role of 3D Bioprinting in Drug Discovery; A review. *Journal of Advanced Biomedical and Pharmaceutical Sciences*, 7, 88–95. https://doi.org/10.21608/JABPS.2024.265010.1213

Hussain, A. (2023c). Probiotics and Vegetable Oil Association: A review. The International Conference on Sustainable Vegetable Oils: Socio- Economic and Ecological Approaches, Bogor, Indonesia, November 30, 2023. http://dx.doi.org/10.13140/RG.2.2.30408.12800

Hussain, A & Ali SA. (2023d). Probiotics Development and CRISPR-Cas System. Conference Presentation at Science Innovation Showcase, 12-14 December, USA, pp 1–18. https://doi.org/10.13140/RG.2.2.23339.00808

Hussain, A. (2024). Understanding the Basics and Major Concepts in Islam. *Blog-Aboutislam*, *January*, 1–9. https://aboutislam.net/Blog/the-Basic-and-Major-Concepts-in-Islam/

Hussain, A., Akram, S., Ahmad, D., Rehman, M., Ahmed, A., and Ali, S. A. (2023). Molecular Assessment and Validation of the Selected Enterococcal Strains as Probiotics. *Probiotics and Antimicrobial Proteins*, *0123456789*. https://doi.org/10.1007/s12602-023-10163-6

Hussain Abrar, S. A. (2024). Understanding the Islamic Perspective of Probiotics. Conference of 10th International Conference on Business, Economy, Management and Social Studies Towards Sustainable Economy (10th BEMSS), June. https://doi.org/10.13140/RG.2.2.30173.22242

Islam, S. U. (2016). Clinical Uses of Probiotics. *Medicine*, *95*(5), e2658. https://doi.org/10.1097/MD.0000000000002658

Kechagia, M., Basoulis, D., Konstantopoulou, S. Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N., and Fakiri, E. M. (2013). Health benefit of probiotic: A review. *Hindawi Publishing Corporation*, *2013*, 1–7.

Khoso, A., Hussain, A., Rehman, M., Akram, S., Ahmad, D., Bin, H., and Sindhu, A. (2024). Molecular Assessments of Antimicrobial Protein Enterocins and Quorum Sensing Genes and Their Role in Virulence of the Genus *Enterococcus*. *Probiotics and Antimicrobial Proteins*, *0123456789*. https://doi.org/10.1007/s12602-024-10278-4

Kimura, K. (2004). Health benefits of probiotics: Probiotics for *Helicobacter pylori* infection. *Food Science and Technology Research*, *10*(1), 1–5. https://doi.org/10.3136/fstr.10.1

Kurniati, K., and Hafsan, H. (2022). Halal Critical Point of Microbial Bioprocess Based-Dairy Products. *Journal of Islam and Science*, *9*(1), 9–16. https://doi.org/10.24252/jis.v9i1.28804

Lodemann, U., Strahlendorf, J., Schierack, P., Klingspor, S., Aschenbach, J. R., and Martens, H. (2015).Effects of the Probiotic *Enterococcus faecium* and Pathogenic *Escherichia coli* Strains in a Pig and Human Epithelial Intestinal Cell Model. *Scientifica*, *2015*, 1–10. https://doi.org/10.1155/2015/235184

Maftei, N. M., Raileanu, C. R., Balta, A. A., Ambrose, L., Boev, M., Marin, D. B., and Lisa, E. L. (2024). The Potential Impact of Probiotics on Human Health: An Update on Their Health-Promoting Properties. *Microorganisms*, *12*(2), 1–29. https://doi.org/10.3390/microorganisms12020234

Mejía-Caballer, A., Salas-Villagrán, V. A., Jiménez-Sern, A., and Farrés, A. (2021). Challenges in the production and use of probiotics as therapeuticals in cancer treatment or prevention. *Journal of Industrial Microbiology and Biotechnology*, *48*(August). https://doi.org/10.1093/jimb/kuab052

Mohd Izhar Ariff, M. K., Muhammad Nazir, A., Diana Mardiana, M. Z., Noor Lizza, M. S., Zamzuri, Z., Ahmad Dahlan, S., and Ezad Azraai, J. (2018). Istihalah and Its Effects on Food: an. *International Journal of Civil Engineering and Technology*, *9*(1), 755–762

Mombelli, B., and Gismondo, M. R. (2000). The use of probiotics in medical practice. *International Journal of Antimicrobial Agents*, *16*(4), 531–536. https://doi.org/10.1016/S0924-8579(00)00322-8

Nami, Y., Haghshenas, B., Abdullah, N., Barzegari, A., Radiah, D., Rosli, R., and Khosroushahi, A. Y. (2015). Probiotics or antibiotics: Future challenges in medicine. *Journal of Medical Microbiology*, *64*(2), 137–146. https://doi.org/10.1099/jmm.0.078923-0

Naseem, C., Bano, M., Hussain, A., Mujahid, U., Maqsood, H., Shams, R., Ahmad, M., and Fazilani, S. A. (2023). Combination of probiotics and phytase supplementation positively affects health of Japanese quails. *International Journal of Agriculture and Biology,* 30, 312-316.

Nasreen, S., Ali, S., Andleeb, S., Summer, M., Hussain, T., Imdad, K., Ara, C., and Tahir, H. M. (2024). Mechanisms of medicinal, pharmaceutical, and immunomodulatory action of probiotics bacteria and their secondary metabolites against disease management: an overview. *Folia Microbiologica*. https://doi.org/10.1007/s12223-024-01155-2

Nueno-Palop, C., and Narbad, A. (2011). Probiotic assessment of *Enterococcus faecalis* CP58 isolated from human gut. *International Journal of Food Microbiology*, *145*(2–3), 390–394. https://doi.org/10.1016/j.ijfoodmicro.2010.12.029

O'Hara, A. M., and Shanahan, F. (2007). Mechanisms of action of probiotics in intestinal diseases. *The Scientific World Journal*, *7*, 31–46. https://doi.org/10.1100/tsw.2007.26

Pandey, K. R., Naik, S. R., and Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics- a review. *Journal of Food Science and Technology*, *52*(12), 7577–7587. https://doi.org/10.1007/s13197-015-1921-1

Piqué, N., Berlanga, M., and Miñana-Galbis, D. (2019). Health benefits of heat-killed (Tyndallized) probiotics: An overview. *International Journal of Molecular Sciences*, *20*(10), 1–30. https://doi.org/10.3390/ijms20102534

Plaza-Diaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M., and Gil, A. (2019). Mechanisms of Action of Probiotics. *Advances in Nutrition*, *10*, S49–S66. https://doi.org/10.1093/advances/nmy063

Poindexter, B., Cummings, J., Hand, I. Adams-Chapman, I., Aucott, S. W., Puopolo, K. M., and Mowitz, M. (2021). Use of probiotics in preterm infants. *Pediatrics*, *147*(6). https://doi.org/10.1542/peds.2021-051485

Quijano, G. (2011). The Benefits of Probiotics on Human Health. *Journal of Microbial and Biochemical Technology*, *s1*(01), 1–6. https://doi.org/10.4172/1948-5948.s1-003

Rabetafika, H. N., Razafindralambo, A., Ebenso, B., and Razafindralambo, H. L. (2023). Probiotics as Antibiotic Alternatives for Human and Animal Applications. *Encyclopedia*, *3*(2), 561–581. https://doi.org/10.3390/encyclopedia3020040

Ranjha, M. M. A. N., Shafique, B., Batool, M. Kowalczewski, P. Ł., Shehzad, Q., Usman, M., and Aadil, R. M. (2021). Nutritional and health potential of probiotics: A review. *Applied Sciences*, *11*(23). https://doi.org/10.3390/app112311204

Reid, G. (2005). The Importance of Guidelines in the Development and Application of Probiotics. *Current Pharmaceutical Design*, *11*(1), 11–16. https://doi.org/10.2174/1381612053382395

Reid, G., Jass, J., Sebulsky, M. T., and McCormick, J. K. (2003). Potential Uses of Probiotics in Clinical Practice. *Clinical Microbiology Reviews*, *16*(4), 658–672. https://doi.org/10.1128/CMR.16.4.658-672.2003

Rijkers, G. T., De Vos, W. M., Brummer, R. J., Morelli, L., Corthier, G., and Marteau, P. (2011). Health benefits nd health claims of probiotics: Bridging science and marketing. *British Journal of Nutrition*, *106*(9), 1291–1296. https://doi.org/10.1017/S000711451100287X

Roobab, U., Batool, Z., Manzoor, M. F., Shabbir, M. A., Khan, M. R., and Aadil, R. M. (2020). Sources, formulations, advanced delivery and health benefits of probiotics. *Current Opinion in Food Science*, *32*, 17–28. https://doi.org/10.1016/j.cofs.2020.01.003

Sanders, M. E., Goh, Y. J., and Klaenhammer, T. R. (2019). Probiotics and prebiotics. *Food Microbiology: Fundamentals and Frontiers*, 831–854. https://doi.org/10.1128/9781555819972.ch32

Sanders, M. E., Merenstein, D., Merrifield, C. A., and Hutkins, R. (2018). Probiotics for human use. *Nutrition Bulletin*, *43*(3), 212–225. https://doi.org/10.1111/nbu.12334

Santosa, S., Farnworth, E., and Jones, P. J. H. (2006). Probiotics and their potential health claims. *Nutrition Reviews*, *64*(6), 265–274. https://doi.org/10.1301/nr.2006.jun.265-274

Sarkar, S. (2013). Potential of probiotics as pharmaceutical agent: A review. *British Food Journal*, *115*(11), 1658–1687. https://doi.org/10.1108/BFJ-06-2011-0163

Shanahan, F. (2003). Probiotics: A perspective on problems and pitfalls. *Scandinavian Journal of Gastroenterology, Supplement*, *38*(237), 34–36. https://doi.org/10.1080/00855910310001476

Sharma, S., Arora, M., and Baldi, A. (2013). Probiotics in India: Current status and future prospects. *PharmAspire*, *1*, 1–11.

Singh, K., Kallali, B., Kumar, A., and Thaker, V. (2011). Probiotics: A review. *Asian Pacific Journal of Tropical Biomedicine*, *1*, S287–S290. https://doi.org/10.1016/S2221-1691(11)60174-3

Snydman, D. R. (2008). The safety of probiotics. *Clinical Infectious Diseases*, *46*. https://doi.org/10.1086/523331

Stavropoulou, E., and Bezirtzoglou, E. (2020). Probiotics in Medicine: A Long Debate. *Frontiers in Immunology*, 11(September), 1–20. https://doi.org/10.3389/fimmu.2020.02192

Suvorov, A., Ermolenko, E., Alechina, G., Chernysh, A., Karaseva, A., Di Pierro, F., and Suvorov, A. (2019). *Enterococcus* as probiotics: what is the advantage? *Nutrafoods*, *1*, 17–25. https://doi.org/10.17470/NF-019-0003

Tachibana, L., Telli, G. S., de Carla Dias, D., Gonçalves, G. S. Ishikawa, C. M., Cavalcante, R. B., and Ranzani-Paiva, M. J. T. (2020). Effect of feeding strategy of probiotic *Enterococcus faecium* on growth performance, hematologic, biochemical parameters and non-specific immune response of Nile tilapia. *Aquaculture Reports*, *16*, 100277. https://doi.org/10.1016/j.aqrep.2020.100277

Tegegne, B. A., and Kebede, B. (2022). Probiotics, their prophylactic and therapeutic applications in human health development: A review of the literature. *Heliyon*, *8*(6), e09725. https://doi.org/10.1016/j.heliyon.2022.e09725

Verna, E. C., and Lucak, S. (2010). Use of probiotics in gastrointestinal disorders: What to recommend? *Therapeutic Advances in Gastroenterology*, *3*(5), 307–319. https://doi.org/10.1177/1756283X10373814

Weichselbaum, E. (2009). Probiotics and health: A review of the evidence. *Nutrition Bulletin*, *34*(4), 340–373. https://doi.org/10.1111/j.1467-3010.2009.01782.x

Wolfe, W., Xiang, Z., Yu, X., Li, P., Chen, H., Yao, M., Fei, Y., Huang, Y., Yin, Y., and Xiao, H. (2023). The Challenge of Applications of Probiotics in Gastrointestinal Diseases. *Advanced Gut and Microbiome Research*, *2023*, 1–10. https://doi.org/10.1155/2023/1984200

Yap, C. K., and Al-Mutairi, K. A. (2023). Effective Microorganisms as Halal-Based Sources for Biofertilizer Production and Some Socio-Economic Insights: A Review. *Foods*, *12*(8). https://doi.org/10.3390/foods12081702

Chapter 05

Beneficial Effects of Probiotics on the Animal Production Cycle: An Overview of Clinical Impacts and Performance

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ABSTRACT

Livestock is a significant source of income for small-scale farming and agriculture. The primary element of livestock farming, which has drawn extra attention for enhancing animal performance, is feed. Numerous studies have been conducted to enhance feed utilization by incorporating feed additives. Antibiotics have long been a common addition to livestock diets to promote growth. The search for substitute feed additives has gotten more intense since they are prohibited in many countries. Probiotics are substitutes that are known to be safe for animals. It has been demonstrated that probiotic use of probiotics in livestock has been demonstrated to enhance immunity, productivity, and animal health. Probiotics increased feed conversion rate, nutrient digestibility, and the rumen microbial ecosystem to improve growth performance. The purpose of this review article is to discuss the role of probiotics in livestock production. This paper reviews the beneficial effects of probiotics on the animal production cycle, encompassing aspects of growth promotion, disease prevention, feed efficiency, and environmental sustainability. Probiotics exert their effects through mechanisms such as competitive exclusion of pathogens, modulation of the gut microbiota, enhancement of nutrient absorption, and modulation of immune responses. In poultry production, probiotics have been shown to improve growth performance, reduce the incidence of digestive disorders, and enhance resistance to pathogens such as *Salmonella* and *Campylobacter*. This review attempts to discuss the potential roles of probiotics on productive performance, health, digestive system and immune system of animals.

KEYWORDS
Animal health, Digestibility, Disease prevention, Livestock

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INTRODUCTION

Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts, have emerged as valuable tools in optimizing animal production cycles across various agricultural sectors (WHO, 2001). Probiotics, which are classified as non-pathogenic microorganisms, are now frequently added to livestock feed. By maintaining a healthy gastrointestinal environment and enhancing intestinal function, their use aims to improve production performance and disease prevention (Chaucheyras-Durand et al., 2008; Mountzouris et al., 2009).

Antibiotics have been utilized for a very long time in animal husbandry, both as treatments for bacterial infections and as growth promoters (Beyene, 2016). Breeders are under increasing pressure to find alternative, more environmentally friendly techniques due to the ban on using antibiotics as growth promoters and the negative effects of overusing them, such as antibiotic resistance and the presence of antibiotic residues in food and the environment (AMCRA, 2020). From long time nutritionists are interested to manipulate the microbial environment of the rumen to improve feed utilization, thereby improving animal production and health and, in recent years, the safety and quality of ruminant food products. Antimicrobial resistance has emerged as a result of the therapeutic use of these antibiotics in animals as a result, antimicrobial medications are less effective in treating human illnesses and can potentially spread antibiotic resistance to humans (Prestinaci et al., 2015). For this reason, probiotics are thought to be a great alternative to antibiotics or antimicrobial agents in the fields of animal health and livestock production.

Probiotics have been the subject of numerous studies suggesting that they may have a significant impact on breeding as a potential replacement for conventional antibiotics or as a straightforward supplement with positive effects on growth (Nikoskelainen et al., 2003; Biavati et al., 2018). Probiotics are beneficial microorganisms that, when taken in sufficient amounts, can change the microflora in the gut of the host leading to better health (Rook and Brunet, 2005). Several types of bacteria (mainly Lactic acid and Non-Lactic Acid Bacteria), yeasts (milk strains) or fungi can be considered probiotics (Tripathi et al., 2008). The traditional uses of probiotics to improve gut health, such as reducing lactose intolerance, boosting intestinal immunity to infections, reducing traveler's diarrhea, and relieving bloating, have been extensively studied and documented (Tellez et al., 2001). Probiotic studies have been conducted in pets, equine and livestock, with chickens and pigs being the main focus of the research. Clinical studies have shown that probiotics can improve the growth of a variety of domestic animals, including cows, newborn calves, piglets and broilers (Kurtoglu et al., 2004).

Beneficial Effects of Probiotics for Livestock

Probiotics enhance growth rate and feed conversion efficiency (Haddad and Goussous, 2005). They also strengthen defense against infectious diseases (Collado et al., 2007; Vanderpool, 2008; Yan and Polk, 2010), improve nutritional absorption and digestion (Soren et al., 2013), and enhance the quality and yield of milk (Kritas et al., 2006; Reklewska et al., 2000). Additionally, probiotics reduce contamination and improve carcass quality (Abdelrahman and Hunaiti, 2008). Probiotics offer numerous benefits, including enhanced nutrient absorption and digestibility, accelerated growth and productivity (Soren et al., 2013), and the inhibition of disease-causing organisms. They help prevent bacterial infection-related diarrhea, reduce stress following vaccination, antibiotic therapy, and travel, and stimulate the immune system (Collado et al., 2007; Vanderpool, 2008). Regular and sensible intake of probiotics significantly impacts the immune system by increasing the production of natural interferons and immunoglobulins, and stimulating cell-mediated immunity (Koenen et al., 2004). The strain should possess the ability to confer advantageous effects on the host animal, such as heightened growth or resilience against illnesses. It must not be harmful or pathogenic. It must exist as live cells, ideally in big quantities. It must be able to survive and undergo metabolism in the gastrointestinal tract; for instance, it must be resistant to low pH and organic acids. It must be stable and able to endure extended periods of time in both field and storage conditions (Ezema, 2013).

Table 1: Microorganisms used as probiotics and their beneficial effect in animals

Genus	Species	Benefits in livestock	References
	L. acidophilus	Reduced scouring	(Retta, 2016)
	L. casei	Improved feed intake	(Rahimoon et al., 2023)
	L. rhamnosus	Enhanced live weight gain	(Fooks and Gibson, 2002)
Lactobacillus	*L. reuteri Bacillus*		(Lodemann et al., 2006)
	L. plantarum		(Seo et al., 2010)
	L. fermentum		
	L. brevis		
	L. helveticus		
	L. delbruckei		
	L. gallinarum		
	L. salivarius		
	B. subtilis	Incease in live weight gain	(Kritas et al., 2006)
	B. cereus	Enhanced milk production	(Qiao et al., 2009)
Bacillus	*B. toyoi*	increased ruminal digestibility	
	B. natto		
	B. mesentericus		
	B. licheniformis		
	B. bifidum	Increased feed efficiency and Reduced	(Abe et al., 1995)
Bifidobacterium	*B. pseudolongum*	incidence of diarrhea.	
	B. breve		
	B. thermophilum		
	S. cerevisiae	enhanced humoral immunity	(Roos et al., 2010)
Saccharomyces	*S. boulardii*		
Aspergillus	*A.oryzae*	Improved feed intake	(Beharka et al., 1991)
	A. niger	Live weight gain	
Enterococcus	*E. faecium*	Increased production of milk	(Nocek and Kautz, 2006)
Streptococcus	*S. thermophiles*	Improved feed intake	(Retta, 2016)
		Reduced scouring	
Pediococcus	*P. acidilactici*	Deffence against Salmonellosis	(Rahimoon et al., 2023)
Lactococcus	*L. lactis*	Deffence against Salmonellosis	(Rahimoon et al., 2023)

Mode of Action of Probiotics

Probiotics may function through the following mechanisms, according to theories: (1) production of anti-pathogenic compounds; (2) competition for nutrients; (3) immune system stimulation; and (4) competition for colonization sites. Antagonism with the pathogen by immunomodulation of the host and prevention of bacterial toxin production (Isolauri et al., 2001; (Guillot, 2003). While yeasts are specifically linked to the final two mechanisms, lactic bacteria are typically responsible for the first three. Probiotic bacteria differ in how they act depending on the host and strain; therefore, combining probiotics with various modes of action may increase the range of protection offered by biotherapeutic preparations (Lima-Filho et al., 2000).

Other action such as bacterial probiotics produce organic acids that can lower the pH of the stomach in monogastric animals improving the environment for the microbiota living there and decreasing the likelihood of pathogen colonization (lactic or acetic acid). It has been demonstrated that some bacteria are capable of producing enzymes that can hydrolyze bacterial toxins or of emitting antimicrobial peptides like bacteriocins, which can stop the growth of harmful bacteria. The ability of certain probiotics to metabolize inhibitory substances like amines or nitrates and assist in their detoxification is crucial for the anaerobic ecology of the gut (Jouany et al., 2008).

Role and Impact of Probiotics on Animal Production

An early increase in the animal's ability to digest and absorb its food has been associated with a change in the makeup of the bacteria that live in the rumen. A higher forage intake can improve live weight gain, milk output, and fat contents of milk, but in dairy cows, the effects are frequently negligible (Cammack et al., 2018). Probiotics have been linked to a decrease in coliform bacteria in newborn calves, according to numerous studies, indicating that they are crucial for establishing and preserving a balanced microbiota in dairy cattle, probiotics can improve milk production. *Enterococcus faecalis, Bacillus sublitis*, and *Saccharomyces cerevisiae* are examples of probiotic microorganisms that can improve milk secretion (Ma et al., 2020). Probiotics have the ability to increase the body weight of ruminants. One probiotic combination that was taken from a fine goat and fed to other goats for about two months included *Lactobacillus reuteri* DDL 19, *Lactobacillus alimentarius* DDL 48, *Enterococcus faecium* DDE 39, and B*ifidobacterium bifidum* DDBA. As a result, the goat's standard bodyweight increased by 9% (Apás et al., 2010). Numerous studies have demonstrated that probiotics have no effect on a carcass's dressing percentage, marbling score, yield grade, or quality grade. Yet, hot carcass weight was generally greater when probiotics were incorporated into the diet. Nonetheless, compared to heifers not given the probiotic, those given a Propionibacterium probiotic both during the receiving and finishing stages had a higher percentage of carcasses graded (Filho-Lima et al., 2000). Increased production of volatile fatty acids, nutrient digestibility, feed conversion rate, and stimulation of lactic acid-dependent protozoa were used to confirm that probiotics improved growth performance (Abd El-Tawab et al., 2016). Probiotics have been used to boost milk production, decrease diarrhea in both pigs and cattle, and prevent Salmonella from colonizing chickens' digestive tracts. They have also been used to improve the efficiency with which feed is utilized (Bernardeau and Vernoux, 2013). According to a study, probiotics improved performance. Sheep that were allowed to graze in the trials showed increased feed intake and growth. Certain animals were classified as having "high" performance (Seo et al., 2010). A small ruminant study found that in a single trial, the number of "low" emitters per unit of feed intake may increase, and it was confirmed in a follow-up trial that these differences in growth rate, nutrient digestibility, and fermentation persisted when the same type of diet was fed (Oetzel et al., 2010).

Role and Impact of Probiotics on Animal Health

Probiotics have been used as livestock feed supplements. Initially, the idea behind adding them to feed was to boost the animal's resistance to illness, thereby promoting growth and overall health (Kesarcodi-Watson et al., 2008). Probiotics have a proven ability to treat a variety of diseases such as cancer prevention, intestinal health enhancement, orodental disease, or hypercholesterolemia (Kechagia et al., 2013). However, these probiotics must be able to be used against other conditions, including lactose intolerance, acute diarrhea, and antibiotic-associated diarrhea. (Nazir et al., 2023). In the first twenty-four hours following feeding, yeast cultures can accelerate the breakdown of rumen fiber, thereby increasing the amount of fodder consumed by ruminants. Moreover, a correlation has been observed between reduced incidence of diarrhea and higher rates of Lactobacillus loss. On the other hand, when animals experience stress, the Lactobacillus population declines, and infant diarrhea becomes more prevalent. Performance responses are probably not as important as reducing the prevalence of diarrhea, which is particularly common in young pre-ruminants (especially within the first three weeks of life). Beef calves must endure several hardships prior to joining the feedlot, such as weaning, transport, fasting, assembly, immunization, castration, and dehorning (Jouany et al., 2008).

Probiotic supplements have been demonstrated in a few recent studies to improve gut microbiota, lower pathogen shedding and disease symptoms, boost gut immunity, and enhance health and disease resistance in animals (Cao et al., 2019, Chaves et al., 2017, Yang et al., 2015; Vendrell et al., 2008; Sorroza et al., 2013). Probiotics also have an antagonistic effect on foodborne pathogens like Salmonella, *Clostridium perfringens, Escherichia coli, Listeria monocytogenes*, and *Staphylococcus aureus*, as well as the capacity to regulate the gut microflora (Van Immerseel et al., 2006; Giannenas et al., 2012; Jungersen et al., 2014)

Role of Probiotics on Digestive System of Livestock

Probiotics help maintain a healthy balance between beneficial and pathogenic bacteria in the stomach, intestine, and cecum, which speeds up an animal's rate of digestion and makes it easier for absorb nutrients. Additionally, they will help broilers' body weight, digestibility of amino acids, and capacity to be stimulated by calcium be improved (Bai et al., 2013). Probiotics can increase the digestibility of food by increasing the activity of the host's digestive tracts enzymes.

Probiotics provide digestible proteins, vitamins, enzymes, and other cofactors; they also produce lactic acid, which aids in better digestion, nutritional metabolism, and nutrient utilization. The amylase, protease, and lipase content of lactic acid contributes to better feed conversion efficiency by facilitating better feed digestion and absorption of fat, protein, and carbohydrates (Awad et al., 2009). Probiotics improve the host's ability to digest food by boosting the activity of digestive enzymes in the GIT. *Lactobacillus acidophilus*, for instance, is present in probiotic feed that has been shown to increase dry matter intake, daily feed conversion efficiency, and apparent digestibility of nutrients in buffalo calves when compared to the control group (Sharma et al., 2018). In animals, probiotics speed up the digestive process. In broilers, probiotics can improve the composition of cecal microorganisms and the way they digest nutrients (Khalid et al., 2021).

Role of Probiotics in the Immune System

Probiotics have a variety of ways to strengthen the host's immunity. Probiotics have been shown in numerous studies to have immunostimulatory properties (Bilal et al., 2021; Kong et al., 2020; Punetha et al., 2018; Terada et al., 2020). *Lactobacillus fermentum* and *Saccharomyces cerevisiae* probiotics stimulated the gut T-cell immunity, as evidenced by the increased production of CD3+, CD4+, and CD8+ T-lymphocytes in the chicken gastrointestinal tract (Bai et al., 2013). When fed food containing probiotics *Lactobacillus jensenii* TL2937 and *Lactobacillus gasseri* TL2919, the expression of CD3+, IL-2, and IFN-γ genes was higher in the small intestine of neonatal chicks that were three and seven days old (Sato et al., 2009). In chickens, probiotics can also raise serum immunoglobulin levels. IgA and IgM serum levels were increased in chickens by a probiotic feed supplement that included *Lactobacillus acidophilus, Bacillus subtilis*, and *Clostridium butyricum* (Zhang and Kim, 2014).

Lactic acid bacteria, or LABs, have the ability to produce a wide variety of antimicrobial compounds that prevent pathogenic invasions. Defensins, organic acids, bacteriocins, ethanol, carbon dioxide, and diacetyl are a few examples of antimicrobial peptides (AMPs) (Liao and Nyachoti, 2017). It has been demonstrated that organic acids such as lactic acid, formic acid, and short-chain fatty acids can suppress potentially harmful microbes that are significant for farm animals. Lactic acid is the primary byproduct of glucose metabolism that can be produced by Lactobacillus bacteria (Russo et al., 2017).

Table 2: Major beneficial effects for probiotics used in livestock (Ahasan et al., 2015, Newbold, 2006)

Animal	Common benefits
Cattle	Increase in efficiency of feed
	enhancing health and preventing acidosis
	Increase milk yield and quality
	Promote weight gain
Young ruminants	Promote maturation of rumen microflora
	Minimize the colonization of pathogen within the body
	Boosting the safety of digestion during weaning
Equine	improved digestibility of the diet
	Reducing diarrhea in foals
	Prevent digestive disorders such as Colic
	Reduce stress in a racing horse
Poultry	Maintain intestinal microbiota healthy.
	Increased weight gain
	Enhance broiler carcass/meat
	Adjust the immune response
	Risk reduction of salmonellosis in layers
Pig	Enhance milk quality and quantity, as well as colostrum quality.
	Boost the vitality and size of the litter
	Reduce the occurence of diarrhea by increasing piglet weight
	Increase meat quality, digestibility, and feed efficiency
	Minimize constipation

Conclusion

Probiotics enhance the health and performance of livestock, which ultimately benefits their productivity. Probiotics help to enhance digestion by lowering clinical and subclinical acidosis, raise ruminal pH, and enhance the ecology of ruminal microflora. Probiotics improve the quality and quantity of milk and meat produced, as well as the growth of many domestic animals. Additionally, probiotics have the potential to defend animals from infections and strengthen the immune system. In simple words use of probiotics enhance production of livestock by enhancing health, digestion and immune system. Particular probiotic strains need to be carefully chosen for each species of animal in a given environment in order to avoid any potential harm. Since every probiotic has a unique behavior, it is crucial to determine the ideal circumstances in a given setting for a probiotic to flourish, colonize, multiply, and benefit the host animals. It is important to have a comprehension knowledge of the immunomodulatory impacts of different probiotics and their viability prior to adding probiotics to farm animals' dietary feed. Furthermore, comprehensive studies that are dependent on dosage should be carried out, and molecular testing at a reliable laboratory should be used to confirm the identity of the organisms. Before creating thorough guidelines for the safe and efficient use of probiotics, more research is required.

Ethics Approval

This study did not require an ethics license.

REFERENCES

Abd El-Tawab, M.M., Youssef, I.M., Bakr, H.A., Fthenakis, G.C. and Giadinis, N.D. (2016) Role of probiotics in nutrition and health of small ruminants. *Pol Journal Veterinary Science*, 19, 893–906.

Abdelrahman, M. M., and Hunaiti, D. A. (2008). The effect of dietary yeast and protected methionine on performance and trace minerals status of growing Awassi lambs. *Livestock Science*, 115(2-3), 235-241.

Abe, F., Ishibashi, N., and Shimamura, S. (1995). Effect of administration of bifidobacteria and lactic acid bacteria to newborn calves and piglets. *Journal of Dairy Science*, 78(12), 2838-2846.

Ahasan, A.S.M.L., Agazzi, A., Invernizzi, G., Bontempo, V., and Savoini, G. (2015). The Beneficial Role of Probiotics in Monogastric Animal Nutrition and Health. *Journal Dairy, Veterinary and Animal Research,* 2.

AMCRA. (2020) Centre de Connaissances Concernant L'utilisation des Antibiotiques et L'antibiorésistance Chez les Animaux. Retrieved on 13/09/2020. Available from: https://www.amcra.be/fr/antibiotiques-et-antibioresistance.

Apas, A. L., Dupraz, J., Ross, R., Gonzalez, S. N., and Arena, M. E. (2010). Probiotic administration effect on fecal mutagenicity and microflora in the goat's gut. *Journal of Bioscience and Bioengineering,* 2010. 06. 005.

Awad, W.A., Ghareeb, K., Abdel-Raheem, S., and Böhm, J. (2009). Effects of dietary inclusion of probiotic and symbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Science*, 88, 49–56.

Bai, S. P., Wu, A. M., Ding, X. M., Lei, Y., Bai, J., Zhang, K. Y., and Chio, J. S. (2013). Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. *Poultry Science*, 92(3), 663-670.

Beharka, A. A., Nagaraja, T. G., and Morrill, J. L. (1991). Performance and ruminal function development of young calves fed diets with Aspergillus oryzae fermentation extract. *Journal of Dairy Science*, 74(12), 4326-4336.

Bernardeau, M. and Vernoux, J.P. (2013) Overview of differences between microbial feed additives and probiotics for food regarding regulation, growth promotion e_ects and health properties and consequences for extrapolation of farm animal results to humans. *Clinical Microbiology Infection*, 19, 321–330.

Beyene, T. (2016) Veterinary drug residues in food-animal products:Its risk factors and potential effects on public health. *Journal Veterinary Science Technology*, 7(1):1–7.

Biavati, B., and Mattarelli, P. (2018). The Bifidobacteria and Related Organisms. Cambridge: Academic Press; Related genera within the family Bifidobacteriaceae; pp. 49–66.

Bilal, M., Si, W., Barbe, F., Chevaux, E., Sienkiewicz, O., and Zhao, X. (2021) Effects of novel probiotic strains of Bacillus pumilus and Bacillus subtilis on production, gut health, and immunity of broiler chickens raised under suboptimal conditions. *Poultry Science Journal,* 2020. 11. 048

Camilleri, G.M., Verger, E.O., Huneau, J.F., Carpentier, F., Dubuisson, C., and Mariotti, F. (2013) Plant and animal protein intakes are differently associated with nutrient adequacy of the diet of French adults. *Journal Nutrition*, 143(9):1466–1473.

Cammack, K. M., Austin, K. J., Lamberson, W. R., Conant, G. C., and Cunningham, H. C. (2018). RUMINNAT NUTRITION SYMPOSIUM: Tiny but mighty: the role of the rumen microbes in livestock production. *Journal of Animal Science*, 96(2), 752-770.

Cao, L., Yang, X.J., Li, Z.J., Sun, F.F., Wu, X.H., and Yao, J.H. (2012) Reduced lesions in chickens with Clostridium perfringens-induced necrotic enteritis by Lactobacillus fermentum 1. 20291. *Poultry Science*, 91(12):3065–3071.

Chaucheyras-Durand, F., Walker, N.D., and Bach, A. (2008) Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. *Animal Feed Science Technology,* 145: 5-26.

Chaves, B.D., and Brashears, M.M., (2017) Nightingale K.K. Applications and safety considerations of Lactobacillus salivarius as a probiotic in animal and human health. *Journal Applied Microbiology*, 123(1):18–28.

Collado, M.C., Meriluoto, J., and Salminen, S. (2007) In vitro analysis of probiotic strain combinations to inhibit pathogen adhesion to human intestinal mucus. *Fd Research Inter*, 40(5):629-636.

Ezema, C. (2013) Probiotics in animal production: A review. Journal of Veterinary Medicine and Animal Health. Vol. 5(11), pp. 308-316.

Food and Agriculture Organization, (2009). Forum D'experts de Haut Niveau L'agriculture Mondiale àl'horizon 2050, 12-13 Octobre. Rome, Italy: Food and Agriculture Organization.

Fooks, L.J., and Gibson, G.R. (2002) Probiotics as modulators of the gut flora. *British Journal Nutrition,* 88: S39-S49.

Fuller, R. (1989) Probiotics in man and animals. *Journal Applied Bacteriology*, 66, 365-378.

Jouany, J.P., (2008). "Effect of live yeast culture supplementation on apparent digestibility and rate of passage in horses fed a high-fiber or high-starch diet.," *Journal of Animal Science*, vol. 1;86, no. (2):339, p. 47.

Giannenas, I., Papadopoulos, E., Tsalie, E., Triantafillou, E., Henikl, S., Teichmann, K., and Tontis, D. (2012) Assessment of dietary supplementation with probiotics on performance, intestinal morphology and microflora of chickens infected with Eimeria tenella. *Veterinary Parasitology*, 188(1-2):31–40.

Guillot, J.F., (2003) Probiotic feed additives. *Journal Veterinary Pharmacology Thereonology*, 26: 52-55.

Haddad, S.G., and Goussous, S.N. (2005) Effect of yeast culture supplementation on nutrient intake, digestibility and growth performance of Awassi lambs. *Animal Feed Science Technology,* 118: 343-348.

Harimurti, S., and Hadisaputro, W. (2015) Beneficial Microorganisms in Agriculture, Aquaculture and Other Areas. Cham: Springer; Probiotics in poultry; pp. 1–19.

Heredia, N. and García, S. (2018) Animals as sources of food-borne pathogens: A review. *Animal Nutrition*, 4, 250 255.

Isolauri, E., Sutas, Y., Kankaanpaa, P., Arvilommi, H. and Salminen, S., (2001) Probiotics: Effects on immunity. *America Journal Clinical Nutrition*, 73: 444-450. PMID: 11157355.

Jungersen, M., Wind, A., Johansen, E., Christensen, J., Stuer-Lauridsen, B., and Eskesen, D. (2014). The science behind the probiotic strain Bifidobacterium animalis subsp. *lactis BB-12® Microorganisms*, 2(2):92–110.

Kesarcodi-Watson, A., (2008) "Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes.," *Aquaculture*. , vol. 31, no. 274(1), pp. 1-4.

Kechagia, M., Basoulis, D., Konstantopoulou, S., Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N., and Fakiri, E. M. (2013). Health benefits of probiotics: a review. *International Scholarly Research Notices*, (1), 481651.

Khalid, A. H., Ullah, K. S., Naveed, S., Latif, F., Pasha, T. N., Hussain, I., and Qaisrani, S. N. (2021) Effects of spray dried yeast (Saccharomyces cerevisiae) on growth performance and carcass characteristics, gut health, cecal microbiota profile and apparent ileal digestibility of protein, amino acids and energy in broilers. *Tropical Animal Hea and Production*, 11250-021- 02684-5

Koenen, M.E., Kramer, J., van der Hulst, R., Heres, L., Jeurissen, S.H. and Boersma, W.J. (2004) Immunomodulation by probiotic lactobacilli in layer- and meat-type chickens. *Br. Poultry Science*, 45: 355-366.

Kong, Y., Gao, C., Du, X., Zhao, J., Li, M., Shan, X., and Wang, G. (2020) Effects of single or conjoint administration of lactic acid bacteria as potential probiotics on growth, immune response and disease resistance of snakehead fish (Channa argus). *Fish and Shellfish Immunology,* 2020. 05. 003

Kritas, S.K., Govaris, A., Christodoulopoulos, G., and Burriel, A.R. (2006) Effect of Bacillus licheniformis and Bacillus subtilis Supplementation of Ewe's Feed on Sheep Milk Production and Young Lamb Mortality. *Journal Veterinary Medicine A Physiology Pathology Clinicla Medicine,* 53(4): 170-173.

Kurtoglu, V., Kurtoglu, F., Seker, E., Coskun, B., Balevi, T. and Polat, E.S., (2004) Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol. *Food Addition Contam*, 21: 817-823.

Liao, S. F., and Nyachoti, M. (2017) Using probiotics to improve swine gut health and nutrient utilization. *Journal Animal Nutrition*, 06. 007.

Filho-Lima, J. V. M., Vieira, E. C., and Nicoli, J. R. (2000). Antagonistic effect of Lactobacillus acidophilus, Saccharomyces boulardii and Escherichia coli combinations against experimental infections with Shigella flexneri and Salmonella enteritidis subsp. typhimurium in gnotobiotic mice. *Journal of Applied Microbiology*, 88(3), 365-370.

Lodemann, U., Hubener, K., Jansen, N., and Martens, H. (2006) Effects of Enterococcus faecium NCIMB 10415 as probiotic supplement on intestinal transport and barrier function of piglets. *Archive Animal Nutrition,* 60: 35-48.

Ma, Z. Z., Cheng, Y. Y., Wang, S. Q., Ge, J. Z., Shi, H. P., and Kou, J. C. (2020) Positive effects of dietary supplementation of three probiotics on milk yield, milk composition and intestinal flora in Sannan dairy goats varied in kind of probiotics. *Journal Animal Physiology and Animal Nutrition,* (berlin).

Mountzouris, K.C., Balaskas, C., Xanthakos, I., Tzivinikou, A., and Fegeros, K. (2009) Effects of a multi-species probiotic on biomarkers of competitive exclusion efficacy in broilers challenged with Salmonella enteritidis. *Br Poultry Science,* 50: 467-478.

Nazir, S., Rasheed, M., Fatima, O., Raazi, E. T., and Fayyaz, M. (2023). Probiotics, An Approach towards Modern Medication. *Journal of Zoo Biology*, 6(1).

Nikoskelainen, S., Ouwehand, A.C., Bylund, G., Salminen, S., and Lilius, E.M. (2003) Immune enhancement in rainbow trout (Oncorhynchus mykiss) by potential probiotic bacteria (Lactobacillus rhamnosus). *Fish Shellfish Immunology*, 15(5):443–452.

Nocek, J. E., and Kautz, W. P. (2006). Direct-fed microbial supplementation on ruminal digestion, health, and performance of pre-and postpartum dairy cattle. *Journal of Dairy Science*, 89(1), 260-266.

Oetzel, G.R., Emery, K.M., Kautz, W.P. and Nocek, J.E., (2007) Direct-fed microbial supplementation and health and performance of pre- and postpartum dairy cattle: A field trial. *Journal Dairy Science*, 90: 2058-2068.

Prestinaci, F., Pezzotti, P. and Pantosti, A. (2015) Antimicrobial resistance: A global multifaceted phenomenon. *Pathology Glob Health*, 109, 309–318.

Punetha, M., Roy, A. K., Ajithakumar, H. M., Para, I. A., Gupta, D., Singh, M., and Bharati, J. (2018) Immunomodulatory effects of probiotics and prilled fat supplementation on immune genes expression and lymphocyte proliferation of transition stage Karan Fries cows. *Veterinary World*, 2018. 209- 214

Qiao, D., Ke, C., Hu, B., Luo, J., Ye, H., Sun, Y. and Zeng, X. (2009). Antioxidant activities of polysaccharides from Hyriopsis cumingii. *Carbohydrate Polymers*, 78(2), 199-204.

Newbold, C.J., (2006) "Dietary additives to control methanogenesis in the rumen.," International congress series. Elsevier., vol. 1293, pp. 138-147.

Rahimoon, Mohsen, M., Mirani. A.H., Sahito, J.K., Bhutto, A.L., Khoso, P.A., Leghari, R.A., Kaka, A., et al. (2023). "Ameliorative effect of probiotics used in animals: A comprehensive review." Pure and Applied Biology (PAB) 13, no. 2 (2023): 179-193.

Reklewska, B., Ryniewicz, Z., Krzyzewski, J., Karaszewska, A., Goralczyk, M., Nalecz-Tarwacka, T., and Strzalkowska, N. (2000) Dietary manipulation of milk protein content in goats. Annals Warsaw Agricultural University, *Animal Science*, pp 133-143.

Retta, K. S. (2016). Role of probiotics in rumen fermentation and animal performance: a review. *International Journal of Livestock Production*, 7(5), 24-32.

Rook, G.A., and Brunet, L.R. (2005) Microbes, immunoregulation, and the gut. *Gut,* 54: 317-320.

Roos, T. B., Tabeleão, V. C., Dümmer, L. A., Schwegler, E., Goulart, M. A., Moura, S. V., and Gil-Turnes, C. (2010). Effect of Bacillus cereus var. Toyoi and Saccharomyces boulardii on the immune response of sheep to vaccines. *Food and Agricultural Immunology*, 21(2), 113-118.

Russo, P., Arena, M. P., Fiocco, D., Capozzi, V., Drider, D., and Spano, G. (2017) Lact*obacillus plantarum* with broad antifungal activity: A promising approach to increase safety and shelf-life of cereal-based products. *International Journal of Food Microbiology*, 2016. 04. 027

Safari, R., Adel, M., Lazado, C.C., Caipang, C.M., and Dadar, M. (2016) Host-derived probiotics Enterococcus casseliflavus improves resistance against Streptococcus iniae infection in rainbow trout (Oncorhynchus mykiss) via immunomodulation. *Fish Shellfish Immunology*, 52:198–205.

Sato, K., Takahashi, K., Tohno, M., Miura, Y., Kamada, T., Ikegami, S., and Kitazawa, H. (2009) Immunomodulation in gut-associated lymphoid tissue of neonatal chicks by immunobiotic diets. *Poultry Science*,

Seo, J.K., Kim, S.W., Kim, M.H., Santi, D., Kam, D.K., and Ha, J.K. (2010) Direct-fed microbials for ruminant animals. *Asian-Aust Journal Animal Science,* 23: 1657-1667.

Sharma, A. N., Kumar, S., and Tyagi, A. K. (2018) Effects of mannan-oligosaccharides and Lactobacillus acidophilus supplementation on growth performance, nutrient utilization and faecal characteristics in Murrah buffalo calves. *Journal Animal Physiology and Animal Nutrition,* (berlin), 102, 679–689.

Soren, N.M., Tripathi, M.K., Bhatt R.S., and Karim, S.A. (2013) Effect of yeast supplementation on the growth performance of Malpura lambs. *Tropical Animal Health Production,* 45: 547-554.

Sorroza, L., Real, F., Acosta, F., Acosta, B., Deniz, S., Roman, L., El Aamri, F., and Padilla, D. (2013) A probiotic potential of Enterococcus gallinarum against Vibrio anguillarum infection. *Fish Pathology,* 48(1):9–12.

Vanbelle, M., (1990) "Probiotics in animal nutrition: a review.," *Archives of Animal Nutrition*, vol. 1;40, no. 7, pp. 543-67.

Tellez, G., Petrone, V.M., Escorcia, M., Morishita, T.Y., Cobb, C.W., Villasenor, L. and Promsopone, B., (2001) Evaluation of avian-specific probiotic and R. Salmonella enteritidis, Salmonella typhimurium and Salmonella heidelberg-specific antibodies on cecal colonization and organ invasion of. enteritidis in broilers. *Journal Food Protection*, 64: 287-291.

Terada, T., Nii, T., Isobe, N., and Yoshimura, Y. (2020) Effects of probiotics Lactobacillus reuteri and Clostridium butyricum on the expression of toll-like receptors, pro- and anti-inflammatory cytokines, and antimicrobial peptides in broiler chick intestine. *Journal Poultry Science,*

Tripathi, M.K., Karim, S.A., Chaturvedi, O.H., and Verma, D.L. (2008) Effect of different liquid cultures of live yeast strains on performance, ruminal fermentation and microbial protein synthesis in lambs. *Journal Animal Physiology Animal Nutriton,* (Berl) 92: 631-639.

Van Immerseel, F., Russell, J.B., Flythe, M.D., Gantois, I., Timbermont, L., Pasmans, F., and Haesebrouck, F. (2006) The use of organic acids to combat Salmonella in poultry:A mechanistic explanation of the efficacy. *Avian Pathology*, 35(3):182–188.

Vanderpool, C., Yan, F., and Polk, B.D. (2008) Mechanisms of probiotic action: implications for therapeutic applications in inflammatory bowel diseases. *Inflammatory Bowel Diseases*, 14(11):1585-1596.

Vendrell, D., Balcazar, J.L., Blas, I.D., Ruiz-Zzarzuela, I., Girones, O., and Muzquiz, J.L. (2008) Protection of rainbow trout (Oncorhynchus mykiss) from lactococcosis by probiotic bacteria. *Comp Immunology Microbiology*, 31(4):337–345.

Yan, F., and Polk, D.B. (2010) Probiotics: progress toward novel therapies for intestinal diseases. *Current Opinion in Gastroenterology*, 26(2):95.

Yang, F., Wang, A., Zeng, X., Hou, C., Liu, H.S., and Qiao, S. (2015) *Lactobacillus reuteri* I5007 modulates tight junction protein expression in IPEC-J2 cells with LPS stimulation and in newborn piglets under normal conditions. *BMC Microbiology,* 15(35):32.

Zhang, Z.F., and Kim, I. H. (2014) Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. *Poultry Science,* 2013- 03314.

Chapter 06

Reduction of Body Fat and Alteration of Intestinal Microbiota with Probiotics and Prebiotics in Patients with Obesity and Overweight

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ABSTRACT

Obesity/overweight is caused by imbalanced energy between intake and expenditure of calories, leading to excessive fat accumulation that impairs healthy. As a lifestyle illness, excess body fat is a determinant for numerous chronic conditions and is linked to significant morbidity and mortality. Scientific evidence has in past decades established a connection between the intestinal microbiota and obesity as individuals with obese conditions have shown altered gut microenvironment that contributes to mild forms of inflammation. Since gut dysbiosis promotes overweight and obesity, prebiotic and probiotic therapy has emerged as a potent therapeutic strategy to normalize the intestinal microbiota composition. In this chapter, the mechanisms of select prebiotics and probiotics have been investigated as part of understanding how they help in treating obesity. By enhancing beneficial bacteria and reducing the composition of pathogenic microorganisms, prebiotic/probiotic therapy has been shown to produce anti-inflammatory effects that is crucial in reducing body fat in patients with obesity. It has also been established that prebiotics and probiotics are involved in the expression of hunger-reducing hormones and promotes satiety that is essential in reducing energy intake.

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INTRODUCTION

According to the World Health Organization (WHO), obesity is an excessive fat buildup capable of impairing health, fundamentally caused by an energy imbalance between caloric consumption and caloric expenditure (Safaei et al., 2021). Obesity and overweight remain a major public health concern globally and represent the main lifestyle illnesses that contribute to further health concerns and are implicated in numerous chronic illnesses like cardiovascular diseases, malignancies, diabetes, and metabolic syndrome. Overweight and obesity rates continue to rise worldwide and are linked with increased chronic morbidity and mortality. The World Obesity Atlas 2023 reports that in 2020, approximately over 2.6 billion people were affected by overweight and obesity globally, and this figure is projected to reach 4 billion by 2035 and representing an increase from 38% to over 50% of the world's population (Lobstein et al., 2023). Figures from the WHO also estimated about 2 billion and 600 million adults with overweight and obesity in 2014 respectively (Simo et al., 2021).

The annual mortality figures show that in 2019, there were an estimated 5 million obesity-related deaths globally, with the age-standardized death rate (ASDR) approximated at 62.59 per 100,000 population (Chong et al., 2023). Despite public health efforts to manage overweight and obesity, the disorders continue to rise and contribute to excess morbidity and mortality. The complex, multifactorial, and relapsing nature of these chronic conditions and associated significant implications for the health of individuals have led scientists to consider novel therapies with the potential to ameliorate the complications (Guerra et al., 2021). Obesity and overweight are significantly linked to premature death and chronic conditions that compromise life expectancy and overall quality of life for patients and the goal of therapy is to improve outcomes for individuals. There is urgency to fully comprehend mediating mechanisms of overweight and obesity since its worldwide prevalence continues to rise as it would inform the discovery of novel targets for safe and effective treatments and to identify biomarkers for tracking the disorder and the efficacy of the strategies to reduce weight (Clark et al., 2023).

Gut Microbiota Role in Obesity

Scientific knowledge of the intestinal microbiome, as well as its intricate relationship to pathophysiology, has grown substantially in recent years. Obesity alters the intestinal microenvironment required for the survival of diverse viral species than those identified in individuals without obesity, leading to susceptibility to detrimental variants capable of causing more serious disorders (Lin and Li, 2021). The gut flora variations alter the weight and metabolism of the host and any microbial population imbalances or 'dysbiosis' results in various diseases. Animal studies show that host-microbiota alteration can lead to mild inflammatory responses marked by moderate increases in proinflammatory gene expression associated with metabolic syndrome, such a Toll-like receptor (TLR) 5, TLR2, and NOD-like receptor family pyrin domain containing 6 genes (NLRP6) (Chassaing et al., 2017). The results of the loss of genes include changed microbiota combination, low levels of inflammation, and a metabolic syndrome-like composition transferrable via fecal transplant. A healthy gut microbiota maintains the metabolism and energy balance of the body by releasing health-beneficial products like neurotoxins, immunotoxins, and carcinogens which infiltrate the blood and directly modulate gene expression and influence the immunity and metabolic processes in humans (Liu et al., 2021). Any imbalance results in metabolic conditions and increased central appetite that cause obesity.

Further animal studies demonstrate the major role gut microbes play in extracting energy from food through diverse mechanisms. Davis (2016) underline how the host cannot digest many polycarbohydrates from plants and starches, and it is the gut microbes that metabolize them to short chain fatty acids (SCFA), such as butyrate, acetate, and propionate that serve as primary energy sources for colonic epithelium and for processes like lipogenesis and glucogenesis in the liver. Germ-free rat models have been used to establish the link between the intestinal bacteria and adiposity, with studies showing conventionally raised mice that ingest less food exhibited 40% higher body fat content and 47% higher gonadal fat content compared with axenic mice. Transplanting the distal colon microorganisms from the normal mice into their gnotobiotic counterparts also resulted in increased body fat by 60% within 2 weeks without food intake increases or obvious variations in energy expenditure. The findings confirmed the role of intestinal bacteria in affecting the phenotype that is linked to host adiposity. Sarmiento-Andrade et al. (2022) confirmed certain groups of bacteria efficiently absorb nutrients and energy and rapidly metabolize nutrients to boost calories absorbed and increase BMI, with examples showing overgrowth of the phylum *Firmicutes* bacteria and *Bacteroidetes* decrease characterize obese mice and human intestines. Through shotgun sequencing, the profile and composition of intestinal microbiota and their effects on human metabolism has been identified. Based on several studies, bacteria number variations in individuals with obesity versus normal weight individuals has been documented, as shown in how gestational obesity alters the gut bacteria where *Bacteroides* increased levels in the third trimester correlates with twice the susceptibility to neonatal obesity (Gorczyca et al., 2022).

Marvasti et al. (2020) study of 50 normal and 50 obese subjects investigated the relative abundance of gut microbiota and their correlative with the body mass index (BMI) of individuals. They examined the ratio of *Firmicutes* and *Bacteroidets* (F/B), which make up the most frequent phyla of gut microbiota, as well as anaerobic intestinal commensal bacteria such as *F. prausnitzii*, *A. muciniphila*, *Bifido-bacterium*, *Roseburia*, and *Prevotella*. The findings showed the F/B ratio was markedly elevated in subjects with obesity versus the control group, and *Firmicutes* abundance significantly increased while *Bacteroidetes* reduced in obese and control groups respectively. *A. muciniphila* relative abundance also significantly reduced in parallel with BMI increase in obese vs. normal weight, and *Bifidobacterium* relative abundance reduced in the obese group. Conversely, there was substantial elevation of *F. prausnitzii* relative abundance in the subjects with adiposity compared to their counterparts with normal weight.

Overview of Probiotics and Prebiotics

Owing to the role of gut dysbiosis in promoting increased weight and adiposity through promoting inflammation, reduced metabolism of fat and cholesterol, and decreased insulin sensitivity, microbial-level intervention with probiotics for gut ecological dysbiosis has been shown to alter gut flora composition. Several novel therapies like prebiotics and probiotics potentially normalize the intestinal flora (Cai et al., 2023). Biomedical research studies of probiotic species have examined the lactic acid bacteria group whereby treatment of subjects with *Lactobacillus* and *Bifidobacterium* have shown to significantly alter the gut microbiota composition (Azad et al., 2018). Probiotics also have effects on appetite and energy homeostasis by increasing SCFA production, with some *Bifidobacterium* spp. and *Lactobacillus* spp. helping the production of prohealthy conjugated linoleic acid (CLA) responsible for body weight control through metabolizing energy and aiding lipolysis (Wiciński et al., 2020). Prebiotics as well have been considered for managing obesity. The prebiotics that are commonly used are carbohydrate-based, with lactose-produced fructans and Galacto-oligosaccharides (GOS) researched widely. Mechanism of prebiotics in metabolic improvement where they underscore their role in modulating the enteroendocrine function representing their systematic effects on lipid and glucose homeostasis and in controlling satiety. Prebiotic supplementation induces heightened glucagon like peptide-1 (GLP-1) and anorexigenic peptide YY (PYY) circulation induced by SCFA, as well inducing the proliferation of commensal bacteria to alter mucosal architecture (Wang et al., 2023). Based on this background, this chapter sets out to evaluate prebiotics and probiotics anti-adiposity action and altering gut bacteria in obese and overweight individuals.

Alteration of Intestinal Microbiota

The intestinal microflora of obese subjects potentially promotes more efficient dietary energy extraction and storage as opposed to lean individuals. There is further evidence from research showing that obesogenic intestinal microbiota is associated with intestinal inflammation as proinflammatory tumor necrosis factor-a (TNF-α) messenger RNA levels in the ileum are strongly correlated with increased weight, fat mass, and the levels of plasma insulin and glucose when exposed to a high-fat diet (HFD) (Klancic and Reimer, 2019). Hence, enteric bacteria are essential for triggering intestinal inflammation with an HFD as TNF-α mRNA levels are only upregulated in conventionally raised animals as opposed to germ-free (GF) animals (Malesza et al., 2021). The mechanisms of prebiotics and probiotics alteration of intestinal microbiota in obesity management is a crucial focus of research. Important considerations should be made for how prebiotic therapy acted on *Bifidobacterium* spp. deficiency and promoted SCFA production by the obesogenic intestinal bacteria (Dahiya et al., 2017).

Enhancement of Beneficial Bacteria

Salazar et al. (2015) investigated 30 obese women to determine how inulin-type fructans (ITF) change gut microbiota composition and activity with the goal of determining fecal SFCA concentration disparities and exploring the correlation of *Bifidobacterium*, SCFA, and host metabolism biological markers. The subjects were categorized in two groups that received 16 g of ITF or maltodextrin (placebo group) over 3 months. According to the findings, the post-intervention period showed that the obese women receiving ITF had significantly increased *Bifidobacterium* genus as well as certain species genus as *B. adolescentis*, *B. longum*, and *B. pseudocatenulatum*. Further, a Spearman correlation analysis revealed an inverse association of the alterations in *B. bifidum* and *B. adolescentis* with the percentage of fat mass. The fecal SCFA profiles also indicated that acetate, propionate, and total SCFA markedly reduced after prebiotic treatment. Overall, the findings confirmed that ITF selectively modulated the composition of the intestinal microbiome as exhibited by the *Bifidobacterium* spp. which significantly increased. Accordingly, prebiotic treatment in obese individuals selectively enhance beneficial bacteria mainly identified in the adult intestinal flora (Cerdó et al., 2019). It was also found that *Bifidobacterium* had a negative correlation with anthropometric/biological parameters such as levels of serum lipopolysaccharides (LPS), meaning prebiotics potentially improve metabolic endotoxemia in obesity as they reduce gut permeability. The reduced fecal SCFA further indicated prebiotic treatment potentially reduced adiposity in obese individuals.

Table 1: Mechanisms of Select Prebiotics and Probiotics in Gut Alteration and Body Fat Reduction

Gut Alteration	Prebiotic	Probiotic	References
Enhancing beneficial bacteria	Inulin-type fructans		(Salazar et al., 2015)
		Lactobacillus salivarius, *Lactobacillus rhamnosus*, *Bifidobacterium animalis*	(Chen et al., 2022)
Gut barrier regulation		*Akkermansia, Bifidobacterium, Lactobacillus, Leucononstoc*	(Fijan et al., 2023; Everard et al., 2013)
		Lactobacillus plantarum bacteriocin plantaricin (PlnEF)	(Heeney et al., 2018)
Short-chain fatty acid production	Inulin and GOS		(Holmes et al., 2020)
	VSL#3		(Yadav et al., 2013)
Reduction of Body Fat			
Satiety	Inulin-type fructans		(Hamilton and Bomhof, 2023)
	Polydextrose		(Olli et al., 2015; Daud et al., 2014)

Additional research has been done examining the reshaping of obesity-related gut dysbiosis using multi-strain probiotic supplementation as well as its effects on lipid metabolism in managing obesity. In a study by Chen et al. (2022), they treated 82 overweight and obese children with three strains of supplementary prebiotics: *Lactobacillus salivarius* AP-32, *L. rhamnosus* bv-77, and *Bifidobacterium animalis* CP-9. The analysis showed that probiotic supplementation significantly reduced BMI, total cholesterol (TC), low-density lipoprotein (LDL), leptin, and TNF-a in the subjects and there was an alteration of the gut microbiota composition, such as significant increase of the beneficial bacteria *B. animalis* after prebiotic administration. Investigations of gut microbial communities after treating obese diabetic mice with prebiotics have also reported increased *Bifidobacterium* spp. and the *Eubacterium* *rectale/Clostridium coccoides* groups while reducing Firmicutes and *Roseburia* spp. (Everard et al., 2011). The gut bacterial populations also showed significant phylum-wide shift where Bacteroides increased while Firmicutes decreased after prebiotic treatment, and Actinobacteria and Proteobacteria also increased. These gut microbiota composition alterations were associated with significantly reduced levels of fasting glycemia and improved glucose tolerance. When treated with dietary α-cyclodextrin prebiotics, HFD-fed obese mice exhibited increased total number of bacteria: *Bacteroides*, *Bifidobacterium*, and *Lactobacillus*, which were reduced in gut microbiota after feeding the HFD (Nihei et al., 2018).

Regulating Gut Barrier Function

Excessive adiposity correlates with gut permeability as exhibited by the loss of epithelial integrity causing upregulated LPS infiltration and the circulation of other inflammation agents (Di Vincenzo et al., 2024). The shift if gut microbiome adversely affect intestinal permeability and probiotic intervention may attenuate this negative impact by promoting beneficial bacteria. Probiotics role in restoring gut permeability after the effects of excess adiposity. They noted that dysbiosis, inflammatory cytokines, immune cell activation, and enterocyte health alter intestinal permeability (DiMattia et al., 2023). Pro-inflammatory agents like interferon-γ (IFN- γ) and TNF-α temporarily compromised the gut barrier integrity through tight junction (TJ) protein rearrangement in the enterocyte membrane via myosin light chain kinase (MLCK) gene activation (Al-Sadi et al., 2016). Evidence shows that probiotic supplementation ameliorates increased intestinal permeability by potentially promoting the production of TJ protein, increasing mucus secretion and the metabolism of ethanolamine, and producing butyrate that enhances enterocyte health.

Heeney et al. (2018) also assessed the ability of *Lactobacillus plantarum* bacteriocin plantaricin EF (PlnEF) to maintain gut barrier integrity when administered to a mouse model of diet-induced obesity. PlnEF, a two-peptide bacteriocin, has been confirmed to induce cell membrane disruption in bacteria. The mice exhibited significantly increased production of the TJ protein Zonula Occludens-1 (ZO-1) when fed with *L. plantarum* with an HFD, and the transcript levels of ZO-1 mRNA were also elevated in whole ileal tissues. Further, PlnEF peptides prevented IFN-γ and TNF-α induced reductions in transepithelial resistance. Overall, LP probiotics fortified TJ between intestinal epithelial cells as PlnEF peptides sufficiently prevented cytokine-induced disruptions to epithelia barrier integrity. *Akkermansia muciniphila* probiotic has also been investigated for safeguarding gut barrier integrity when a prebiotic like oligofructose is administered (Everard et al., 2013). The evidence showed that *A. muciniphila* normalized metabolic endotoxemia, adiposity, and the CD11c adipose tissue marker. The enhancement of gut barrier function by *A. muciniphila* was linked with epithelia signaling mechanisms by acting on the intestinal mucosa that secretes antimicrobial peptides for innate immunity to maintain the gut barrier.

Production of Short-Chain Fatty Acids

SCFAs are carboxylic acids produced when microorganisms ferment various dietary compounds, majorly fibers. Propionate (Prop), butyrate (Bu), and acetate (Ac) are the most extensively studied as they are produced in the colon (Ilyés et al., 2022). Studies have shown that microbial action of fermenting indigestible dietary carbohydrates to produce SCFAs has proven to be critical in protecting against pediatric obesity and metabolic syndrome. Holmes et al. (2020) conducted an *in vitro* study to examine fecal microbiota production of SCFAs from 17 children with obesity treated with over-the counter (OTC) prebiotic supplements. From the assessment of diverse prebiotic supplements like inulin and GOS, it was proven that administration showed efficacy in increasing the total SCFAs. SCFA production after administering prebiotics also correlated with the relative abundance of beneficial bacterial genera, including *Akkermansia*, *Methanobrevibacter*, and *Lactobbacillus*.

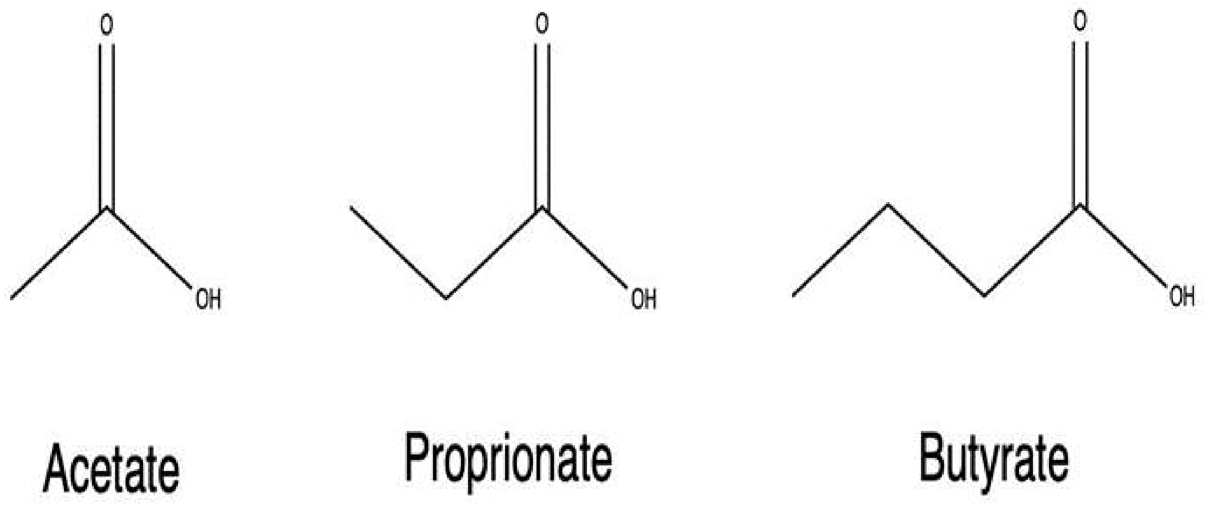

Fig. 1: Short-Chain Fatty Acid Substrates

Yadav et al. (2013) investigated VSL#3 probiotic beneficial metabolic effects when administered to HFD mice to counter obesity and diabetes. Their findings showed that probiotic supplementation reduced the gain in weight corresponding to feeding with a low-fat diet (LFD) while further decreasing fat depot size, fat mass, and adipocyte size without alteration in lean mass. The mechanism of enhancing metabolism in obesity/overweight was linked to significant decrease in the circulating levels of IL-6, TNF-a, and MCP-1, further resulting in decreased food intake, body weight gain, and enhanced glucose homeostasis. The enhanced metabolic function was also exhibited by the decreased insulin, triglycerides, free fatty acids (FFAs), and resistin while adiponectin was elevated and anti-inflammation improved. It was additionally noted that VSL#3 probiotics promoted GLP-1 secretion, mediated by butyrate, from the intestinal L-cells as it modified gut microbiota and altered the level of gut hormones involved in food intake regulation. GLP-1 is a hunger reducing hormone, which was significantly increased with probiotic supplementation. The process causing the upregulation of GLP-1 was the marked decrease in Firmicutes and an upsurge in Bacteriodetes and Bifidobacteria (Cabral et al.,2021). Gut flora alterations were linked to the increased levels of SCFA butyrate as the gut flora composition alterations contributed to the transformed gut metabolic environment.

Prebiotic and Probiotic Reduction of Body Fat

A. Stimulating the Expression of Satiety Hormones

Scientists have documented the role of inulin-type fructans (ITFs) in stimulating anorectic gut hormones release that act to reduce appetite and energy intake. In a randomized crossover study of ITFs mechanisms of inducing satiety, Hamilton and Bomhof (2023) administered sweetened milk (SM) or sweetened milk plus oligofructose-enriched inulin (OI) to participants and recorded the perceived measures of hunger after intervention. In this human trial involving 14 participants between the ages of 18 and 50 years, the subjects exhibited increased GLP-1 and PYY concentrations post-treatment. The appetite perceptions also showed that SM+OI reduced overall hunger relative to SM, and a tendency towards reduced satisfaction with prebiotic supplementation. There was further increased flatulence for SM+OI compared to SM, and increased abdominal discomfort was marked with SM+OI. Hence, the study reported the acute effect of OI after exercise as long-term physical activity is usually related to increased hunger and energy compensation. Overall, OI prebiotics elevated GLP-1 and PYY while reducing acyl-gherin post-exercise, with evidence of OI fermentative activity in the gut.

In another study, Olli et al. (2015) investigated the polydextrose (PDX) prebiotic for its postprandial effects on satiety hormone responses in obese participants with subjective feelings of appetite. The trial involved 18 subjects who consumed a high-fat meal with or without PDX (15 g) assessed postprandial concentrations of peptides ghrelin, cholecystokinin (CCK), GLP-1, and PYY, as well as SCFAs and lactic acid. The subjects exhibited elevated levels of GLP-1 release into the plasma after PDX consumption compared to the placebo, while the rest of the peptides did not show statistically different variations. Lactic acid concentrations significantly decreased post-treatment, and acetic acid marginally reduced. PDX significantly reduced hunger during the satiety period (40.4%), and marginally enhanced satisfaction by 22.5% compared to the placebo. Overall, the downregulated concentrations of postprandial plasma lactate after PDX in obesity implied that prebiotics have a lipolytic effect after consuming an HFD. The reduced food intake due to prebiotic promotion of satiety translates too reduced body fat for individuals and improved obesity outcomes. Daud et al. (2014) investigate 22 healthy overweight and obese participants, oligofructose prebiotics reduced hunger and motivation to eat and decrease energy intake (EI). Slight reductions in intrahepatocellular lipids (IHCL) and glutamyl transferase gamma (γGT) suggested how prebiotics protected against fat accumulation in the liver. More trials can be done to determine the fat-reducing effects of prebiotics and probiotics in obesity.

B. Gut-Brain Axis Modulation

Gut microbiota interacts with diverse organs such as the brain as they might target the brain directly through vagal stimulation or indirectly via immune-neuroendocrine mechanisms (Asadi et al., 2022). Research supports the bidirectional signaling within the gut-brain axis (GBA) in obesity pathophysiology facilitated by mechanisms like the metabolic, endocrine, neural, and immune system. The role of intestinal microbiota in obesity is also linked with regulating adiposity, homeostasis and energy balance, and central appetite and food reward signaling (Rautmann and de La Serre, 2021). The nervous system is instrumental among the probiotic pathways in lipid metabolism regulation, with SCFAs and secondary bile acids induced by probiotic metabolism triggering the intestinal-brain axis by prompting intestinal hormone production. The hypothalamus activation by gut hormones like GLP-1 and PYY alters the intake and expenditure of energy to achieve metabolism balance (Song et al., 2023). Additionally, leptin hormones secreted by the adipose tissue affects appetite when probiotics are administered as the hormones synthesize fat by preventing fatty acid synthase expression and suppressing appetite by stimulating hypothalamic receptors, which also alter the consumption of energy, and enhancing nervous system lipid metabolism.

Wang et al. (2020) have reported on the effects of intestinal bacteria in neuroendocrine modulating carbohydrate and lipid metabolism, specifically focused on the microbiota-gut-brain-liver axis. They noted that intestinal microbiota affected intestinal movement, metabolism, immune responses, and behaviors through the mediation of enteric neurons. SCFAs, such as acetate, propionate, and butyrate activate the vagal afferent pathway and suppress consumption of food. The intestinal LPS influence the vagal pathway and instigates inflammatory responses and obesity, with TLR4 receptor expressed on the afferent fibers capable of sensing LPS and transferring the signal to the brain. Prebiotic and probiotics induce gut peptides that act through the vagal and non-vagal neuronal relays that trigger the gut-to-brain signaling pathways to reduce the ingestion of food and increase energy expenditure (Bauer et al., 2015).

Conclusion

Obesity and overweight remain a significant public health concern worldwide and is one of the major lifestyle diseases contributing to further health concerns and numerous chronic illnesses. The burden of disease has made it an imperative to consider the mediating mechanisms of obesity as this would inform novel therapies after the discovery of new targets. The association between the gut microbiome and obesity has been documented in research, which makes prebiotics and probiotics potential therapeutic candidates for reducing body fat and altering intestinal microbiota. SCFA production and elevated levels of GLP-1 and PYY peptides, as well as proliferation of beneficial bacteria are likely mechanisms through which prebiotics and probiotics modulate obesity outcomes. Future research focused on the metabolic effects of different strains of prebiotics and probiotics can be considered, as well as combination therapies.

REFERENCES

Al-Sadi, R., Guo, S., Ye, D., Rawat, M., and Ma, T. Y. (2016). TNF-α modulation of intestinal tight junction permeability is mediated by NIK/IKK-α axis activation of the canonical NF-κB pathway. *The American Journal of Pathology*, *186*(5), 1151-1165.

Asadi, A., Shadab Mehr, N., Mohamadi, M. H., Shokri, F., Heidary, M., Sadeghifard, N., and Khoshnood, S. (2022). Obesity and gut–microbiota–brain axis: A narrative review. *Journal of Clinical Laboratory Analysis*, *36*(5), e24420.

Azad, M. A. K., Sarker, M., Li, T., and Yin, J. (2018). Probiotic species in the modulation of gut microbiota: An overview. *BioMed Research International*, *2018*.

Bauer, P. V., Hamr, S. C., and Duca, F. A. (2016). Regulation of energy balance by a gut–brain axis and involvement of the gut microbiota. *Cellular and Molecular Life Sciences*, *73*, 737-755.

Cabral, L. Q. T., Ximenez, J. A., Moreno, K. G. T., and Fernandes, R. (2021). Probiotics have minimal effects on appetite-related hormones in overweight or obese individuals: A systematic review of randomized controlled trials. *Clinical Nutrition*, *40*(4), 1776-1787.

Cai, Y., Liu, P., Zhou, X., Yuan, J., and Chen, Q. (2023). Probiotics therapy show significant improvement in obesity and neurobehavioral disorders symptoms. *Frontiers in Cellular and Infection Microbiology*, *13*, 1178399.

Cerdó, T., García-Santos, J. A., G. Bermúdez, M., and Campoy, C. (2019). The role of probiotics and prebiotics in the prevention and treatment of obesity. *Nutrients*, *11*(3), 635.

Chassaing, B., Raja, S. M., Lewis, J. D., Srinivasan, S., and Gewirtz, A. T. (2017). Colonic microbiota encroachment correlates with dysglycemia in humans. *Cellular and Molecular Gastroenterology and Hepatology*, *4*(2), 205-221.

Chen, A. C., Fang, T. J., Ho, H. H., Chen, J. F., Kuo, Y. W., Huang, Y. Y., and Yeh, Y. T. (2022). A multi-strain probiotic blend reshaped obesity-related gut dysbiosis and improved lipid metabolism in obese children. *Frontiers in Nutrition*, *9*, 922993.

Chong, B., Jayabaskaran, J., Kong, G., Chan, Y. H., Chin, Y. H., Goh, R., and Chew, N. W. (2023). Trends and predictions of malnutrition and obesity in 204 countries and territories: an analysis of the Global Burden of Disease Study 2019. *EClinicalMedicine*, *57*.

Clark, J. M., Garvey, W. T., Niswender, K. D., Schmidt, A. M., Ahima, R. S., Aleman, J. O., and Wells, Q. (2023). Obesity and overweight: probing causes, consequences, and novel therapeutic approaches through the American Heart Association's strategically focused research network. *Journal of the American Heart Association*, *12*(4), e027693.

Daud, N. M., Ismail, N. A., Thomas, E. L., Fitzpatrick, J. A., Bell, J. D., Swann, J. R., and Frost, G. S. (2014). The impact of oligofructose on stimulation of gut hormones, appetite regulation and adiposity. *Obesity*, *22*(6), 1430-1438.

Dahiya, D. K., Renuka, Puniya, M., Shandilya, U. K., Dhewa, T., Kumar, N., and Shukla, P. (2017). Gut microbiota modulation and its relationship with obesity using prebiotic fibers and probiotics: a review. *Frontiers in Microbiology*, *8*, 563.

Davis, C. D. (2016). The gut microbiome and its role in obesity. *Nutrition Today*, *51*(4), 167-174.

DiMattia, Z., Damani, J. J., Van Syoc, E., and Rogers, C. J. (2023). Effect of probiotic supplementation on intestinal permeability in overweight and obesity: A systematic review of randomized controlled trials and animal studies. *Advances in Nutrition*, 100162.

Di Vincenzo, F., Del Gaudio, A., Petito, V., Lopetuso, L. R., and Scaldaferri, F. (2024). Gut microbiota, intestinal permeability, and systemic inflammation: a narrative review. *Internal and Emergency Medicine*, *19*(2), 275-293.

Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J. P., Druart, C., Bindels, L. B., and Cani, P. D. (2013). Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences*, *110*(22), 9066-9071.

Everard, A., Lazarevic, V., Derrien, M., Girard, M., Muccioli, G. G., Neyrinck, A. M., and Cani, P. D. (2011). Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Dabetes*, *60*(11), 2775-2786.

Fijan, S. (2023). Probiotics and their antimicrobial effect. *Microorganisms*, *11*(2), 528.

Guerra, J. V., Dias, M. M., Brilhante, A. J., Terra, M. F., Garcia-Arevalo, M., and Figueira, A. C. M. (2021). Multifactorial basis and therapeutic strategies in metabolism-related diseases. *Nutrients*, *13*(8), 2830.

Gorczyca, K., Obuchowska, A., Kimber-Trojnar, Ż., Wierzchowska-Opoka, M., and Leszczyńska-Gorzelak, B. (2022). Changes in the gut microbiome and pathologies in pregnancy. *International journal of Environmental Research and Public Health*, *19*(16), 9961.

Hamilton, C. C., and Bomhof, M. R. (2023). Oligofructose-Enriched Inulin Consumption Acutely Modifies Markers of Postexercise Appetite. *Nutrients*, *15*(24), 5017.

Heeney, D. D., Zhai, Z., Bendiks, Z., Barouei, J., Martinic, A., Slupsky, C., and Marco, M. L. (2019). Lactobacillus plantarum bacteriocin is associated with intestinal and systemic improvements in diet-induced obese mice and maintains epithelial barrier integrity in vitro. *Gut Microbes*, *10*(3), 382-397.

Holmes, Z. C., Silverman, J. D., Dressman, H. K., Wei, Z., Dallow, E. P., Armstrong, S. C., and David, L. A. (2020). Short-chain fatty acid production by gut microbiota from children with obesity differs according to prebiotic choice and bacterial community composition. *MBio*, *11*(4), 10-1128.

Ilyés, T., Silaghi, C. N., and Crăciun, A. M. (2022). Diet-related changes of short-chain fatty acids in blood and feces in obesity and metabolic syndrome. *Biology*, *11*(11), 1556.

Klancic, T., and Reimer, R. A. (2020). Gut microbiota and obesity: Impact of antibiotics and prebiotics and potential for musculoskeletal health. *Journal of Sport and Health Science*, *9*(2), 110-118.

Lin, X., and Li, H. (2021). Obesity: Epidemiology, pathophysiology, and therapeutics. *Frontiers in Endocrinology*, *12*, 706978.

Liu, B. N., Liu, X. T., Liang, Z. H., and Wang, J. H. (2021). Gut microbiota in obesity. *World Journal of Gastroenterology*, *27*(25), 3837.

Lobstein, T., Jackson-Leach, R., Powis, J., Brinsden, H., and Gray, M. (2023). World obesity atlas 2023. World Obesity Federation. https://data.worldobesity.org/publications/?cat=19

Marvasti, F. E., Moshiri, A., Taghavi, M. S., Riazi, S., Taati, M., Sadati, S. F., and Siadat, S. D. (2020). The first report of differences in gut microbiota composition between obese and normal weight Iranian subjects. *Iranian Biomedical Journal*, *24*(3), 148.

Malesza, I. J., Malesza, M., Walkowiak, J., Mussin, N., Walkowiak, D., Aringazina, R., and Mądry, E. (2021). High-fat, western-style diet, systemic inflammation, and gut microbiota: a narrative review. *Cells*, *10*(11), 3164.

Nihei, N., Okamoto, H., Furune, T., Ikuta, N., Sasaki, K., Rimbach, G., and Terao, K. (2018). Dietary α-cyclodextrin modifies gut microbiota and reduces fat accumulation in high-fat-diet-fed obese mice. *Biofactors*, *44*(4), 336-347.

Olli, K., Salli, K., Alhoniemi, E., Saarinen, M., Ibarra, A., Vasankari, T., and Tiihonen, K. (2015). Postprandial effects of polydextrose on satiety hormone responses and subjective feelings of appetite in obese participants. *Nutrition Journal*, *14*, 1-12.

Rautmann, A. W., and de La Serre, C. B. (2021). Microbiota's role in diet-driven alterations in food intake: satiety, energy balance, and reward. *Nutrients*, *13*(9), 3067.

Safaei, M., Sundararajan, E. A., Driss, M., Boulila, W., and Shapi'i, A. (2021). A systematic literature review on obesity: Understanding the causes and consequences of obesity and reviewing various machine learning approaches used to predict obesity. *Computers in Biology and Medicine*, *136*, 104754.

Salazar, N., Dewulf, E. M., Neyrinck, A. M., Bindels, L. B., Cani, P. D., Mahillon, J., and Delzenne, N. M. (2015). Inulin-type fructans modulate intestinal Bifidobacterium species populations and decrease fecal short-chain fatty acids in obese women. *Clinical Nutrition*, *34*(3), 501-507.

Sarmiento-Andrade, Y., Suárez, R., Quintero, B., Garrochamba, K., and Chapela, S. P. (2022). Gut microbiota and obesity: New insights. *Frontiers in Nutrition*, *9*, 1018212.

Simo, L. P., Agbor, V. N., Temgoua, F. Z., Fozeu, L. C. F., Bonghaseh, D. T., Mbonda, A. G. N., and Mbanya, D. (2021). Prevalence and factors associated with overweight and obesity in selected health areas in a rural health district in Cameroon: a cross-sectional analysis. *BMC Public Health*, *21*, 1-12.

Song, X., Liu, Y., Zhang, X., Weng, P., Zhang, R., and Wu, Z. (2023). Role of intestinal probiotics in the modulation of lipid metabolism: implications for therapeutic treatments. *Food Science and Human Wellness*, *12*(5), 1439-1449.

Wang, Y., Salonen, A., and Jian, C. (2023). Can prebiotics help tackle the childhood obesity epidemic? *Frontiers in Endocrinology*, *14*, 1178155.

Wang, S. Z., Yu, Y. J., and Adeli, K. (2020). Role of gut microbiota in neuroendocrine regulation of carbohydrate and lipid metabolism via the microbiota-gut-brain-liver axis. *Microorganisms*, *8*(4), 527.

Wiciński, M., Gębalski, J., Gołębiewski, J., and Malinowski, B. (2020). Probiotics for the treatment of overweight and obesity in humans—a review of clinical trials. *Microorganisms*, *8*(8), 1148.

Yadav, H., Lee, J. H., Lloyd, J., Walter, P., and Rane, S. G. (2013). Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. *Journal of Biological Chemistry*, *288*(35), 25088-25097

Chapter 07

Use of Probiotics and Prebiotics against Clostridial Diseases in Poultry

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ABSTRACT

Despite significant efforts in prevention and control, bacterial illnesses such as *Clostridium perfringens*-induced necrotic enteritis, continue to pose a serious threat to poultry producers in terms of economic losses and market limitations. Antibiotics have been utilized for growth promotion in addition to the prevention and management of such illnesses. Thus, these behaviours have been connected to the development and spreading of microorganisms that are resistant to antibiotics, posing a serious worldwide risk to people, animals, and the environment. Probiotics, prebiotics, and synbiotics are being used with great expectations. Primarily utilized to preserve the balance of the intestinal microbiota in cattle, they prove to be a successful strategy in combating infections that endanger both consumers and animals. This chapter provides a quick overview of the role that antimicrobial resistance plays in combating various bacterial infections, particularly clostridial infections, as well as the replacement of the antibiotics with probiotics, prebiotics, and synbiotics.

KEYWORDS		
Clostridium perfringens, Antibiotics, Probiotics, Prebiotics, Synbiotics, Illness, Microbiota	Received: 18-May-2024 Revised: 14-July-2024 Accepted: 17-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Finding high-quality feed that is both sustainable and reasonably priced is the largest obstacle to commercial chicken production. Despite this obstacle, commercial chick farming is one of the best ways to obtain animal protein (Iyayi, 2008). Compared to other animal sectors that produce food, the poultry business has grown at a higher rate. Parallel to the sharp rise in the world's output of chicken meat and eggs, there has been a rise in the volume of poultry goods traded (Windhorst, 2006).

The information at hand suggests that during the past few years, the chicken meat sector has experienced greater growth than the egg sector (Windhorst, 2006). In the chicken business, feed accounts for a large portion of the entire cost of production for producing meat and eggs. Under better conditions, five to six weeks can be required for broiler chickens to reach a weight of 2-3 kg. It is now difficult to prevent bacterial infections of the intestines without the need for medication in poultry production in Europe and America due to the ban on antibiotic growth boosters. Infection-related mortality is an important problem for the chicken sector.These illnesses cause decreased growth rates in poultry, which leads to financial losses. The primary instruments used to treat or prevent these diseases are antibiotics. The integrity of gastrointestinal tract (GIT) is vital to the health of birds because it acts as their intial defence against foreign invaders and facilitates the absorption of nutrients (Pan and Yu, 2014).

Numerous types of bacteria can be found in the GIT that make up the gut microbiota. These include *Lactobacillus, Clostridium, Ruminococcus, Salmonella enterica serovars*, Enterococcus spp., and *Escherichia coli*. They also maintain homeostasis and aid in the processing of nutrients. Mobile genetic elements like plasmids and transposons have the ability to horizontally transfer genes, hence introducing antimicrobial resistance genes (ARGs) into the gut microbiota. Antibiotics can alter the gut microbiota of chickens by lowering the number of harmful bacteria present, improving intestinal absorption of nutrients, and eventually improving growth characteristics (Diarra et al., 2007). Therefore, it's critical to comprehend how dietary habits affect the intestinal microbiota of chickens (Pan and Yu, 2014; Islam et al., 2019). Necrotizing enteritis (NE), a prevalent avian disease, is caused by the Gram-positive, spore-forming, obligate anaerobic bacterium C. perfringens and the global poultry business loses six billion dollars annually as a result of it(McDevitt et al., 2006). This bacterium is environmental pathogen and normally present in the digestive tracts of humans and animals, and when body undergo stress, it also poses a threat to food safety (Collier et al., 2003). Subclinical NE causes poor feed conversion ratios and decreased weight growth, which in turn causes production losses (Stutz and Lawton, 1984). When *C. perfringens* causes intestinal injury, the germs have access to the bile duct and bloodstream, which destroys other organs in birds (Timbermont et al., 2011). Common antibiotics used to prevent NE in poultry include avilamycin and bacitracin methylene disalicylate. Controlling this infection has therefore become extremely important due to the AMU limits in poultry, not only for the gut health of the birds but also for the sake of food safety (McDevitt et al., 2006). Unfortunately, extended and irrational use of antibiotics in animals can ultimately select for strains or species resistant to the drugs (Aarestrup, 1999). Because genes encoding this resistance can spread to other bacteria that were previously vulnerable, the health of humans and animals may be in danger (Montagne et al., 2003). Consequently, several countries like European Union in January 2000 have limited or completely forbidden (Sweden, January 1986) the use of in-feed medications as growth promoters for animals (Montagne et al., 2003)**.** To enhance performance and control illness, Antibiotic growth promotants (Nagpal et al.) are added to the diets of food animals. The use of enzymes, probiotics, prebiotics, symbiotic products, and even nutrition to improve chicken gut health and prevent or reduce production losses due to enteric illnesses has been investigated by researchers as a potential more effective feed additive than AGP.

Probiotics

The Greek terms "pro" and "bios" give rise to the English word "probiotic," which means "for life." It is likely that Mechnikov, who observed that the initial concept of probiotics was introduced in 1907, suggesting that bacteria could positively impact the normal microflora in the intestinal tract (Miecznikow, 1907). Ferdinand Vergin is credited with coining the word "probiotic" in his 1954 study "Anti- und Probiotika," which compared the beneficial effects ("probiotica") of specific bacteria with the harmful effects of antibiotics and other antimicrobial drugs on the gut microbiota (Vergin, 1954).

Probiotics are defined as “live isolates of strictly specified microbes which, when supplied in sufficient quantities, confer beneficial health effects on the host” in the definition that was developed in 2002 by experts from the FAO and WHO working groups (Joint, 2002). In 2013, ISAPP upheld the idea. A recipe or product is only considered "probiotic" if it satisfies certain tight requirements. The three most crucial of these requirements are: a suitable number of functional cells; a positive impact on the host's health (which may include growth promotion); and a positive impact on the alimentary tract's performance. The effectiveness of probiotic supplements is determined by various factors. It is therefore essential to select the appropriate bacterial strains and utilize the appropriate dosage.Probiotics are beneficial to health and promote growth, that's why they are frequently utilized in animal feed, particularly for chickens and pigs. These kinds of formulas could include one or more carefully selected bacterial isolates, and their administration methods may include powder, suspension, capsules, pellets, gels, or pastes, contingent on the host animals' age and species. These are added to feed and premixes or utilized as a direct peros supplement on a periodic or continuous basis. When probiotic cultures are added to feed, they have to fulfil certain requirements (Markowiak and Śliżewska, 2018).

Probiotic Microorganisms

Products that contain probiotics may contain one or more particular bacterial isolates. In the EU, the majority of microbes utilized as feed additives are bacteria. *Pediococcus, Lactobacillus, Enterococcus, Bacillus, and Streptococcus* are the genera from which they are usually Gram-positive bacteria. Probiotics include certain yeast strains from Kluyveromyces species and Saccharomyces cerevisiae. Bacteria from the genera *Lactobacillus and Enterococcus,* which are normally found in levels of 107–108 and 105–106 CFU/g, respectively, make up the natural microflora of the animal gastrointestinal tract. However, the digestive system typically does not include yeast or bacteria from the *Bacillus* genus. Most of the microbes listed above ought to be potentially harmless to the host. Some of them, though, could be problematic. For example, antibiotic resistance may spread due to bacteria belonging to the *Enterococcus* genus and certain strains of *B. cereus* could create the endotoxins and emetic toxins (Anadón et al., 2006).

Mode of Action of Probiotics

Diseases may arise based on how the host and microorganism interact (Garcia et al., 2010). The exact mode of action of SFPs remains unclear. Nonetheless, it has been found that SFPs function via the same mechanism as regular probiotic microbes (Cartman et al., 2008). Probiotics' working principle in poultry has been described in a variety of ways (Figure no 1). The first method was termed as "competitive exclusion."; it works by preventing harmful germs from colonizing sites of adhesion through competition for them (Chichlowski et al., 2007). Beneficial bacteria's adherence allowed for this competitive exclusion to occur (Chichlowski et al., 2007). The capacity of the SFP strain to cling to the intestinal wall is necessary for the continuation of this mechanism of action (Fuller, 1989). The metabolites produced by gut bacteria compete with pathogenic substances for adherence in the epithelium of the intestine and regulate their growth. These metabolites include hydrogen peroxides, bacteriocins, and short-chain organic acids. (Dankowiakowska et al., 2013) assist in adhesion to the mucosal layer of the intestine (Buck et al., 2005). The newborn's digestive tract is sterile, and germs from the surroundings begin to infiltrate the gut before its organs can manufacture antibodies. As a result, using probiotics helps to naturally inhibit the microorganisms that cause sickness because of their capacity to adhere to the gut mucosa (Dankowiakowska et al., 2013). Probiotics have been shown to have a second mode of action that involves regulating dysbiosis. Enteric dysbiosis has the potential to modify host-microbe interactions, resulting in medical disorders (Byndloss and Bäumler, 2018; Plaza-Díaz et al., 2018). Probiotics may be able to treat dysbiosis and balance out the disturbed or unbalanced microflora, according to recent research (Vieira et al., 2016; Mendes et al., 2018). Probiotics' antagonistic action enabled the third mechanism to function. A class of tiny antimicrobial compounds produced by probiotics (called bacteriocins, mucins, and defensins) prevents infections from colonizing (Khan and Naz, 2013). The most significant advantage of probiotics is thought to be their ability to modify GI immunity, which is known to be the fourth mode of action. Numerous investigations have demonstrated that probiotics stimulate the production of anti-inflammatory mediators, including transforming growth factor beta (TGF-β), thymic stromal lymphopoietin (TSLP), and interleukin-10 (IL-10). Additionally, concurrently reduces the generation of pro-inflammatory cytokines such as TNF-α and interleukin 8 (IL-8) (Georgieva et al., 2015). The ways in which probiotics work are:

1. Competitive exclusion of harmful bacteria by probiotics.
2. Probiotics restore dysbiosis resulting from any cause.
3. Probiotics generate bactericidal chemicals that lyse infections, whereas probiotic surface receptors deactivate and disrupt microbes.
4. When intestinal cells come into contact with pathogens, they release pro-inflammatory substances such as TNF-α, interleukin-8, and interleukin- 12. By producing more anti-inflammatory mediators like TGF-β and TSLP, probiotics reduce the synthesis of pro-inflammatory chemicals and help transform premature dendritic cells into regulatory dendritic cells. TGF-β, or transforming growth factor-β; TSLP, lymphopoietin in stroma of thymus (Khalid et al., 2022).

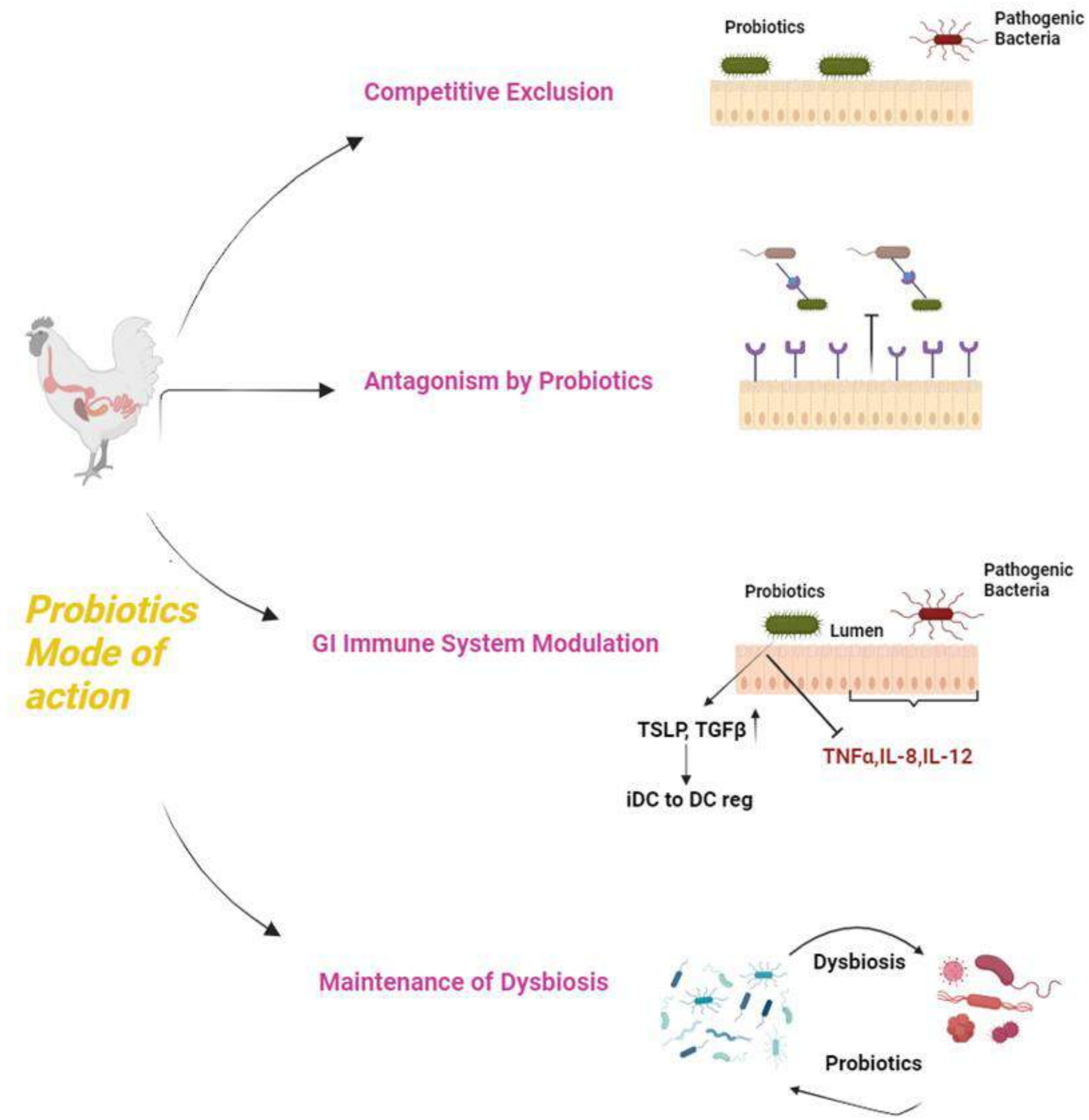

Fig. 1: Modes of action of probiotics.

Prebiotics

Prebiotics are another type of natural feed additive used in addition to probiotics. The addition of lactic bacteria to the human gut microbiota after carbohydrate eating was originally documented by Rettger and Cheplin in 1921.(Rettger, 1921). The notion of prebiotics was initially introduced in 1995 (Gibson and Roberfroid, 1995). The definition used nowadays was coined in December 2016 by ISAPP. Prebiotics, by definition, are a class of substances that function outside of the digestive tract. These substances include molecules other than carbohydrates, like polyphenols and polyunsaturated fatty acids that have been changed into fatty acids that are conjugated that are comparable. They may now be considered for other groups, such as nourishment for animals, and are no longer just for human consumption, which is another significant development. However, standards regarding the specific processes of microflora manipulation and the requirement of proven positive impacts on host health have been upheld (Gibson et al., 2017). Numerous substances, including cellulose, xylanes, and pectins, promote the growth of diverse gut microbes. Prebiotics should stimulate specific metabolic pathways rather than being heavily metabolized in order to promote the host's ecosystem's health. Benefits from using indigestible oligosaccharides, like fructans and galactans, have been well-documented (Rastall and Gibson, 2015).

Prebiotic Substances

Prebiotic compounds include proteins, lipids, peptides, and carbohydrates that are not absorbed (oligosaccharides and polysaccharides). Fruit, cereals, and legumes are organic sources of prebiotic bacteria. However, commercial chemical and enzymatic processes are used to produce numerous comparable compounds (Śliżewska et al., 2013). Several prebiotics are often used, including FOS, oligofructose, gluco-oligosaccharides, glico-oligosaccharides, lactulose, lactitol, malto-oligosaccharides, xylo-oligosaccharides, stachyose, and raffinose. (Monsan and Paul, 1995; Orban et al., 1997; Patterson et al., 1997; Collins and Gibson, 1999; Patterson and Burkholder, 2003). Upon arrival in the large intestine, those materials serve as nourishing substrates for healthy intestinal microorganisms (Grajek et al., 2005). These are the classes into which prebiotics fall based on characteristics that indicate a positive impact on the health of host: not broken down (or only partially broken down), not absorbed in the small intestine, inadequately fermented by bacteria in the mouth, well-fermented by gut microbes that seem to be beneficial, and poorly fermented by possible intestinal microbes (Markowiak and Śliżewska, 2018). Lactol, lactulose, cereal fiber, isomalto-oligosaccharides (IMO), xylo-oligosaccharides (XOS), FOS, and GOS are the prebiotics that are most frequently used in animal feeding.Determining the right dosage is crucial when creating prebiotic formula compositions. Flatulence and diarrhoea may result from taking too many prebiotics. However, a big benefit of such kinds of formulae is that they have no known side effects and can be used long-term as a preventative measure (Olveira and González-Molero, 2016; Markowiak and Śliżewska, 2018).

Mode of Action of Prebiotics

As was previously indicated, there are many different kinds of prebiotics; in this case, we will only talk about fructans and how they work. Fructans, which are usually produced by microbes or hydrolyzed from polysaccharides, have been added to broiler diets recently. There are three different groups of fructans: the branching group, the levan group, and the inulin group. Initially, according to DP (degrees of polymerization), the inulin group, commonly referred to as fructooligosaccharides (FOS), can be categorized into the following groups: The DP of inulin, which is typically derived from chicory roots (Cichorium intybus L.), ranges from 3 to 60. Oligofructose (OF), on the other hand, has a DP of 2 to 10 and can be produced by partial breakdown of inulin, lactose or sucrose conversion via catalysis (Ritsema and Smeekens, 2003; Rossi et al., 2005). Oligosaccharides containing β-2,1 fructosyl-fructose linkage and a glucose terminal unit are found in plants, which makes up the majority of the inulin group. Second, there is another group of fructans called the levan group that are mainly linked by β-2,6 fructosyl fructose bonds. Last but not least, fructans, which are members of the branching group, have fair proportions of both β-2,1 fructosyl-fructose and β-2,6 fructosyl fructose linkages (Zhao et al., 2013). Because of their β-glycosidic link, fructans are protected from being broken down by the digestive enzymes of fowl, which increases the amount of good bacteria (like *Bifidobacteria and Lactobacilli*) and decreases the amount of bad bacteria (like *E. coli and C. pefringens*) in the broiler's intestine (Xu et al., 2003; Kim et al., 2011; Ricke, 2015). Long-chain fructans can cross the small intestine and ferment in the distal sections of the intestine, despite their slow breakdown in the animal gut. As a result, the inulin group with higher DP may not have a substantial impact on the jejunum's microbiota, rather, it could change the composition of microorganisms and raise the levels of lactic acid or SCFA in broiler ceca. (Rehman et al., 2008). The effects of fructans on intestinal microbiota are demonstrated by the increased levels of two important beneficial microbes, *Bifidobacteria* and *Lactobacillus*, in the feed of broilers and hens that contain fructans (Rada et al., 2001; Xu et al., 2003; Rebolé et al., 2010; Zhao et al., 2013). In addition to producing extracellular enzymes to break down fructooligosaccharides (FOS), *Lactobacillus* and *Bifidobacteria* also acted as competitors with other gut microbe species, stop harmful microbes from multiplication (Rossi et al., 2005). For example, broilers given FOS had lower levels of *Campylobacter* titers in the large intestine and ceca (Zhao et al., 2013). In the ileocecal junction or ceca of broilers, a decrease in *C. perfringens* titers was seen irrespective of the addition of long- or short-chain FOS (Xu et al., 2003; Biggs et al., 2007; Zhao et al., 2013). In contrast, the colonization of cecal *C. perfringens* and *Salmonella typhimurium* in *E. coli* and FOS alone or in combination with the products that cause the removal of harmful pathogens, reduced the number of chickens challenged with *Salmonella*(Yang et al., 2008; Telg and Caldwell, 2009). Additionally, diets ranging in FOS concentration from 0.25 to 1% may lessen the amount of *Salmonella* and *E. Coli* in broiler cecum (Xu et al., 2003; Li et al., 2008; Kim et al., 2011; Zhao et al., 2013). Lactic acid and cecal SCFA may have contributed to the decrease in these harmful bacteria. Similar to in vitro findings, broilers fed inulin had much higher levels of lactic acid and cecal butyric acid (Rehman et al., 2008; Rebolé et al., 2010). Butyrate is the primary short-chain fatty acid that is metabolized by epithelial cells in the intestine, serving as a vital fuel for the growth of intestinal epithelium (Topping and Clifton, 2001). It has been proposed that improved mucosal structure is related to increased butyric acid levels. According to earlier research, In the ceca, FOS enhanced the villus to crypt depth ratio.and the height of microvillus in the ileum and jejunum. Yet, fructan additions, might have detrimental effects on broilers (Xu et al., 2003; Rebolé et al., 2010). The intestine's bacteria may ferment food too quickly, producing an excess of SCFA that damages intestinal mucosal barriers and increases intestinal permeability. This can lead to diarrhoea, poor development, and invasion by pathogens (Wu et al., 1999; Wu et al., 2005). Yet, High fructan additions, might have detrimental effects on broilers. The intestine's bacteria may ferment food too quickly, producing an excess of SCFA that damages intestinal mucosal barriers and increases intestinal permeability. This can lead to diarrhoea, poor development, and invasion by pathogens (Wu et al., 1999; Wu et al., 2005). Through these two main pathways, fructans enhanced the immunological responses of the immune system of body and lymphoid tissue present in gut. First of all, fructans can raise Bifidobacteria levels, which may alter cytokine or antibody production. Second, after leukocytes' receptors react to metabolites of fructans, like SCFA, leukocytes may become activated. All things taken into account, fructans may affect intestinal microbes, intestinal SCFA levels, mucosal architecture, and immunological responses. In the intestinal microbiota of broilers, there will be an increase in *Lactobacillus*, *Bifidobacteria* and there will be a decrease in pathogens like *E. coli*, *C. perfringens,* and *Salmonella (*Figure 2). There will be immune system modulation due to *lactobacillus* and *bifidobacteria* production. Fermentation of fructans will give rise to (SCFA's) short-chain fatty acids, which will cause an increase in MUC gene expression, an increase in microvillus height, decrease in depth of crypt, increase in height of Villus: crypt depth and these changes will also inhibit the pathogen colonization (Teng and Kim, 2018).

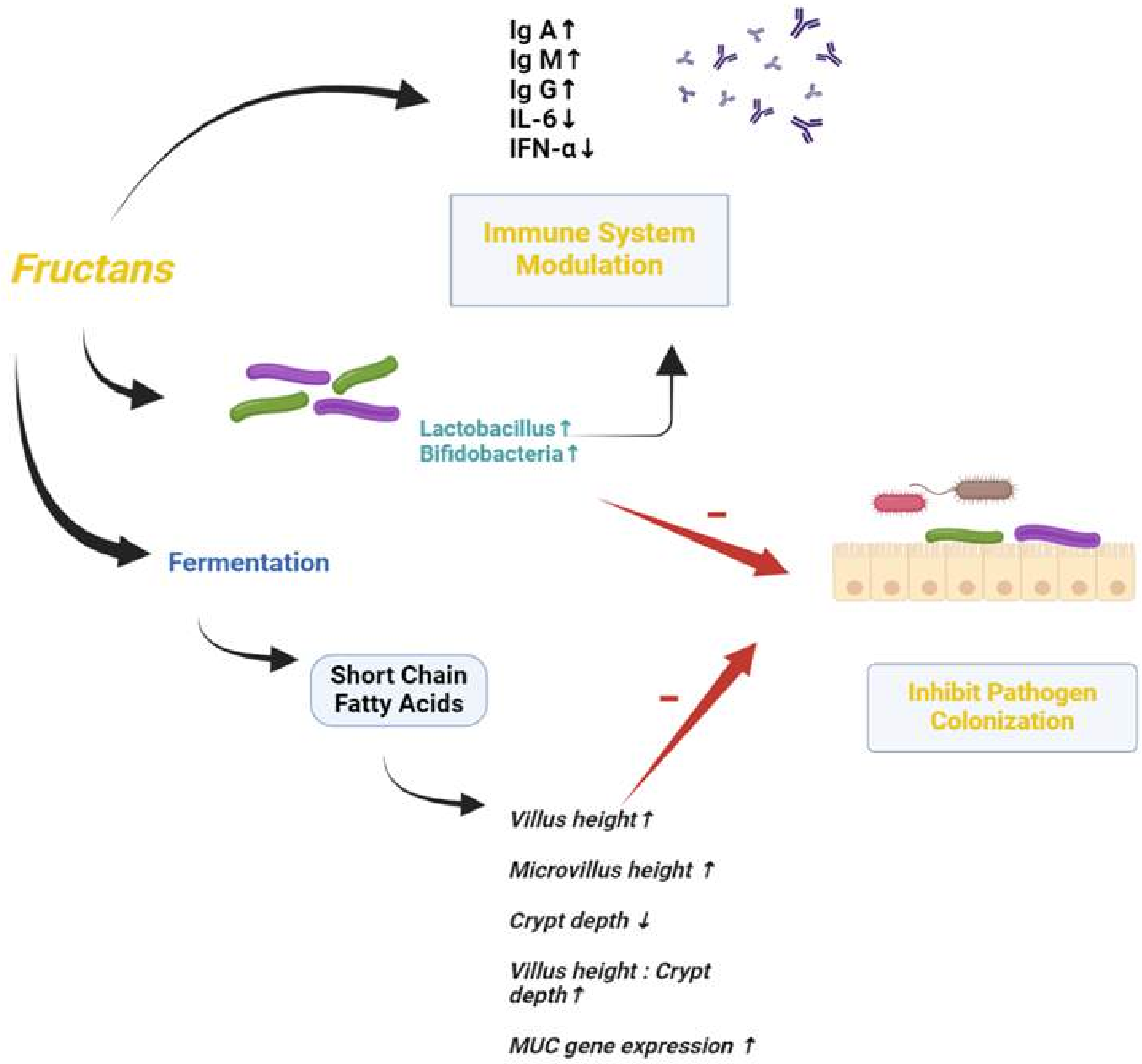

Fig. 2: Mode of action of prebiotics.

Synbiotics

In animal nutrition, the combination of probiotics and prebiotic are also utilized. When they used the term "synbiotic" in 1995, Gibson and Roberfroid described it as " a probiotic-prebiotic blend that enhances the host's ability to survive and introduce nutritional supplements containing live microorganisms into the gastrointestinal system,by enhancing host welfare by the targeted stimulation of one or a small development of numerous microorganisms that promote health and metabolism" (Gibson and Roberfroid, 1995). The term "synbiotic" should only be applied to products where a prebiotic component preferentially benefits a probiotic bacterium since it suggests synergy (Cencic and Chingwaru, 2010). Enhancing the survival of probiotic bacteria in the gastrointestinal system is the main goal of that kind of combination. Synbiotics were developed to address potential obstacles to probiotic survival in the gastrointestinal system. They possess both probiotic and prebiotic qualities (Rioux et al., 2005). Probiotics operate as a barrier to protect the gastrointestinal tract and have a favorable impact on intestinal balance. Conversely, probiotic bacteria receive their energy and nourishment from prebiotics (Gibson and Roberfroid, 1995). In light of this, a product containing both ingredients in a suitable ratio ought to have a greater impact than just the probiotic or prebiotic acting individually (Panesar et al., 2009).

Synbiotics in Use

Probiotic bacteria and prebiotic materials most frequently utilized in animal feeding were covered in earlier sections. The most widely used combination in synbiotic products appears to be FOS with bacteria from the Lactobacillus or Bifidobacterium genera (Markowiak and Śliżewska, 2018).

Synbiotics for Animals

Conventional DNA-based molecular tools have made significant strides in recent years in terms of both research and application, giving microbiologists new and unparalleled capabilities for characterizing and comprehending microbial populations (Pontes et al., 2007). Microbiologists can examine a more comprehensive picture of environmental microbiological communities through metagenomic investigations, which include clone library construction and screening as well as the isolation of entire microbial community genomes. This helps them to better understand the interactions between microbes and their surroundings (Singh et al., 2008). Metagenomics appears to be a viable method for evaluating the synbiotic impact of animal gut microbiota.

It has been confirmed that FOS and *Lactobacillus paracasei* bacteria have a synergistic effect on piglets' gut microbiome (Nemcova et al., 1999). An increase in the total count of obligate aerobes and as well as an increase in the quantity of beneficial *Lactobacillus and Bifidobacterium* genus bacteria has been observed in the animal group by researches. Simultaneously, the number of bacteria belonging to the species *Clostridium, E. coli,* and *Enterobacteriaceae* reduced in the stool of the piglets under study (Nemcova et al., 1999). The impact of the product that contain *Enterococcus faecium* bacteria as probiotic and fructooligosaccharides (FOS) as prebiotic, and immune-modulating compounds derived from marine algae (ficophytic chemicals) on the health of broiler chickens was investigated by (Awad et al., 2009). Five weeks of breeding 600 broiler chickens were examined. FCR, BW gain on daily basis, and carcass ratio, all showed a discernible increase in comparison to control animals (Awad et al., 2009). In summary, researchers agree that applying probiotics and prebiotics separately is less beneficial than utilizing synbiotic products (Biggs et al., 2007; Awad et al., 2009; Revolledo et al., 2009).

Conclusion

Prebiotics, probiotics help in maintaining a healthy gut microbiota in poultry. Prebiotics function as a dietary source for beneficial bacteria and probiotics introduce beneficial bacteria in the gut. To strengthen poultry's defenses against a variety of intestinal ailments, probiotics and prebiotics are recommended. They cause the activation of cytokines, other immune mediators and induce the synthesis of immunoglobins, thus enhancing the competitive exclusions of enteric bacteria by occupying attachment sites in the gut lining, thereby reducing the colonization and proliferation of enteric pathogens. Prebiotics and probiotics offer a natural and sustainable approach to disease prevention without contributing to the development of antibiotic-resistance strains. Prebiotics and probiotics can improve the feed efficiency, nutrient utilization and growth performance in poultry. They contribute to the sustainability of poultry production summary. In summary, a comprehensive strategy for boosting gut health, boosting immunity, lowering pathogen load, and boosting overall performance while addressing antibiotic resistance problems is provided by the use of prebiotics and probiotics in chicken production.

REFERENCES

Aarestrup, F. M. (1999). Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. *International Journal of Antimicrobial Agents*, *12*(4), 279-285. https://doi.org/10.1016/S0924-8579(99)90059-6

Anadón, A., Martínez-Larrañaga, M. R., and Martínez, M. A. (2006). Probiotics for animal nutrition in the European Union. Regulation and safety assessment. *Regulatory Txicology and Parmacology*, *45*(1), 91-95. https://doi.org/10.1016/j.yrtph.2006.02.004

Awad, W., Ghareeb, K., Abdel-Raheem, S., and Böhm, J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Science*, *88*(1), 49-56. https://doi.org/10.3382/ps.2008-00244

Biggs, P., Parsons, C., and Fahey, G. (2007). The effects of several oligosaccharides on growth performance, nutrient digestibilities, and cecal microbial populations in young chicks. *Poultry Science*, *86*(11), 2327-2336. https://doi.org/10.3382/ps.2007-00427

Buck, B. L., Altermann, E., Svingerud, T., and Klaenhammer, T. R. (2005). Functional analysis of putative adhesion factors in Lactobacillus acidophilus NCFM. *Applied and Environmental Microbiology*, *71*(12), 8344-8351. https://doi.org/10.1128/AEM.71.12.8344-8351.2005

Byndloss, M. X., and Bäumler, A. J. (2018). The germ-organ theory of non-communicable diseases. *Nature Reviews Microbiology*, *16*(2), 103-110. https://doi.org/10.1038/nrmicro.2017.158

Cartman, S. T., La Ragione, R. M., and Woodward, M. (2008). Bacillus subtilis spores germinate in the chicken gastrointestinal tract. *Applied and Environmental Microbiology*, *74*(16), 5254-5258. https://doi.org/10.1128/AEM.00580-08

Cencic, A., and Chingwaru, W. (2010). The role of functional foods, nutraceuticals, and food supplements in intestinal health. *Nutrients*, *2*(6), 611-625. http://dx.doi.org/10.3390/nu2060611

Chichlowski, M., Croom, J., McBride, B., Havenstein, G., and Koci, M. (2007). Metabolic and physiological impact of probiotics or direct-fed-microbials on poultry: a brief review of current knowledge. *International Journal of Poultry Science*, *6*(10), 694-704. http://dx.doi.org/10.3923/ijps.2007.694.704

Collier, C., Van der Klis, J., Deplancke, B., Anderson, D., and Gaskins, H. (2003). Effects of tylosin on bacterial mucolysis, Clostridium perfringens colonization, and intestinal barrier function in a chick model of necrotic enteritis. *Antimicrobial Agents and Chemotherapy*, *47*(10), 3311-3317. https://doi.org/10.1128/aac.47.10.3311-3317.2003

Collins, M. D., and Gibson, G. R. (1999). Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *The American Journal of Clinical Nutrition*, *69*(5), 1052S-1057S. https://doi.org/10.1093/ajcn/69.5.1052s

Dankowiakowska, A., Kozłowska, I., and Bednarczyk, M. (2013). Probiotics, prebiotics and snybiotics in Poultry–mode of action, limitation, and achievements. *Journal of Central European Agriculture*, *14*(1), 467-478. https://doi.org/10.5513/JCEA01/14.1.1222

Diarra, M. S., Silversides, F. G., Diarrassouba, F., Pritchard, J., Masson, L., Brousseau, R., and Skura, B. J. (2007). Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, Clostridium perfringens and enterococcus counts, and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in Escherichia coli isolates. *Applied and Environmental Microbiology*, *73*(20), 6566-6576. https://doi.org/10.1128/AEM.01086-07

Fuller, R. (1989). Probiotics in man and animals. *The Journal of Applied Bacteriology*, *66*(5), 365-378.

Garcia, C. C., Guabiraba, R., Soriani, F. M., and Teixeira, M. M. (2010). The development of anti-inflammatory drugs for infectious diseases. *Discovery Medicine*, *10*(55), 479-488.

Georgieva, M., Georgiev, K., and Dobromirov, P. (2015). Probiotics and immunity. In K. Metodiev (Ed.), *Immunopathology and Immunomodulation* (pp. 312). IntechOpen.

Gibson, G., and Roberfroid, M. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of Nutrition*, *125*(6), 1401-1412. https://doi.org/10.1093/jn/125.6.1401

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., and Cani, P. D. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology and Hepatology*, *14*(8), 491-502. https://doi.org/10.1038/nrgastro.2017.75

Grajek, W., Olejnik, A., and Sip, A. (2005). Probiotics, prebiotics and antioxidants as functional foods. *Acta Biochimica Polonica*, *52*(3), 665-671. http://dx.doi.org/10.18388/abp.2005_3428

Islam, M. R., Lepp, D., Godfrey, D. V., Orban, S., Ross, K., Delaquis, P., and Diarra, M. S. (2019). Effects of wild blueberry (Vaccinium angustifolium) pomace feeding on gut microbiota and blood metabolites in free-range pastured broiler chickens. *Poultry Science*, *98*(9), 3739-3755. https://doi.org/10.3382/ps/pez062

Iyayi, E. (2008). Prospects and challenges of unconventional poultry feedstuffs. *Nigerian Poultry Science Journal*, *5*(4), 186-194.

Joint, F. (2002). *WHO Working Group: Guidelines for the evaluation of probiotics in food: report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food*.

Khalid, A., Khalid, F., Mahreen, N., Hussain, S. M., Shahzad, M. M., Khan, S., and Wang, Z. (2022). Effect of spore-forming probiotics on the poultry production: a review. *Food Science of Animal Resources*, *42*(6), 968. https://doi.org/10.5851%2Fkosfa.2022.e41

Khan, R., and Naz, S. (2013). The applications of probiotics in poultry production. *World's Poultry Science Journal*, *69*(3), 621-632.

Kim, G.-B., Seo, Y., Kim, C., and Paik, I. (2011). Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. *Poultry Science*, *90*(1), 75-82. https://doi.org/10.3382/ps.2010-00732

Li, X., Qiang, L., Liu, and Xu, C. (2008). Effects of supplementation of fructooligosaccharide and/or Bacillus subtilis to diets on performance and on intestinal microflora in broilers. *Archives Animal Breeding*, *51*(1), 64-70.

https://doi.org/10.5194/aab-51-64-2008

Markowiak, P., and Śliżewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut pathogens*, *10*, 1-20. https://doi.org/10.1186/s13099-018-0250-0

McDevitt, R., Brooker, J., Acamovic, T., and Sparks, N. (2006). Necrotic enteritis; a continuing challenge for the poultry industry. *World's Poultry Science Journal*, *62*(2), 221-247. https://doi.org/10.1079/WPS200593

Mendes, M. C. S., Paulino, D. S., Brambilla, S. R., Camargo, J. A., Persinoti, G. F., and Carvalheira, J. B. C. (2018). Microbiota modification by probiotic supplementation reduces colitis associated colon cancer in mice. *World Journal of Gastroenterology*, *24*(18), 1995. https://doi.org/10.3748%2Fwjg.v24.i18.1995

Miecznikow, E. (1907). O naturze ludzkiej—Zarys Filozofii Optymistycznej. *Wydawnictwo Biblioteka Naukowa*.

Monsan, P. F., and Paul, F. (1995). Oligosaccharide feed additives. *Biotechnology in Animal Feeds and Animal Feeding*, 233-245. http://dx.doi.org/10.1002/9783527615353.ch11

Montagne, L., Pluske, J., and Hampson, D. (2003). A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Animal Feed Science and Technology*, *108*(1-4), 95-117. https://doi.org/10.1016/S0377-8401(03)00163-9

Nagpal, R., Mainali, R., Ahmadi, S., Wang, S., Singh, R., Kavanagh, K., and Yadav, H. (2018). Gut microbiome and aging: Physiological and mechanistic insights. *Nutr Healthy Aging*, *4*(4), 267-285. https://doi.org/10.3233/NHA-170030

Nemcova, R., Bomba, A., Gancarcikova, S., Herich, R., and Guba, P. (1999). Study of the effect of Lactobacillus paracasei and fructooligosaccharides on the faecal microflora in weanling piglets. *Berliner und Munchener Tierarztliche Wochenschrift*, *112*(6-7), 225-228.

Olveira, G., and González-Molero, I. (2016). An update on probiotics, prebiotics and symbiotics in clinical nutrition. *Endocrinología y Nutrición*, *63*(9), 482-494. https://doi.org/10.1016/j.endoen.2016.10.011

Orban, J., Patterson, J., Sutton, A., and Richards, G. (1997). Effect of sucrose thermal oligosaccharide caramel, dietary vitamin-mineral level, and brooding temperature on growth and intestinal bacterial populations of broiler chickens. *Poultry Science*, *76*(3), 482-490. https://doi.org/10.1093/ps/76.3.482

Pan, D., and Yu, Z. (2014). Intestinal microbiome of poultry and its interaction with host and diet. *Gut Microbes*, *5*(1), 108-119. https://doi.org/10.4161/gmic.26945

Panesar, P. S., Kaur, G., Panesar, R., and Bera, M. B. J. (2009). Synbiotics: potential dietary supplements in functional foods. *International Food Information Service*, *2009*.

Patterson, J., and Burkholder, K. (2003). Application of prebiotics and probiotics in poultry production. *Poultry science*, *82*(4), 627-631. https://doi.org/10.1093/ps/82.4.627

Patterson, J., Orban, J., Sutton, A., and Richards, G. (1997). Selective enrichment of bifidobacteria in the intestinal tract of broilers by thermally produced kestoses and effect on broiler performance. *Poultry Science*, *76*(3), 497-500. https://doi.org/10.1093/ps/76.3.497

Plaza-Díaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M., and Gil, A. (2018). Immune-mediated mechanisms of action of probiotics and synbiotics in treating pediatric intestinal diseases. *Nutrients*, *10*(1), 42. https://doi.org/10.3390/nu10010042

Pontes, D. S., Lima-Bittencourt, C. I., Chartone-Souza, E., and Amaral Nascimento, A. M. (2007). Molecular approaches: advantages and artifacts in assessing bacterial diversity. *Journal of Industrial Microbiology and Biotechnology*, *34*(7), 463-473. https://doi.org/10.1007/s10295-007-0219-3

Rada, V., Dušková, D., Marounek, M., and Petr, J. (2001). Enrichment of Bifidobacteria in the hen caeca by dietary inulin. *Folia Microbiologica*, *46*, 73-75. https://doi.org/10.1007/BF02825891

Rastall, R. A., and Gibson, G. R. (2015). Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. *Current Opinion in Biotechnology*, *32*, 42-46. https://doi.org/10.1016/j.copbio.2014.11.002

Rebolé, A., Ortiz, L., Rodríguez, M. L., Alzueta, C., Treviño, J., and Velasco, S. (2010). Effects of inulin and enzyme complex, individually or in combination, on growth performance, intestinal microflora, cecal fermentation characteristics, and jejunal histomorphology in broiler chickens fed a wheat-and barley-based diet. *Poultry Science*, *89*(2), 276-286. https://doi.org/10.3382/ps.2009-00336

Rehman, H., Hellweg, P., Taras, D., and Zentek, J. (2008). Effects of dietary inulin on the intestinal short chain fatty acids and microbial ecology in broiler chickens as revealed by denaturing gradient gel electrophoresis. *Poultry science*, *87*(4), 783-789. https://doi.org/10.3382/ps.2007-00271

Rettger, L. F. (1921). *A Treatise on the Transformation of the Intestinal Flora*. Yale University Press.

Revolledo, L., Ferreira, C., and Ferreira, A. J. (2009). Prevention of Salmonella Typhimurium colonization and organ invasion by combination treatment in broiler chicks. *Poultry science*, *88*(4), 734-743. https://doi.org/10.3382/ps.2008-00410

Ricke, S. (2015). Potential of fructooligosaccharide prebiotics in alternative and nonconventional poultry production systems. *Poultry science*, *94*(6), 1411-1418. https://doi.org/10.3382/ps/pev049

Rioux, K. P., Madsen, K. L., and Fedorak, R. N. (2005). The role of enteric microflora in inflammatory bowel disease: human and animal studies with probiotics and prebiotics. *Gastroenterology Clinics*, *34*(3), 465-482. https://doi.org/10.1016/j.gtc.2005.05.005

Ritsema, T., and Smeekens, S. (2003). Fructans: beneficial for plants and humans. *Current Opinion in Plant Biology*, *6*(3), 223-230. http://dx.doi.org/10.1016/S1369-5266(03)00034-7

Rossi, M., Corradini, C., Amaretti, A., Nicolini, M., Pompei, A., Zanoni, S., and Matteuzzi, D. (2005). Fermentation of fructooligosaccharides and inulin by bifidobacteria: a comparative study of pure and fecal cultures. *Applied and*

Environmental Microbiology, *71*(10), 6150-6158. http://dx.doi.org/10.1128/AEM.71.10.6150-6158.2005

Singh, B., Gautam, S. K., Verma, V., Kumar, M., and Singh, B. (2008). Metagenomics in animal gastrointestinal ecosystem: Potential biotechnological prospects. *Anaerobe*, *14*(3), 138-144. https://doi.org/10.1016/j.anaerobe.2008.03.002

Śliżewska, K., Nowak, A., Barczyńska, R., and Libudzisz, Z. (2013). Prebiotyki-definicja, właściwości i zastosowanie w przemyśle. *Żywność Nauka Technologia Jakość*, *1*(86), 5-20.

Stutz, M., and Lawton, G. (1984). Effects of diet and antimicrobials on growth, feed efficiency, intestinal Clostridium perfringens, and ileal weight of broiler chicks. *Poultry science*, *63*(10), 2036-2042. https://doi.org/10.3382/ps.0632036

Telg, B., and Caldwell, D. (2009). Efficacy testing of a defined competitive exclusion product in combination with fructooligosaccharide for protection against Salmonella Typhimurium challenge in broiler chicks. *Journal of Applied Poultry Research*, *18*(3), 521-529. http://dx.doi.org/10.3382/japr.2009-00003

Teng, P.-Y., and Kim, W. K. (2018). Roles of prebiotics in intestinal ecosystem of broilers. *Frontiers in veterinary science*, *5*, 245. https://doi.org/10.3389/fvets.2018.00245

Timbermont, L., Haesebrouck, F., Ducatelle, R., and Van Immerseel, F. (2011). Necrotic enteritis in broilers: an updated review on the pathogenesis. *Avian pathology*, *40*(4), 341-347. https://doi.org/10.1080/03079457.2011.590967

Topping, D. L., and Clifton, P. M. (2001). Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews*, *81*(3), 1031-1064. http://dx.doi.org/10.1152/physrev.2001.81.3.1031

Vergin, F. (1954). Anti-und Probiotika [Antibiotics and probiotics]. *Hippokrates*, *25*(4), 116–119.

Vieira, A. T., Fukumori, C., and Ferreira, C. M. (2016). New insights into therapeutic strategies for gut microbiota modulation in inflammatory diseases. *Clinical and Translational Immunology*, *5*(6), e87. https://doi.org/10.1038/cti.2016.38

Windhorst, H.-W. (2006). Changes in poultry production and trade worldwide. *World's Poultry Science Journal*, *62*(4), 585-602. https://doi.org/10.1017/S0043933906001140

Wu, T., Dai, X., and Wu, L. (1999). Effects of fructooligosaccharide on the broiler production. *Acta Agriculturae Zhejiangensis*, *11*(2), 85-87.

Wu, T., Malinverni, J., Ruiz, N., Kim, S., Silhavy, T. J., and Kahne, D. (2005). Identification of a multicomponent complex required for outer membrane biogenesis in Escherichia coli. *Cell*, *121*(2), 235-245. https://doi.org/10.1016/j.cell.2005.02.015

Xu, Z., Hu, C., Xia, M., Zhan, X., and Wang, M. (2003). Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Science*, *82*(6), 1030-1036. https://doi.org/10.1093/ps/82.6.1030

Yang, Y., Iji, P., Kocher, A., Mikkelsen, L., and Choct, M. (2008). Effects of mannanoligosaccharide and fructooligosaccharide on the response of broilers to pathogenic Escherichia coli challenge. *British poultry science*, *49*(5), 550-559. https://doi.org/10.1080/00071660802290408

Zhao, P., Wang, J., and Kim, I. (2013). Effect of dietary levan fructan supplementation on growth performance, meat quality, relative organ weight, cecal microflora, and excreta noxious gas emission in broilers. *Journal of Animal Science*, *91*(11), 5287-5293.

Chapter 08

Microbial Matrimony: Exploring the Potential of Prebiotics and Probiotics for Optimal Reproductive Health

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ABSTRACT

The symbiotic relationship between the microbiome and reproductive health is among the most discussed topics of the last decade. Live microorganisms, probiotics with multiple physiological functions, are considered alternative therapeutic agents in improving reproductive ability. It has been evidenced that there is a great diversity of microbes in the reproductive systems of both sexes, suggesting the gut microbiome is a factor in maintaining reproductive health. Dysbiosis of the reproductive microbiome is linked with various reproductive disorders and adverse pregnancy outcomes, indicating that probiotic therapy may be an option for microbiome balance restoration and reproductive function mitigation. Probiotics work by adjusting the bacterial population and the microbiota's activity, regulating the host's metabolism and immune response. Prebiotics, which are non-living substrates that preferentially support the development of beneficial microbes, are a complementary method of promoting reproductive health by promoting the growth of probiotic strains. This chapter explores the potential of prebiotics and probiotics to be utilized as novel therapeutic modalities to improve reproductive health, emphasizing their clinical applications and future research and practice trends.

KEYWORDS Probiotics, Prebiotics, Testicular function, Follicular development, Pregnancy outcomes, Reproductive diseases	Received: 28-Jun-2024 Revised: 03-July-2024 Accepted: 07-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Probiotics are live microbes with an extensive variety of the effects they have on their hosts. The food, especially fermented products such as yogurt, makes the majority of the commercial probiotics. Probiotics have several health benefits that can be categorized into four key domains: reduction of pathogen growth (Yan et al., 2007), enhancement of intestinal barrier function (Yan et al., 2011), regulation of the immune system (La Fata et al., 2018), and control of pain perception (Rousseaux et al., 2007). Thus, use of probiotics may be helpful therapeutically when treating the vastness of diseases. It has been comprehensively recognized that there exists a diverse microbiome in the animal system and that it plays the role of maintaining a normal physiology and favorable health of an organism (Dominguez-Bello et al., 2019). Scientific research has also focused on the discovery of microbes in the reproductive organs of both male and female sexes. The reproductive microbiota were primarily detected in semen from males (Lundy et al., 2021), whereas microbiomes were found everywhere in the reproductive tract from females (Heil et al., 2019), and each part or tissue of the reproductive system was colonized by a distinctive microbiome with a particular composition. With commensal microorganisms, the ecosystem is balanced in the reproductive process, thereby, increasing host fertility and performance (Rowe et al., 2020).

Dysbiosis of the microbiota of the reproductive tract may promote various diseases, aberrant pregnancy outcomes, and embodiment in the female reproductive system (Schoenmakers et al., 2019). Furthermore, numerous studies have demonstrated that gut microbiota also plays an important role in the regulation of some relatable diseases and preservation of the reproductive system basal state of health (Quaranta et al., 2019). Probiotic treatments, which address the microbiome, are becoming another logical alternative treatment modality when conclusive evidence reveals that the microbiome is linked to reproductive health and related conditions. Lots of recent researches have established a probiotic-based medicine or supplements prevent related diseases and reproductive disorders (Helli et al., 2022). Given the correlation between metabolic health and reproductive function, probiotics may enhance host reproductive function by controlling host metabolism (Palmer et al., 2012). Probiotics can affect the various membrane structures associated with reproduction. They also support epithelial barrier function and membrane integrity, which are necessary for successful blastulation and the development of the amnion, chorion, and placenta (Reid et al., 2013). Additionally, numerous studies have demonstrated the immunomodulatory effect of probiotics, and because some probiotics can disrupt the inflammatory cascade, they may be beneficial in certain reproductive functions and diseases related to them (Sanz, 2011).

On the other hand, prebiotics are described as "non-viable substrates that act as nutrients for beneficial microbes possessed by the host, such as administered probiotic strains and indigenous (resident) microorganisms" (Gibson et al., 2017) by the International Scientific Association for Probiotics and Prebiotics (ISAPP). This is a substance that the human body cannot break down, as it is resistant to gastric acid and is not broken down by enzymes found in mammals or consumed by the gastrointestinal system. The intestinal flora digests prebiotics and specifically activates some of the colon's bacteria, changing their development and activity in the host's favor (Gibson and Roberfroid, 1995). According to another study prebiotics are substances that can be deliberately fermented to change the structure and activity of the gut's beneficial host health flora, often known as "bifidogenic factors." (Gibson et al., 2004). Prebiotics were redefined in 2016 by the International Scientific Association for Probiotics and Prebiotics as compounds that the host intestinal flora may utilize and alter selectively, expecting to improve host health. The term "prebiotics" has been redefined to encompass non-carbohydrates, and its mode of action is no longer restricted to the gastrointestinal system or food (Gibson et al., 2017).

Typically, they are plant-based products like polysaccharides (FOS, GOS, fructose- or galactooligosaccharides, inulin) or non-sugar molecules that are fermented by the microbiota instead of being broken down by the host's enzymes and enhance the host's health. Another critical source of prebiotics in the human diet may be dairy products, most notably yogurt. The following substances have prebiotic qualities: Lactose, phosphates, oligosaccharides, especially those that contain N-acetylglucosamine, lactoperoxidase enzymes, nucleotides, lysozyme enzymes and alfa-lactoalbumin, glycomacropeptide (GMP) and lactoferrin (Vega-Bautista et al., 2019). This chapter will profile the prebiotic and probiotic novel therapeutic modalities that can play a pivotal role in improving reproductive health. It will focus on their clinical applications and future trend specialization for research and practice.

Diversity of Probiotics and Prebiotics

Probiotics

Probiotics are a diverse group of organisms with a broad distribution range that can be classified into three main categories: *Lactobacilli, Bifidobacteria,* and some others. Considering the fact that *L. acidophilus*, the most widely consumed as well as studied due to its probiotic characteristics, forms the largest group within the LAB group, the majority of investigation in respect of probiotic species deal with this group. Notably, *Lactobacillus* goes to open the way for good flora dominance by inhibiting the growth of the pathogens. Such microbes are one of the most important probiotics, therefore generating lots of interest in the research on the human gastrointestinal microbiome, which is closely related to human health. *Lactobacillus* also functions to synthesize vitamins and amino acids that are essential yet, it facilitates the intake and absorption of minerals (Milani et al., 2017).

The word bifidobacterium is a translation of the genus of the bacteria which frequently possess the branching ends (the term " bifidobacterium " comes from the Greek word "bi" which means two and "defidia" which means splitting) (Henrick et al., 2018). It is the set of microorganisms that is essential physiological bacteria to the human health and are often found in large amounts in dietary supplements. For proper intestinal health, Bifidobacterium species can grow and metabolize in both the middle and end of the small and large intestine and adapt to being anaerobic, and also secrete bifidogenic substances specific to the type of probiotics (Bested et al., 2013). The diversity of the Bifidobacterium class, which currently consists of 32 species and nine subspecies, includes 14 species that were isolated by human feces (Klaassens et al., 2009).

Gram-positive parthenococci as well as *Enterococcus, Lactobacillus* and *Bifidobacterium* are extensively applied in food production. As a significant feature of *Enterococcus* strains that constitute a probiotic, they can concurrently coexist, compete, and adhere to cells in the digestive tract. Besides, *Enterococcus* has a great tolerance to a wide range of temperatures and pH values because of its ability to produce the bacteriocins, the natural antibacterial agent useful in the food sector (Hanchi et al., 2018).

As technology is advancing, *Saccharomyces cerevisiae* is used by the industries because of its historical safety record and the identification of favourable strains. For instance, *S. boulardii* are popularly used in the treatment of digestive disorders, among others, diarrhea symptoms. It has been thoroughly studied for its probiotic effect; it is beneficial when used with antibiotic therapy. Furthermore, it possesses immunomodulatory properties that help regulate immune pathways in the context of infectious or chronic conditions (Czerucka and Rampal, 2019). Other more widely available probiotic groups are S*treptococcus* species, *Bacillus* species, and *E. coli*, alongside the yeasts and *Enterococci* already discussed. The pictorial representation of probiotics diversity is shown in Fig. 1.

Prebiotics

According to previous investigations, prebiotics are oligosaccharide carbohydrates, primarily xylooligosaccharides (XOS), lactulose, galactooligosaccharides (GOS) inulin, and the fructose-oligosaccharides (FOS) (Yin et al., 2022). But recent investigation indicates that prebiotics aren't just carbs—they can also be other non-carbohydrate substances that comply with the prebiotic profile, like polyphenols that are extracted from fruits like blueberries 21 and black raspberries (Jiao et al., 2019). New prebiotic species are continually being generated due to ongoing optimization processes for prebiotic production; these mainly consist of polyphenols, polysaccharides, and polypeptide polymers, all of which have promising future directions for research. The pictorial representation of prebiotics diversity is shown in Fig. 2. The primary sources of emerging prebiotics are microorganisms from a variety of sources: fruit juices, waste products from fruits, algae, and herbal remedies (Rezende et al., 2021). Even while the understanding of these prebiotics is not as advanced as that of GOS and FOS, their potential warrants further investigation and appears to have an exciting future.

Fig. 1: A pictorial representation of probiotics diversity

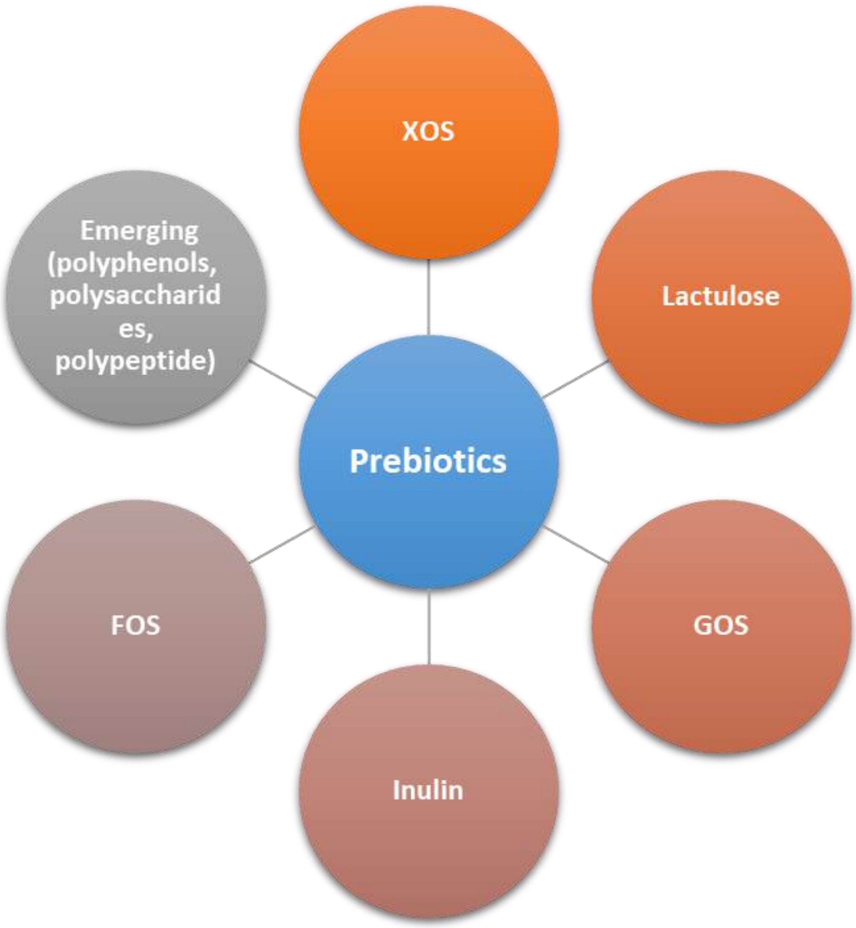

Fig. 2: A pictorial representation of prebiotics diversity

Harnessing the Potential of Probiotics

Probiotics, have recently been under focus for their benefits on the gut and now the researchers are looking into their effect on fertility. Recent findings propose that these beneficial microorganisms can help improve fertility and reproductive health in the male and female. Through enhancing hormonal balance and reproductive organs, probiotics could be a natural approach to treating several reproductive concerns.

Effect of Probiotics on Male Reproductive Health

Probiotics can definitely play a role in male reproductive health since they have a potential to enhance sperm quality and movement. They also aid in the regulation of the flora in the gastrointestinal tract with beneficial impact in relation to testosterone and reproductive system. Recent works have also indicated the benefits of using probiotics in reducing oxidative stress, inflammation and therefore male fertility.

Semen Quality and Spermatogenesis

Although, there is a lack of research on the effects of probiotics on male fertility. Probiotic strains, however, have been found to improve semen mobility and kinematic characteristics in vitro and in vivo, as well as in specific disease models. When *Lactobacillus rhamnosus* PB01 was added to diet-induced obese mice, the percentage of progressively motile sperm increased and there were beneficial implications for weight loss as well as reproductive hormones (Dardmeh et al., 2017). In asthenozoospermic males, on the other hand, *Lactobacillus* and *Bifidobacterium* improved sperm motility and decreased the percentage of DNA fragmentation in sperm. It was also discovered that mice fed alginate oligosaccharide, which promotes the growth of probiotic bacteria in the gut, had higher sperm quality and spermatogenesis after fecal microbiota transplantation (FMT) (Zhang et al., 2021).

Probiotic supplementation can improve spermatogenesis, testosterone levels, and seminiferous tubule cross-sectional profiles in aged mice, suggesting that probiotics may influence testicular function and therefore, semen quality (Poutahidis et al., 2014). Studies have suggested that the gut microbiota may be responsible for controlling the testicular function and also change the blood-testis barrier (BTB) permeability (Al-Asmakh et al., 2014). If the data above are proven to be so through further study, it might herald great advances in treating infertility. In this respect, various theories have emerged to explain better the role of probiotics in improving spermatozoa function. By conducting the in vitro experiment, Zhang et al. (2020) found out the inhibitory effect of the harmful species (*Pseudomonas aeruginosa*) and the helpful species (*Lactobacillus casei*) in the poultry serum. They found that, although *L. casei* treatment alone did not improve these parameters, it significantly lowered the decline in sperm motility and the impairment of mitochondrial activity caused by *P. aeruginosa*. This suggests that *Lactobacillus* may improve semen quality by resisting the detrimental effects of predominantly harmful bacteria on sperm.

More in vivo research has shown that probiotic administration may influence spermatogenesis and testicular function by altering the gut flora and serving as an antioxidant. Spearman's correlation analysis was used to examine the links between testicles and essential gut microbiota (Tian et al., 2019). According to studies, giving sperm *L. rhamnosus* CECT8361 and Bacteroidetes longum CECT7347 enhanced sperm motility, lowered sperm intracellular H2O2, and decreased DNA fragmentation. Therefore, by functioning as antioxidants, these probiotic strains may improve sperm quality (Valcarce et al., 2017).

Prostatic Health

The effects of probiotics on the prostate have been rarely examined, with only a few studies in recent years. Several in vitro experiments have shown that treatment of human prostate cancer cells with specific probiotic strains (*L. rhamnosus* GG, *L. acidophilus* La-05, *L. casei*-01, and Bifidobacterium animals Bb12) strongly induced apoptosis (Rosa et al., 2020), indicating the potential of probiotics to suppress prostate cancer. Furthermore, probiotics have been demonstrated to improve the prevention of episodes and alleviate symptoms in chronic bacterial prostatitis caused by *Enterobacteriaceae* (Chiancone et al., 2019). In addition, decreased bacterial load of *E. coli* and *Enterococcus faecalis* in urine cultures was observed after probiotic administration in prostatitis (Pacifici et al., 2021). However, no clear etiology exists for prostate disease; thus, the relationship between prostate microbiota, prostatitis, and prostate cancer, as well as the potential role of probiotics in alleviating them, is worth further investigation.

Effects of Probiotics on Female Reproductive Health

Follicular Dynamics

The impact of probiotics on follicular growth in women having healthy ovaries has not been extensively studied. In menopausal women, Probiotic supplements are believed to help prevent related symptoms, such as obesity and dyslipidemia, among others, by delaying the decline in estradiol production and ovarian activity. Probiotics derived from the feces of healthy women were given to ovariectomized menopausal rats and they settled in the gut, which changed some metabolites and increased estrogen circulation (Chen et al., 2021). Probiotics are found to profoundly impact the post-partum estrus in cows, particularly for the sows. The investigation revealed that sows with diverse parities (Hayakawa et al., 2016) would have shorter estrus intervals when using probiotics (single or in a cocktail). From these results, the probiotics could cause changes in weaning estrus periods through the regulation of hormone secretion or gut microbiota.

Placenta

During the fetus development, a transient tissue connected to the mother called placenta joins the fetus to the uterus. In order to support proper growth of the foetus, the placenta's function is to effectively transfer oxygen and nutrients from the mother to the foetus. Various published studies have shown that oral probiotics may play a role in the regulation of the placental function. Dietary intake of probiotics might result in altering the composition of the placental microbiota and thus have any impact on the placental function as the studies have revealed that probiotics are able to cross the placental barrier from the gut to amniotic fluid by genetically labeled *E. faecium* strain (Voreades et al., 2014). The in vitro studies proved that *L. rhamnosus* GR-1 decreased the secretion of TNF-α from human placental trophoblast cell following activation by lipopolysaccharide. Probiotics also play a special role in mitigating placental inflammatory responses, thereby minimizing the chances of developing severe preeclampsia (Brantsæter et al., 2011). Hence, the probiotics may influence the placental function by changing the microbiota profile, stimulating the immune system, and improving the placental metabolic regulation which might occur during pregnancy.

Pregnancy

Research has proven that expecting mothers, who take probiotic supplements, have improved metabolism and composition of gut microbiota that, in turn, have a direct impact on improved fetal development (Jarrett et al., 2019). The studies conducted on probiotics have evaluated a positive influence of the gut microbiota in the immune development of the fetus. Specific probiotics like *Bifidobacterium lactis*, *B. longum* and *L. rhamnosus* particularly, have been identified to show prominent changes in the expression of TLR-related genes in the fetal gut during the period of pregnancy (Rautava et al., 2012). Concerning the pigs after weaning, and neonatal piglet mortality rate, the maternal fetus growth during pregnancy defines also the birth weight (Rootwelt et al., 2012). Studies have demonstrated the probiotic treatment for pregnant sows during the last trimester of the pregnancy improves litter and the birth weight in sows of their early and a higher parity (Böhmer et al., 2006). Moreover, the concurrent use of Bacillus and prebiotics (isomaltooligosaccharide) has been reported in improving fetal growth, thereby enhancing serum levels of growth hormone (Gu et al., 2019) and the placental antioxidant capacity.

Reproductive Diseases

Probiotic consumption is recommended as a therapeutic strategy to modify the gut microbiota structure in the treatment of the PCOS (Shirvani-Rad et al., 2021). The results of a recent study show that the frequency of remission in PCOS can be reduced when FMT re-establishes eubiotics in the gut flora after dysbiosis (Corrie et al., 2021). This indicates the possibility of using beneficial probiotic bacteria in PCOS. Probiotics are believed to be one of the effective modalities of bacterial vaginosis (BV) treatment as they increase the rate of colonization of lactobacillus and help with the elimination of discomfort and the restoration of the vaginal flora. Oral administration of *L. crispatus* will contribute to the decrease of abundance in patients with BV after enteric probiotic administration (Rostok et al., 2019), and application of vaginal probiotic supplementation will help to colonization of *Lactobacilli* in BV patients, thus lowering vaginal pH and increasing the production of antimicrobial substances that both prevent probiotics have a long list of attributes as well on endometrium. A lab investigation demonstrated that endometrial epithelial cells wounded by HIV-1 could restore their barrier function when treated with probiotic *Lactobacilli* (Dizzell et al., 2019). Moreover, probiotics fortify the uterine barrier function and lower inflammation, which are key in preventing endometritis induced by *Staphylococcus aureus* (Hu et al., 2019). The functioning of *Lactobacillus* in managing the endometriosis-associated pain has been confirmed (Leonardi et al., 2020)). The research cited indicates that probiotic activate the epithelial integrity, restructure the endometrial biome and minimizes inflammation to cure endometritis and endometriosis, respectively.

Harnessing the Potential of Prebiotics

A healthy environment within the genital tract prevents infection and inflammation. It is currently understood that treating diseases and infections of the reproductive system requires restoring the balance of this intricate ecosystem. Employing probiotic bacteria, which promote the growth of advantageous microbes, can help accomplish this goal. Prebiotics that are intended to improve the state of resident microorganisms and used probiotics may also have an extra benefit. Table 4.1 summarizes the application of prebiotics to increase probiotic levels and eventually improve reproductive health.

Future Directions

In the field of reproduction, we are constantly amazed by the discoveries in prebiotics and probiotics that can benefit overall health. In the future, more attention should be paid to developing specialized interventions, tapping into the latest discoveries in microbiome science, and creating prebiotic and probiotic supplementations to suit individual microbiome composition. Besides that, more detailed clinical trials and studies would clarify the efficiency and safety of the treatments in different populations. The integration of microbiome engineering with precision synthetic biology and targeted therapy at the level of reproductive health challenges may be the future of reproductive health treatment, contributing to the development of therapeutics for reproductive diseases. In the end, the amusement of microbial matrimony, which deals with the combination of prebiotics and probiotics, depicts a new era in reproductive medicine. Exploiting the symbiosis among microbes and hosts which provides an all-round solution is a new method of optimizing reproductive health through genetic engineering. It is possible that the clinical practice will be transformed radically, thereby producing successful results for individuals globally.

Table 4.1: A Summary of Clinical Application of Prebiotics to Enhance Reproductive Health

Prebiotic Treatment	Specie	Clinical Effects	References
FOS+XOS	Pregnant women	Reduced oxidative stress, enhanced mitochondrial and cholinergic function in fetal and maternal brain	(Krishna et al., 2015)
XOS	Hy-Line brown laying hens	Increased ovarian weight and follicular size, increased level of reproductive hormones (LH, FSH, P4), improved lipid metabolism	(Wen et al., 2022)
Short chain GOS+ long chain FOS	Pregnant mice	Increased tolerogenic immune reaction, decreased Th-1 dependent delayed-type hypersensitivity response	(Van Vlies et al., 2012)
GOS	Pregnant women	Decreased level of LachnospiraceaeUCG_001 but increased level of Paraprevotella and Dorea. No significant effect on gestational week, birth weight, chest circumference, head circumference, and delivery mode	(Van Vlies et al., 2012)
Mannan-oligosaccharides (MOS)	Zebrafish	Increased sperm production, no significant effect on oocyte maturation and levels of 17b-estradiol and testosterone.	(Forsatkar et al., 2018)
MOS	Male Rats	Decreased level of corticosterone, increased level of testosterone, increased seminiferous tubules' radii and sperm production	(Rodrigues et al., 2021)
Bovine lactoferrin+*L. acidophilus* La-14+*L. rhamnosus* HN001	Women	Increased levels of vaginal *L. acidophilus* and *L. rhamnosus* on days 14 and 21	(De Alberti et al., 2015)
Bovine lactoderrin+*L. acidophilus* GLA-14+ *L. rhamnosus* HN001	Women	Decreased vaginal pH, decreased vaginal discharge, itching and fishy odor, increased vaginal colonization of these two probiotics	(Russo et al., 2018)
Lactoferrin	Women	Optimization of vaginal flora (significantly increased level of *Lactobacillus*)	(Otsuki and Imai, 2017)
Lactoferrin	Women	Increased level of vaginal *Lactobacillus helveticus,* decrease in the levels of bacterial species causing BV, decreased vaginal pH	(Pino et al., 2017)
Lactoferrin	Women	Increased level of vaginal *Lactobacillus*	(Otsuki et al., 2014)

Conclusion

There has been growing interest in the link between the microbiome and reproductive health, and probiotics as therapeutic candidates. There are different microbes in female and male reproductive systems, which proves that the gut microbiome affects reproductive health. Probiotics offer potential for promoting health and reducing disorders, as dysbiosis correlates with adverse pregnancy outcomes. They modulate bacterial concentrations, control the metabolic rate and are involved in immune defence mechanisms. Prebiotics work hand in hand with probiotics in that they support growth of desirable bacteria. In males, probiotics positively affect sperm characteristics such as quality, motility and testicular functionality, while in females, they help maintain estradiol levels and promote normal growth of the follicles in addition to enhancing the functionality of the placenta. Specifically, prebiotics such as oligosaccharides promote probiotic activity, anti-oxidant protection, and hormonal balance. Subsequent studies should concentrate on specific therapy for the specific microbial composition, advancing microbiota modulation to precision medicine, and performing clinical trials on safety and efficiency in relation to infertility, which can expand the opportunities for solving reproductive issues.

REFERENCES

Al-Asmakh, M., Stukenborg, J.-B., Reda, A., Anuar, F., Strand, M.-L., Hedin, L., Pettersson, S., and Söder, O. (2014). The gut microbiota and developmental programming of the testis in mice. *PLoS One*, *9*(8), e103809.

Bested, A. C., Logan, A. C., and Selhub, E. M. (2013). Intestinal microbiota, probiotics and mental health: from Metchnikoff to modern advances: part III–convergence toward clinical trials. *Gut Pathogens*, *5*, 1-13.

Böhmer, B., Kramer, W., and Roth-Maier, D. (2006). Dietary probiotic supplementation and resulting effects on performance, health status, and microbial characteristics of primiparous sows. *Journal of Animal Physiology and Animal Nutrition*, *90*(7-8), 309-315.

Brantsæter, A. L., Myhre, R., Haugen, M., Myking, S., Sengpiel, V., Magnus, P., Jacobsson, B., and Meltzer, H. M. (2011). Intake of probiotic food and risk of preeclampsia in primiparous women: the Norwegian Mother and Child Cohort Study. *American Journal of Epidemiology*, *174*(7), 807-815.

Chen, Q., Wang, B., Wang, S., Qian, X., Li, X., Zhao, J., Zhang, H., Chen, W., and Wang, G. (2021). Modulation of the gut microbiota structure with probiotics and isoflavone alleviates metabolic disorder in ovariectomized mice. *Nutrients*, *13*(6), 1793.

Chiancone, F., Carrino, M., Meccariello, C., Pucci, L., Fedelini, M., and Fedelini, P. (2019). The use of a combination of Vaccinium macracarpon, Lycium barbarum L. and probiotics (Bifiprost®) for the prevention of chronic bacterial prostatitis: a double-blind randomized study. *Urologia Internationalis*, *103*(4), 423-426.

Corrie, L., Gulati, M., Vishwas, S., Kapoor, B., Singh, S. K., Awasthi, A., and Khursheed, R. (2021). Combination therapy of curcumin and fecal microbiota transplant: Potential treatment of polycystic ovarian syndrome. *Medical Hypotheses*, *154*, 110644.

Czerucka, D., and Rampal, P. (2019). Diversity of Saccharomyces boulardii CNCM I-745 mechanisms of action against intestinal infections. *World Journal of Gastroenterology*, *25*(18), 2188.

Dardmeh, F., Alipour, H., Gazerani, P., van der Horst, G., Brandsborg, E., and Nielsen, H. I. (2017). Lactobacillus rhamnosus PB01 (DSM 14870) supplementation affects markers of sperm kinematic parameters in a diet-induced obesity mice model. *PLoS One*, *12*(10), e0185964.

De Alberti, D., Russo, R., Terruzzi, F., Nobile, V., and Ouwehand, A. C. (2015). Lactobacilli vaginal colonisation after oral consumption of Respecta® complex: a randomised controlled pilot study. *Archives of Gynecology and Obstetrics*, *292*, 861-867.

Dizzell, S., Nazli, A., Reid, G., and Kaushic, C. (2019). Protective effect of probiotic bacteria and estrogen in preventing HIV-1-mediated impairment of epithelial barrier integrity in female genital tract. *Cells*, *8*(10), 1120.

Dominguez-Bello, M. G., Godoy-Vitorino, F., Knight, R., and Blaser, M. J. (2019). Role of the microbiome in human development. *Gut*, *68*(6), 1108-1114.

Forsatkar, M. N., Nematollahi, M. A., Rafiee, G., Farahmand, H., and Lawrence, C. (2018). Effects of the prebiotic mannan-oligosaccharide on feed deprived zebrafish: Growth and reproduction. *Aquaculture Research*, *49*(8), 2822-2832.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., and Cani, P. D. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology and Hepatology*, *14*(8), 491-502.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., and Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews*, *17*(2), 259-275.

Gibson, G. R., and Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of Nutrition*, *125*(6), 1401-1412.

Gu, X., Li, H., Song, Z., Ding, Y., He, X., and Fan, Z. (2019). Effects of isomaltooligosaccharide and Bacillus supplementation on sow performance, serum metabolites, and serum and placental oxidative status. *Animal Reproduction Science*, *207*, 52-60.

Hanchi, H., Mottawea, W., Sebei, K., and Hammami, R. (2018). The genus Enterococcus: between probiotic potential and safety concerns—an update. *Frontiers in Microbiology*, *9*, 346489.

Hayakawa, T., Masuda, T., Kurosawa, D., and Tsukahara, T. (2016). Dietary administration of probiotics to sows and/or their neonates improves the reproductive performance, incidence of post-weaning diarrhea and histopathological parameters in the intestine of weaned piglets. *Animal Science Journal*, *87*(12), 1501-1510.

Heil, B. A., Paccamonti, D. L., and Sones, J. L. (2019). Role for the mammalian female reproductive tract microbiome in pregnancy outcomes. *Physiological Genomics*, *51*(8), 390-399.

Helli, B., Kavianpour, M., Ghaedi, E., Dadfar, M., and Haghighian, H. K. (2022). Probiotic effects on sperm parameters, oxidative stress index, inflammatory factors and sex hormones in infertile men. *Human Fertility*, *25*(3), 499-507.

Henrick, B. M., Hutton, A. A., Palumbo, M. C., Casaburi, G., Mitchell, R. D., Underwood, M. A., Smilowitz, J. T., and Frese, S. A. (2018). Elevated fecal pH indicates a profound change in the breastfed infant gut microbiome due to reduction of Bifidobacterium over the past century. *MSphere*, *3*(2), 10.1128/msphere. 00041-00018.

Hu, X., Guo, J., Xu, M., Jiang, P., Yuan, X., Zhao, C., Maimai, T., Cao, Y., Zhang, N., and Fu, Y. (2019). Clostridium tyrobutyricum alleviates Staphylococcus aureus-induced endometritis in mice by inhibiting endometrial barrier disruption and inflammatory response. *Food and Function*, *10*(10), 6699-6710.

Jarrett, P., Meczner, A., Costeloe, K., and Fleming, P. (2019). Historical aspects of probiotic use to prevent necrotising enterocolitis in preterm babies. *Early Human Development*, *135*, 51-57.

Jiao, X., Wang, Y., Lin, Y., Lang, Y., Li, E., Zhang, X., Zhang, Q., Feng, Y., Meng, X., and Li, B. (2019). Blueberry polyphenols extract as a potential prebiotic with anti-obesity effects on C57BL/6 J mice by modulating the gut microbiota. *The Journal of Nutritional Biochemistry*, *64*, 88-100.

Klaassens, E. S., Boesten, R. J., Haarman, M., Knol, J., Schuren, F. H., Vaughan, E. E., and De Vos, W. M. (2009). Mixed-species genomic microarray analysis of fecal samples reveals differential transcriptional responses of bifidobacteria in breast- and formula-fed infants. *Applied and Environmental Microbiology*, *75*(9), 2668-2676.

Krishna, G., Divyashri, G., Prapulla, S., and Muralidhara, (2015). A combination supplement of fructo-and xylo-oligosaccharides significantly abrogates oxidative impairments and neurotoxicity in maternal/fetal milieu following gestational exposure to acrylamide in rat. *Neurochemical Research*, *40*, 1904-1918.

La Fata, G., Weber, P., and Mohajeri, M. H. (2018). Probiotics and the gut immune system: indirect regulation. *Probiotics and Antimicrobial Proteins*, *10*, 11-21.

Leonardi, M., Hicks, C., El-Assaad, F., El-Omar, E., and Condous, G. (2020). Endometriosis and the microbiome: a systematic review. *BJOG: An International Journal of Obstetrics and Gynaecology*, *127*(2), 239-249.

Lundy, S. D., Sangwan, N., Parekh, N. V., Selvam, M. K. P., Gupta, S., McCaffrey, P., Bessoff, K., Vala, A., Agarwal, A., and Sabanegh, E. S. (2021). Functional and taxonomic dysbiosis of the gut, urine, and semen microbiomes in male infertility. *European Urology*, *79*(6), 826-836.

Milani, C., Duranti, S., Bottacini, F., Casey, E., Turroni, F., Mahony, J., Belzer, C., Delgado Palacio, S., Arboleya Montes, S., and Mancabelli, L. (2017). The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. *Microbiology and Molecular Biology Reviews*, *81*(4), 10.1128/mmbr. 00036-00017.

Otsuki, K., and Imai, N. (2017). Effects of lactoferrin in 6 patients with refractory bacterial vaginosis. *Biochemistry and Cell Biology*, *95*(1), 31-33.

Otsuki, K., Tokunaka, M., Oba, T., Nakamura, M., Shirato, N., and Okai, T. (2014). Administration of oral and vaginal prebiotic lactoferrin for a woman with a refractory vaginitis recurring preterm delivery: appearance of lactobacillus in vaginal flora followed by term delivery. *Journal of Obstetrics and Gynaecology Research*, *40*(2), 583-585.

Pacifici, L., Santacroce, L., Dipalma, G., Haxhirexha, K., Topi, S., Cantore, S., Altini, V., Pacifici, A., De Vito, D., and Pettini, F. (2021). Gender medicine: The impact of probiotics on male patients. *La Clinica Terapeutica*, *172*(1), 8-15.

Palmer, N. O., Bakos, H. W., Owens, J. A., Setchell, B. P., and Lane, M. (2012). Diet and exercise in an obese mouse fed a high-fat diet improve metabolic health and reverse perturbed sperm function. *American Journal of Physiology-Endocrinology and Metabolism*, *302*(7), E768-E780.

Pino, A., Giunta, G., Randazzo, C. L., Caruso, S., Caggia, C., and Cianci, A. (2017). Bacterial biota of women with bacterial vaginosis treated with lactoferrin: an open prospective randomized trial. *Microbial Ecology in Health and Disease*, *28*(1), 1357417.

Poutahidis, T., Springer, A., Levkovich, T., Qi, P., Varian, B. J., Lakritz, J. R., Ibrahim, Y. M., Chatzigiagkos, A., Alm, E. J., and Erdman, S. E. (2014). Probiotic microbes sustain youthful serum testosterone levels and testicular size in aging mice. *PLoS One*, *9*(1), e84877.

Quaranta, G., Sanguinetti, M., and Masucci, L. (2019). Fecal microbiota transplantation: a potential tool for treatment of human female reproductive tract diseases. *Frontiers in Immunology*, *10*, 481536.

Rautava, S., Collado, M. C., Salminen, S., and Isolauri, E. (2012). Probiotics modulate host-microbe interaction in the placenta and fetal gut: a randomized, double-blind, placebo-controlled trial. *Neonatology*, *102*(3), 178-184.

Reid, J. N., Bisanz, J. E., Monachese, M., Burton, J. P., and Reid, G. (2013). The rationale for probiotics improving reproductive health and pregnancy outcome. *American Journal of Reproductive Immunology*, *69*(6), 558-566.

Rezende, E. S. V., Lima, G. C., and Naves, M. M. V. (2021). Dietary fibers as beneficial microbiota modulators: A proposed classification by prebiotic categories. *Nutrition*, *89*, 111217.

Rodrigues, L. E., Kishibe, M. M., Keller, R., dos Santos Caetano, H. R., Rufino, M. N., de Carvalho Sanches, O., Giometti, I. C., Giuffrida, R., and Bremer-Neto, H. (2021). Prebiotics mannan-oligosaccharides accelerate sexual maturity in rats: A randomized preclinical study. *Veterinary World*, *14*(5), 1210.

Rootwelt, V., Reksen, O., Farstad, W., and Framstad, T. (2012). Associations between intrapartum death and piglet, placental, and umbilical characteristics. *Journal of Animal Science*, *90*(12), 4289-4296.

Rosa, L. S., Santos, M. L., Abreu, J. P., Balthazar, C. F., Rocha, R. S., Silva, H. L., Esmerino, E. A., Duarte, M. C. K., Pimentel, T. C., and Freitas, M. Q. (2020). Antiproliferative and apoptotic effects of probiotic whey dairy beverages in human prostate cell lines. *Food Research International*, *137*, 109450.

Rostok, M., Hütt, P., Rööp, T., Smidt, I., Štšepetova, J., Salumets, A., and Mändar, R. (2019). Potential vaginal probiotics: safety, tolerability and preliminary effectiveness. *Beneficial Microbes*, *10*(4), 385-393.

Rousseaux, C., Thuru, X., Gelot, A., Barnich, N., Neut, C., Dubuquoy, L., Dubuquoy, C., Merour, E., Geboes, K., and Chamaillard, M. (2007). Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. *Nature Medicine*, *13*(1), 35-37.

Rowe, M., Veerus, L., Trosvik, P., Buckling, A., and Pizzari, T. (2020). The reproductive microbiome: an emerging driver of sexual selection, sexual conflict, mating systems, and reproductive isolation. *Trends in Ecology and Evolution*, *35*(3), 220-234.

Russo, R., Edu, A., and De Seta, F. (2018). Study on the effects of an oral lactobacilli and lactoferrin complex in women with intermediate vaginal microbiota. *Archives of Gynecology and Obstetrics*, *298*, 139-145.

Sanz, Y. (2011). Gut microbiota and probiotics in maternal and infant health. *The American Journal of Clinical Nutrition*, *94*, S2000-S2005.

Schoenmakers, S., Steegers-Theunissen, R., and Faas, M. (2019). The matter of the reproductive microbiome. *Obstetric Medicine*, *12*(3), 107-115.

Shirvani-Rad, S., Tabatabaei-Malazy, O., Mohseni, S., Hasani-Ranjbar, S., Soroush, A.-R., Hoseini-Tavassol, Z., Ejtahed, H.-S., and Larijani, B. (2021). Probiotics as a complementary therapy for management of obesity: a systematic review. *Evidence-Based Complementary and Alternative Medicine*, *2021*.

Tian, X., Yu, Z., Feng, P., Ye, Z., Li, R., Liu, J., Hu, J., Kakade, A., Liu, P., and Li, X. (2019). Lactobacillus plantarum TW1-1 alleviates diethylhexylphthalate-induced testicular damage in mice by modulating gut microbiota and decreasing inflammation. *Frontiers in Cellular and Infection Microbiology*, *9*, 221.

Valcarce, D., Genovés, S., Riesco, M., Martorell, P., Herráez, M., Ramón, D., and Robles, V. (2017). Probiotic administration improves sperm quality in asthenozoospermic human donors. *Beneficial Microbes*, *8*(2), 193-206.

Van Vlies, N., Hogenkamp, A., Thijssen, S., Dingjan, G., Knipping, K., Garssen, J., and Knippels, L. (2012). Effects of short-chain galacto-and long-chain fructo-oligosaccharides on systemic and local immune status during pregnancy. *Journal of Reproductive Immunology*, *94*(2), 161-168.

Vega-Bautista, A., de la Garza, M., Carrero, J. C., Campos-Rodríguez, R., Godínez-Victoria, M., and Drago-Serrano, M. E. (2019). The impact of lactoferrin on the growth of intestinal inhabitant bacteria. *International Journal of Molecular Sciences*, *20*(19), 4707.

Voreades, N., Kozil, A., and Weir, T. L. (2014). Diet and the development of the human intestinal microbiome. *Frontiers in Microbiology*, *5*, 108740.

Wen, F., Wang, F., Li, P., Shi, H., and Liu, N. (2022). Effect of xylo-oligosaccharides on reproduction, lipid metabolism, and adipokines of hens during the late egg-laying period. *Animal Bioscience*, *35*(11), 1744.

Yan, F., Cao, H., Cover, T. L., Washington, M. K., Shi, Y., Liu, L., Chaturvedi, R., Peek, R. M., Wilson, K. T., and Polk, D. B. (2011). Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGFR-dependent mechanism. *The Journal of Clinical Investigation*, *121*(6), 2242-2253.

Yan, F., Cao, H., Cover, T. L., Whitehead, R., Washington, M. K., and Polk, D. B. (2007). Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology*, *132*(2), 562-575.

Yin, X., Cai, T., Liu, C., Ma, Y., Hu, J., Jiang, J., and Wang, K. (2022). A novel solvothermal biorefinery for production of lignocellulosic xylooligosaccharides, fermentable sugars and lignin nano-particles in biphasic system. *Carbohydrate Polymers*, *295*, 119901.

Zhang, J., Liu, H., Yang, Q., Li, P., Wen, Y., Han, X., Li, B., Jiang, H., and Li, X. (2020). Genomic sequencing reveals the diversity of seminal bacteria and relationships to reproductive potential in boar sperm. *Frontiers in Microbiology*, *11*, 1873.

Zhang, P., Feng, Y., Li, L., Ge, W., Yu, S., Hao, Y., Shen, W., Han, X., Ma, D., and Yin, S. (2021). Improvement in sperm quality and spermatogenesis following faecal microbiota transplantation from alginate oligosaccharide dosed mice. *Gut*, *70*(1), 222-225.

Chapter 09

Probiotics as Regulator of a Healthy Gut Environment in Dairy Animals

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ABSTRACT

The goals of veterinary medicine are on getting maximum production and maintaining the health of dairy animals. The use of probiotics is one of the methods to achieve this goal. The probiotics are required in adequate amounts to get the maximum benefit. They are also a solution to antimicrobial resistance because they limit the irrational use of antibiotics. A variety of chemicals are released by these probiotics that are harmful to pathogenic microbes. These chemicals include proteases, hydrogen peroxide, and bacteriocins. They also help in the regulation of the immune system by regulating the expression of cytokines. Various gram-positive and gram-negative bacteria are sources of probiotics. Similarly, some species of fungi are also potential probiotics. They improve the health of dairy animals by maintaining the microflora in the gut of these animals and help in the maximum absorption of nutrients from the intestines of these dairy animals. They improve the normal physiological processes of the gut, thus helping a dairy animal to reach its maximum production. However, their exact mechanisms of actions at molecular levels are still unknown to us. So, there is a need for further research studies in this field, so that probiotics can be efficiently utilized.

KEYWORDS
Probiotics, dairy, Animals, Resistance, Microbes, Chemicals

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INTRODUCTION

Probiotics maintain the microbial population in the gastrointestinal tract (GIT). They are nonpathogenic microorganisms (Williams, 2010). They are living organisms (Gupta and Garg, 2009). They are microorganisms with health benefits. Their dosages depend upon the product (Kligler and Cohrssen, 2008). Their adequate amounts are required for a proper benefit to a host's body (Baumgardner et al., 2021). Probiotics use can be a promising approach to prevent a number of diseases by improving the immune system (Stavropoulou and Bezirtzoglou, 2020). They can also have functions such as immuno-regulatory functions (Wieërs et al., 2020). The probiotics produce lysozymes, proteases, hydrogen peroxides, and bacteriocins which limit the multiplication of other harmful microbes (El-Saadony et al., 2021). The bacteriocins produced by probiotics can help us to combat with the problem of antimicrobial resistance for example, nisin is a bacteriocin produced by the probiotics. It has been used in the treatment of mastitis caused by gram-positive bacteria such as *Staphylococcus* and *Streptococcus* species (Hernández-González et al., 2021). The probiotics in animals regulate the expression of cytokines and interact with immune system of the animal's body (Refeld et al., 2020). These probiotics have the ability to survive in the challenging environment within the host's body such as gastric acidity and pH variations to give benefits to the animal's body (Melara et al., 2022). Various feed additives are being added to the feed of dairy animals, either nutritional or non-nutritional, and they are maintaining the balance of gut microbiota, thus improving the health nutrient utilization capacity, and productivity of dairy animals. Since the emergence of antimicrobial resistance in dairy animals, they have gained great value. Two types of probiotics are being used in the dairy animals. Some of these are monostrain probiotics containing a single strain of probiotics, while some of the administrated probiotics are multistrain probiotics having two or three strains of probiotics (Lambo et al., 2021). The probiotics improve the feed conversion ratio in dairy animals (Maake et al., 2021). As it has been established that probiotics also regulate the production of volatile fatty acids and nitrogen flow, their molecular and metabolic mechanism of action is still unknown to us (Nalla et al., 2022). It is suggested that probiotics improve mucosal immunity by inhibiting the attachment of pathogens to the mucosa of the gastrointestinal tract of animals (Uyeno et al., 2015). Furthermore, the health benefits of probiotics for the animal's body include the control of acidosis, reduction of methanogenesis, enhanced growth of epithelium, and increased nutrient uptake (Abd El-Trwab et al. 2016). This chapter describes the importance of probiotics for the animal's health and also explains how they regulate the gut of dairy animals.

Important Probiotics of Dairy Animals of Bacterial and Fungal Origin

Probiotics that are beneficial for the animal microbiota have been listed in Table 1. Most of these are bacterial in origin and a few of them are fungal in nature.

Table 1: Probiotics of dairy animals

Organism	Species	Reference
Lactobacillus	*L. acidophilus*	Sharma et al., 2018b
	L. alimentarius	Apás et al., 2014
	L. amylorvous	Maldonado et al., 2012
	L. animalis	Ayala et al., 2018
	L. casei	Ayala-Monter et al., 2019
	L. mucosae	Royan et al., 2021
	L. plantarum	Izuddin et al., 2019
	L. amylovorus	Fernández et al., 2018
	L. rhamnosus	Maake et al., 2021
	L. salivarius	Stefańska et al., 2021
	L. sporogenes	Shreedhar et al., 2016
	L. sakei	Sasazaki et al., 2020
Other lactic acid bacteria	*Streptococcus bovis*	Aphale et al., 2019
	Lactococcus lactis	Armas et al., 2017
	Enterococcus faecalis	Maake et al., 2021
	Pediococcus acidilactici	Reddy et al., 2011
	Propriobacterium freudenreichii	Vasconcelos et al., 2008
Bacillus	*B. licheniformis*	Devyatkin et al., 2021
	B. subtilis	Devyatkin et al., 2021
	B. subtilis natto	Chang et al., 2021
	B. toyonensis	Santos et al., 2021
	B. amyloliquefaciens	Schofield et al., 2018
Other	*E. coli*	Tkalcic et al., 2003
	Megasphaera elsdenii	Carey et al., 2021
	Butyrivibrio fibrisolvens	Fukuda et al., 2006
	Prevotella bryantii	Chiquette et al., 2012
Fungi	*Aspergillus oryzae*	Sucu et al., 2018
	Candida rugosa, Candida pararugosa	Fernandes et al., 2019
	Debaryomyces hansenii	Angulo et al., 2019
	Saccharomyces cerevisiae	Shakira et al., 2018
	Candida tropicalis	Suntara et al., 2021a
Bifidobacterium	*B. animalis*	Bunešová et al., 2012
	B. pseudolongum	Maake et al., 2021
	B. ruminantium	Vlková et al., 2009
	B.bifidum	Apás et al. 2014

Probiotics as Gut Regulators in Dairy Animals

Probiotics have different roles like antimicrobial, gut homeostasis, enhancement of digestion, productivity and growth of the dairy animals. It is summarized in Table 2.

Future Perspectives and Challenges

Livestock is a growing economy of the world. Among the livestock sector, the dairy sector has a significant impact on the economy. The developed countries are now towards the peak production of their dairy animals as they are using the latest products such as probiotics as feed additives. There is a lack of proper knowledge about using these probiotics in developing countries. However, some commercial dairy farms are adding probiotics in the feed of animals as feed additives to get maximum milk production from their dairy animals but household farmers lack proper knowledge about these products and are not using them. As a result, milk production of the dairy animals in most of the developing countries is not according to the nutritional requirements of the people living there. So, it is need of the hour that farmers should be given knowledge about the adequate use of probiotics to keep their animals healthy and get proper production from their dairy animals.

Table 2: Probiotics as gut regulators in dairy animals

Probiotic	Function	Reference
Lactobacillus johnsonii, *Lactobacillus reuteri*	Increase the beneficial microflora in the gut of young calves.	Zhang et al., 2016
Lactobacillus casei, Streptococcus faecalism, Bacillus cerevisiae	Population of opportunistic pathogens in the gut declines	Guo et al., 2022
Bifidobacterium adolescentis, Lactobacillus acidophilus, Bifidobacterium longum	Digestion of dry feed increases when used in combination with vanillin	Kondrashova et al., 2020
Bacillus subtilis natto	Improvement in concentrations of ammonia nitrogen, volatile fatty acids, and microbial protein	Chang et al., 2021
Live yeast	Stabilize rumen pH	Maamouri and Ben Salem, 2022
Paenibacillus fortis	Reduce nitrite toxicosis	Latham et al., 2019
Lactobacillus	Assistance in body defence mechanisms	Pyar and Peh, 2014
Saccharomyces cerevisiae, Lactococcus	Reduction in inflammation of mammary glands	Gao et al., 2020
Lactobacillus casei	Reduction in infections caused by *Staphylococcus aureus*	Souza et al., 2018
Lactobacillus gasseri	Reduction in infections caused by *E. coli* and *Staphylococcus aureus*	Blanchet et al., 2021
Lactobacillus casei, Lactobacillus salivarius, Lactobacillus sakei	Improved ruminal fermentation	Stefańska et al., 2021
Bacillus amyloliquefaciens, Bacillus subtilis	Concentration of intestinal fibre degrading bacteria increases	Du et al., 2018
Bacillus amyloliquefaciens	Methane emission decreases	Schofield et al., 2018
Bacillus licheniformis, Lactobacillus plantarum, Bacillus subtilis	Protein fermentation increased	Chen et al., 2021
Saccharomyces cerevisiae	Decreases the protozoa population	Phesatcha et al., 2021
Lactobacillus casei	Improved milk production by the increased absorption of nutteints	So et al., 2021
Pichia kudriavzevii, Candida tropicalis	Increase in milk protein contents	Suntara et al., 2021b
Saccharomyces cerevisiae	High fat contents in milk	Sun et al., 2021
Lactobacillus plantarum	Antibacterial activity	Angelescu et al., 2019; Beck et al., 2019
Lactobacillus paracasei	Antibacterial activity	Mulaw et al., 2019
Weissella confusa	Cholesterol removing properties	Sharma et al., 2018a
Bacillus amyloliquefaciens	Antimicrobial properties	Lee et al., 2017a
Lactobacillus fermentum	Antibacterial activity against *E. coli*	Owusu-Kwarteng et al., 2015
Lactobacillus plantarum	Antibacterial activity against *Salmonella enterica*	Oguntoyinbo and Narbad, 2015
Lactobacillus paraplantarum	Antimicrobial activity against food-borne microbes	Peres et al., 2014
Pediococcus pentosaceus	Prevention of invasion of *Salmonella*	Chiu et al., 2008
Pediococcus acidilactici	Antibacterial activity against *Mycobacterium smegmatis, Bacillus subtilis, Proteus vulgaris, Staphylococcus aureus,* and *Escherichia coli*	Bhagat et al., 2020
Enterococcus lactis	Antimicrobial activity against *Lactobacillus sakei, Enterococcus faecalis, Listeria monocytogenes,* and *Staphylococcus aureus*	Uymaz Tezel, 2019
Lactobacillus rhamnosus	Adherence to epithelial cells	Kumar and Kumar, 2015
Lactobacillus fermentum	Antimicrobial properties	Pan et al., 2011
Enterococcus faecalis	Adhesion to epithelial cells	Kook et al., 2019
Bacillus amyloliquefaciens	Antibacterial activity against *Bacillus cereus, E.coli, Listeria monocytogenes,* and *Salmonella enterica*	Lee et al., 2017b
Bacillus amyloliquefaciens	Antimicrobial activity against *Staphylococcus aureus, E. coli*, and *Bacillus cereus*	Zulkhairi Amin et al., 2020
Lactobacillus kunkeei	Antibacterial activity against *Salmonella typhimurium* and *E. coli*	Sakandar et al., 2019

Conclusion

The probiotics are living microorganisms and are nonpathogenic in nature. They improve the health of animals by regulating the growth of harmful microbes. They help a dairy animal reach its maximum production by allowing the maximum absorption of nutrients from the intestines. They attach to the gut mucosa of animals, thus inhibiting the attachment of pathogens to the mucosa and limiting their pathogenesis. They release various chemicals that are toxic to harmful bacteria such as hydrogen peroxide, bacteriocins etc. Both the fungi and several species of bacteria are potential probiotics for the animals. Some of these fungi are *Aspergillus oryzae, Debaryomyces hansenii,* and *S. cerevisiae*. The bacterial classes include both gram-positive and gram-negative bacteria. The Gram-positive bacteria include *Lactobacillus, Bacillus,* and other lactic acid-producing bacteria. On the other hand, important gram-negative bacteria include nonpathogenic strains of *E. coli* and *Prevotella bryantii*. Some of the very important functions of these probiotics include antibacterial activity against pathogenic strains of *E. coli, Salmonella enterica, Salmonella typhimurium*, and *Staphylococcus aureus*. Similarly, they also regulate the normal physiological processes ongoing in the gut of dairy animals like reducing the chance of nitrite toxicity, improving microbial fermentation, enhancing the metabolism of dry feed, and increasing the milk production of dairy animals. These probiotics are helping dairy animals reach their maximum production capacity. However, there is a lack of proper knowledge about the use of these probiotics in developing countries, as a result of which their dairy animals lack proper health and adequate nutrition. They should be given information about the use of these probiotics.

REFERENCES

Abd El-Trwab, M. M., Youssef, I. I., Bakr, H. A., Fthenakis, G. C., and Giadinis, N. D. (2016). Role of probiotics in nutrition and health of small ruminants. *Polish Journal of Veterinary Sciences*, *19*(4). DOI 10.1515/pjvs-2016-0114

Angelescu, I. R., Zamfir, M., Stancu, M. M., and Grosu-Tudor, S. S. (2019). Identification and probiotic properties of lactobacilli isolated from two different fermented beverages. *Annals of Microbiology*, *69*, 1557-1565. https://doi.org/10.1007/s13213-019-01540-0

Angulo, M., Reyes-Becerril, M., Cepeda-Palacios, R., Tovar-Ramírez, D., Esteban, M. Á., and Angulo, C. (2019). Probiotic effects of marine Debaryomyces hansenii CBS 8339 on innate immune and antioxidant parameters in newborn goats. *Applied Microbiology and Biotechnology*, *103*, 2339-2352. https://doi.org/10.1007/s00253-019-09621-5

Apás, A. L., González, S. N., and Arena, M. E. (2014). Potential of goat probiotic to bind mutagens. *Anaerobe*, *28*, 8-12. https://doi.org/10.1016/j.anaerobe.2014.04.004

Aphale, D., Natu, A., Laldas, S., and Kulkarni, A. (2019). Administration of Streptococcus bovis isolated from sheep rumen digesta on rumen function and physiology as evaluated in a rumen simulation technique system. *Veterinary World*, *12*(9), 1362. https://doi.org/10.14202%2Fvetworld.2019.1362-1371

Armas, F., Camperio, C., and Marianelli, C. (2017). In vitro assessment of the probiotic potential of Lactococcus lactis LMG 7930 against ruminant mastitis-causing pathogens. *PLoS One*, *12*(1), e0169543. https://doi.org/10.1371/journal.pone.0169543

Ayala, D. I., Chen, J. C., Bugarel, M., Loneragan, G. H., den Bakker, H. C., Kottapalli, K. R., and Nightingale, K. K. (2018). Molecular detection and quantification of viable probiotic strains in animal feedstuffs using the commercial direct fed microbial Lactobacillus animalis NP51 as a model. *Journal of Microbiological Methods*, *149*, 36-43. https://doi.org/10.1016/j.mimet.2018.04.012

Ayala-Monter, M. A., Hernández-Sánchez, D., González-Muñoz, S., Pinto-Ruiz, R., Martínez-Aispuro, J. A., Torres-Salado, N., and Gloria-Trujillo, A. (2019). Growth performance and health of nursing lambs supplemented with inulin and Lactobacillus casei. *Asian-Australasian Journal of Animal Sciences*, *32*(8), 1137. https://doi.org/10.5713%2Fajas.18.0630

Baumgardner, R. M., Berreta, A., and Kopper, J. J. (2021). Evaluation of commercial probiotics for antimicrobial resistance genes. *The Canadian Veterinary Journal*, *62*(4), 379. https://pubmed.ncbi.nlm.nih.gov/33867550

Beck, B. R., Park, G. S., Lee, Y. H., Im, S., Jeong, D. Y., and Kang, J. (2019). Whole genome analysis of Lactobacillus plantarum strains isolated from kimchi and determination of probiotic properties to treat mucosal infections by Candida albicans and Gardnerella vaginalis. *Frontiers in Microbiology*, *10*, 433. https://doi.org/10.3389/fmicb.2019.00433

Bhagat, D., Raina, N., Kumar, A., Katoch, M., Khajuria, Y., Slathia, P. S., and Sharma, P. (2020). Probiotic properties of a phytase producing Pediococcus acidilactici strain SMVDUDB2 isolated from traditional fermented cheese product, Kalarei. *Scientific Reports*, *10*(1), 1926. https://doi.org/10.1038/s41598-020-58676-2

Blanchet, F., Rault, L., Peton, V., Le Loir, Y., Blondeau, C., Lenoir, L., and Even, S. (2021). Heat inactivation partially preserved barrier and immunomodulatory effects of Lactobacillus gasseri LA806 in an in vitro model of bovine mastitis. *Beneficial Microbes*, *12*(1), 95-106. https://doi.org/10.3920/BM2020.0146

Bunešová, V., Domig, K. J., Killer, J., Vlková, E., Kopečný, J., Mrázek, J., and Rada, V. (2012). Characterization of bifidobacteria suitable for probiotic use in calves. *Anaerobe*, *18*(1), 166-168. https://doi.org/10.1016/j.anaerobe.2011.09.008

Carey, M. A., Medlock, G. L., Alam, M., Kabir, M., Uddin, M. J., Nayak, U., and Gilchrist, C. A. (2021). Megasphaera in the stool microbiota is negatively associated with diarrheal cryptosporidiosis. *Clinical Infectious Diseases*, *73*(6), e1242-e1251. https://doi.org/10.1093/cid/ciab207

Chang, M., Ma, F., Wei, J., Liu, J., Nan, X., and Sun, P. (2021). Live Bacillus subtilis natto promotes rumen fermentation by modulating rumen microbiota in vitro. *Animals*, *11*(6), 1519. https://doi.org/10.3390/ani11061519

Chen, H., Guo, B., Yang, M., Luo, J., Hu, Y., Qu, M., and Song, X. (2021). Response of growth performance, blood biochemistry indices, and rumen bacterial diversity in lambs to diets containing supplemental probiotics and Chinese medicine polysaccharides. *Frontiers in Veterinary Science*, *8*, 681389. https://doi.org/10.3389/fvets.2021.681389

Chiquette, J., Allison, M. J., and Rasmussen, M. (2012). Use of Prevotella bryantii 25A and a commercial probiotic during subacute acidosis challenge in midlactation dairy cows. *Journal of Dairy Science*, *95*(10), 5985-5995. https://doi.org/10.3168/jds.2012-5511

Chiu, H. H., Tsai, C. C., Hsih, H. Y., and Tsen, H. Y. (2008). Screening from pickled vegetables the potential probiotic strains of lactic acid bacteria able to inhibit the Salmonella invasion in mice. *Journal of Applied Microbiology*, *104*(2), 605-612. https://doi.org/10.1111/j.1365-2672.2007.03573.x

Devyatkin, V., Mishurov, A., and Kolodina, E. (2021). Probiotic effect of Bacillus subtilis B-2998D, B-3057D, and Bacillus licheniformis B-2999D complex on sheep and lambs. *Journal of Advanced Veterinary and Animal Research*, *8*(1), 146. https://doi.org/10.5455%2Fjavar.2021.h497

Du, R., Jiao, S., Dai, Y., An, J., Lv, J., Yan, X., and Han, B. (2018). Probiotic Bacillus amyloliquefaciens C-1 improves growth performance, stimulates GH/IGF-1, and regulates the gut microbiota of growth-retarded beef calves. *Frontiers in Microbiology*, *9*, 2006. https://doi.org/10.3389/fmicb.2018.02006

El-Saadony, M. T., Alagawany, M., Patra, A. K., Kar, I., Tiwari, R., Dawood, M. A., and Abdel-Latif, H. M. (2021). The functionality of probiotics in aquaculture: An overview. *Fish and Shellfish Immunology*, *117*, 36-52. https://doi.org/10.1016/j.fsi.2021.07.007

Fernandes, T., Carvalho, B. F., Mantovani, H. C., Schwan, R. F., and Ávila, C. L. S. (2019). Identification and characterization of yeasts from bovine rumen for potential use as probiotics. *Journal of Applied Microbiology*, *127*(3), 845-855. https://doi.org/10.1111/jam.14350

Fernández, S., Fraga, M., Silveyra, E., Trombert, A. N., Rabaza, A., Pla, M., and Zunino, P. (2018). Probiotic properties of native Lactobacillus spp. strains for dairy calves. *Beneficial Microbes*, *9*(4), 613-624. https://doi.org/10.3920/BM2017.0131

Fukuda, S., Suzuki, Y., Murai, M., Asanuma, N., and Hino, T. (2006). Isolation of a novel strain of Butyrivibrio fibrisolvens that isomerizes linoleic acid to conjugated linoleic acid without hydrogenation, and its utilization as a probiotic for animals. *Journal of Applied Microbiology*, *100*(4), 787-794. https://doi.org/10.1111/j.1365-2672.2006.02864.x

Gao, J., Liu, Y. C., Wang, Y., Li, H., Wang, X. M., Wu, Y., and Qi, Z. L. (2020). Impact of yeast and lactic acid bacteria on mastitis and milk microbiota composition of dairy cows. *AMB Express*, *10*, 1-12. https://doi.org/10.1186/s13568-020-0953-8

Guo, Y., Li, Z., Deng, M., Li, Y., Liu, G., Liu, D., and Sun, B. (2022). Effects of a multi-strain probiotic on growth, health, and fecal bacterial flora of neonatal dairy calves. *Animal Bioscience*, *35*(2), 204. https://doi.org/10.5713%2Fab.21.0084

Gupta, V., and Garg, R. (2009). Probiotics. *Indian Journal of Medical Microbiology*, *27*(3), 202-209. https://doi.org/10.4103/0255-0857.53201

Hernández-González, J. C., Martínez-Tapia, A., Lazcano-Hernández, G., García-Pérez, B. E., and Castrejón-Jiménez, N. S. (2021). Bacteriocins from lactic acid bacteria. A powerful alternative as antimicrobials, probiotics, and immunomodulators in veterinary medicine. *Animals*, *11*(4), 979. https://doi.org/10.3390/ani11040979

Izuddin, W. I., Loh, T. C., Samsudin, A. A., Foo, H. L., Humam, A. M., and Shazali, N. (2019). Effects of postbiotic supplementation on growth performance, ruminal fermentation and microbial profile, blood metabolite and GHR, IGF-1 and MCT-1 gene expression in post-weaning lambs. *BMC Veterinary Research*, *15*, 1-10. https://doi.org/10.1186/s12917-019-2064-9

Kligler, B., and Cohrssen, A. (2008). Probiotics. *American Family Physician*, *78*(9), 1073-1078. https://www.aafp.org/pubs/afp/issues/2008/1101/p1073.html

Kondrashova, K., Duskaev, G., and Kvan, O. (2020). Evaluation of effects of rumen fluid in combination with probiotic preparations and vanillin on the luminescence of a recombinant strain E. coli. In *E3S Web of Conferences* (Vol. 143, p. 02034). EDP Sciences. https://doi.org/10.1051/e3sconf/202014302034

Kook, S. Y., Chung, E. C., Lee, Y., Lee, D. W., and Kim, S. (2019). Isolation and characterization of five novel probiotic strains from Korean infant and children faeces. *PLoS One*, *14*(10), e0223913. https://doi.org/10.1371/journal.pone.0223913

Kumar, A., and Kumar, D. (2015). Characterization of Lactobacillus isolated from dairy samples for probiotic properties. *Anaerobe*, *33*, 117-123. https://doi.org/10.1016/j.anaerobe.2015.03.004

Lambo, M. T., Chang, X., and Liu, D. (2021). The recent trend in the use of multistrain probiotics in livestock production: An overview. *Animals*, *11*(10), 2805. https://doi.org/10.3390/ani11102805

Latham, E. A., Pinchak, W. E., Trachsel, J., Allen, H. K., Callaway, T. R., Nisbet, D. J., and Anderson, R. C. (2018). Isolation, characterization and strain selection of a Paenibacillus species for use as a probiotic to aid in ruminal methane mitigation, nitrate/nitrite detoxification and food safety. *Bioresource Technology*, *263*, 358-364. https://doi.org/10.1016/j.biortech.2018.04.116

Latham, E. A., Pinchak, W. E., Trachsel, J., Allen, H. K., Callaway, T. R., Nisbet, D. J., and Anderson, R. C. (2019). Paenibacillus 79R4, a potential rumen probiotic to enhance nitrite detoxification and methane mitigation in nitrate-treated ruminants. *Science of the Total Environment*, *671*, 324-328. https://doi.org/10.1016/j.scitotenv.2019.03.390

Lee, A., Cheng, K. C., and Liu, J. R. (2017a). Isolation and characterization of a Bacillus amyloliquefaciens strain with zearalenone removal ability and its probiotic potential. *PloS one*, *12*(8), e0182220. https://doi.org/10.1371/journal.pone.0182220

Lee, S., Lee, J., Jin, Y. I., Jeong, J. C., Chang, Y. H., Lee, Y., and Kim, M. (2017b). Probiotic characteristics of Bacillus strains isolated from Korean traditional soy sauce. *LWT-Food Science and Technology*, *79*, 518-524. https://doi.org/10.1016/j.lwt.2016.08.040

Maake, T. W., Adeleke, M., and Aiyegoro, O. A. (2021). Effect of lactic acid bacteria administered as feed supplement on the weight gain and ruminal pH in two South African goat breeds. *Transactions of the Royal Society of South Africa*, *76*(1), 35-40. https://doi.org/10.1080/0035919X.2020.1870018

Maamouri, O., and Ben Salem, M. (2022). The effect of live yeast Saccharomyces cerevisiae as probiotic supply on growth performance, feed intake, ruminal pH and fermentation in fattening calves. *Veterinary Medicine and Science*, *8*(1), 398-404. https://doi.org/10.1002/vms3.631

Maldonado, N. C., de Ruiz, C. S., Otero, M. C., Sesma, F., and Nader-Macías, M. E. (2012). Lactic acid bacteria isolated from young calves–characterization and potential as probiotics. *Research in Veterinary Science*, *92*(2), 342-349. https://doi.org/10.1016/j.rvsc.2011.03.017

Melara, E. G., Avellaneda, M. C., Valdivié, M., García-Hernández, Y., Aroche, R., and Martínez, Y. (2022). Probiotics: Symbiotic relationship with the animal host. *Animals*, *12*(6), 719. https://doi.org/10.3390/ani12060719

Mulaw, G., Sisay Tessema, T., Muleta, D., and Tesfaye, A. (2019). In vitro evaluation of probiotic properties of lactic acid bacteria isolated from some traditionally fermented Ethiopian food products. *International Journal of Microbiology*, *2019*. https://doi.org/10.1155/2019/7179514

Nalla, K., Manda, N. K., Dhillon, H. S., Kanade, S. R., Rokana, N., Hess, M., and Puniya, A. K. (2022). Impact of probiotics on dairy production efficiency. *Frontiers in Microbiology*, *13*, 805963. https://doi.org/10.3389/fmicb.2022.805963

Oguntoyinbo, F. A., and Narbad, A. (2015). Multifunctional properties of Lactobacillus plantarum strains isolated from fermented cereal foods. *Journal of Functional Foods*, *17*, 621-631. https://doi.org/10.1016/j.jff.2015.06.022

Owusu-Kwarteng, J., Tano-Debrah, K., Akabanda, F., and Jespersen, L. (2015). Technological properties and probiotic potential of Lactobacillus fermentum strains isolated from West African fermented millet dough. *BMC Microbiology*, *15*, 1-10. https://doi.org/10.1186/s12866-015-0602-6

Pan, D. D., Zeng, X. Q., and Yan, Y. T. (2011). Characterisation of Lactobacillus fermentum SM-7 isolated from koumiss, a potential probiotic bacterium with cholesterol-lowering effects. *Journal of the Science of Food and Agriculture*, *91*(3), 512-518. https://doi.org/10.1002/jsfa.4214

Peres, C. M., Alves, M., Hernandez-Mendoza, A., Moreira, L., Silva, S., Bronze, M. R., and Malcata, F. X. (2014). Novel isolates of lactobacilli from fermented Portuguese olive as potential probiotics. *LWT-Food Science and Technology*, *59*(1), 234-246. https://doi.org/10.1016/j.lwt.2014.03.003

Phesatcha, K., Phesatcha, B., Wanapat, M., and Cherdthong, A. (2021). The effect of yeast and roughage concentrate ratio on ruminal pH and protozoal population in Thai native beef cattle. *Animals*, *12*(1), 53. https://doi.org/10.3390/ani12010053

Pyar, H., and Peh, K. K. (2014). Characterization and identification of Lactobacillus acidophilus using biolog rapid identification system. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(1), 189-193. https://web.archive.org/web/20180413015158id_/http://ijppsjournal.com/Vol6Issue1/7982.pdf

Reddy, P. V. M., Reddy, K. K., Kumar, M. S., Harikrishna, C., and Raghunandan, T. (2011). Effect of feeding Pediococcus acidilactici and Saccharomyces boulardii as probiotics in lambs. *Indian Journal of Small Ruminants (The)*, *17*(1), 53-58. https://www.indianjournals.com/ijor.aspx?target=ijor:ijsrandvolume=17andissue=1andarticle=006

Refeld, A., Bogdanova, A., Prazdnova, E., Beskopylny, A., Olshevskaya, A., Maltseva, T., and Zubtsov, V. (2020). Immunobiotics mechanisms of action and prospects of use in veterinary medicine. In *E3S Web of Conferences* (Vol. 210, p. 06017). EDP Sciences. https://doi.org/10.1051/e3sconf/202021006017

Royan, M., Seighalani, R., Mortezaei, F., and Pourebrahim, M. (2021). In vitro assessment of safety and functional probiotic properties of Lactobacillus mucosae strains isolated from Iranian native ruminants intestine. *Italian Journal of Animal Science*, *20*(1), 1187-1200. https://doi.org/10.1080/1828051X.2021.1947908

Sakandar, H. A., Kubow, S., and Sadiq, F. A. (2019). Isolation and in-vitro probiotic characterization of fructophilic lactic acid bacteria from Chinese fruits and flowers. *Lwt*, *104*, 70-75. https://doi.org/10.1016/j.lwt.2019.01.038

Santos, F. D. S., Maubrigades, L. R., Gonçalves, V. S., Ferreira, M. R. A., Brasil, C. L., Cunha, R. C., and Leite, F. P. L. (2021). Immunomodulatory effect of short-term supplementation with Bacillus toyonensis BCT-7112T and Saccharomyces boulardii CNCM I-745 in sheep vaccinated with Clostridium chauvoei. *Veterinary Immunology and Immunopathology*, *237*, 110272. https://doi.org/10.1016/j.vetimm.2021.110272

Sasazaki, N., Obi, T., Aridome, C., Fujimoto, Y., Furumoto, M., Toda, K., and Takagi, M. (2020). Effects of dietary feed supplementation of heat-treated Lactobacillus sakei HS-1 on the health status, blood parameters, and fecal microbes of Japanese Black calves. *Journal of Veterinary Medical Science*, *82*(10), 1428-1435. https://doi.org/10.1292/jvms.20-0181

Schofield, B. J., Lachner, N., Le, O. T., McNeill, D. M., Dart, P., Ouwerkerk, D., and Klieve, A. V. (2018). Beneficial changes in rumen bacterial community profile in sheep and dairy calves as a result of feeding the probiotic Bacillus amyloliquefaciens H57. *Journal of Applied Microbiology*, *124*(3), 855-866. https://doi.org/10.1111/jam.13688

Shakira, G., Qubtia, M., Ahmed, I., Hasan, F., Anjum, M. I., and Imran, M. (2018). Effect of indigenously isolated Saccharomyces cerevisiae probiotics on milk production, nutrient digestibility, blood chemistry and fecal microbiota in lactating dairy cows. http://www.thejaps.org.pk/docs/v-28-02/06.pdf

Sharma, S., Kandasamy, S., Kavitake, D., and Shetty, P. H. (2018a). Probiotic characterization and antioxidant properties of Weissella confusa KR780676, isolated from an Indian fermented food. *LWT*, *97*, 53-60. https://doi.org/10.1016/j.lwt.2018.06.033

Sharma, A. N., Kumar, S., and Tyagi, A. K. (2018b). Effects of mannan-oligosaccharides and Lactobacillus acidophilus supplementation on growth performance, nutrient utilization and faecal characteristics in Murrah buffalo calves. *Journal of Animal Physiology and Animal Nutrition*, *102*(3), 679-689. https://doi.org/10.1111/jpn.12878

Shreedhar, J. N., Patil, M., and Kumar, P. (2016). Effect of probiotics supplementation on milk yield and its composition in lactating Holstein Fresien and Deoni cross bred cows. *Journal of Medical and Bioengineering Vol*, *5*(1). http://www.jomb.org/uploadfile/2015/0601/20150601031025717.pdf

So, S., Wanapat, M., and Cherdthong, A. (2021). Effect of sugarcane bagasse as industrial by-products treated with Lactobacillus casei TH14, cellulase and molasses on feed utilization, ruminal ecology and milk production of mid-lactating Holstein Friesian cows. *Journal of the Science of Food and Agriculture*, *101*(11), 4481-4489. https://doi.org/10.1002/jsfa.11087

Souza, R. F. S., Rault, L., Seyffert, N., Azevedo, V., Le Loir, Y., and Even, S. (2018). Lactobacillus casei BL23 modulates the innate immune response in Staphylococcus aureus-stimulated bovine mammary epithelial cells. *Beneficial Microbes*, *9*(6), 985-995. https://doi.org/10.3920/BM2018.0010

Stavropoulou, E., and Bezirtzoglou, E. (2020). Probiotics in medicine: a long debate. *Frontiers in Immunology*, *11*, 554558. https://doi.org/10.3389/fimmu.2020.02192

Stefańska, B., Sroka, J., Katzer, F., Goliński, P., and Nowak, W. (2021). The effect of probiotics, phytobiotics and their combination as feed additives in the diet of dairy calves on performance, rumen fermentation and blood metabolites during the preweaning period. *Animal Feed Science and Technology*, *272*, 114738. https://doi.org/10.1016/j.anifeedsci.2020.114738

Sucu, E., Moore, C., VanBaale, M. J., Jensen, H., Sanz-Fernandez, M. V., and Baumgard, L. H. (2018). Effects of feeding Aspergillus oryzae fermentation product to transition Holstein cows on performance and health. *Canadian Journal of Animal Science*, *99*(2), 237-243. https://doi.org/10.1139/cjas-2018-0037

Sun, X., Wang, Y., Wang, E., Zhang, S., Wang, Q., Zhang, Y., and Li, S. (2021). Effects of Saccharomyces cerevisiae culture on ruminal fermentation, blood metabolism, and performance of high-yield dairy cows. *Animals*, *11*(8), 2401. https://doi.org/10.3390/ani11082401

Suntara, C., Cherdthong, A., Wanapat, M., Uriyapongson, S., Leelavatcharamas, V., Sawaengkaew, J., and Foiklang, S. (2021a). Isolation and characterization of yeasts from rumen fluids for potential use as additives in ruminant feeding. *Veterinary Sciences*, *8*(3), 52. https://doi.org/10.3390/vetsci8030052

Suntara, C., Cherdthong, A., Uriyapongson, S., Wanapat, M., and Chanjula, P. (2021b). Novel Crabtree negative yeast from rumen fluids can improve rumen fermentation and milk quality. *Scientific Reports*, *11*(1), 6236. https://doi.org/10.1038/s41598-021-85643-2

Tkalcic, S., Zhao, T., Harmon, B. G., Doyle, M. P., Brown, C. A., and Zhao, P. (2003). Fecal shedding of enterohemorrhagic Escherichia coli in weaned calves following treatment with probiotic Escherichia coli. *Journal of Food Protection*, *66*(7), 1184-1189. https://doi.org/10.4315/0362-028X-66.7.1184

Uyeno, Y., Shigemori, S., and Shimosato, T. (2015). Effect of probiotics/prebiotics on cattle health and productivity. *Microbes and Environments*, *30*(2), 126-132. https://doi.org/10.1264/jsme2.ME14176

Uymaz Tezel, B. (2019). Preliminary in vitro evaluation of the probiotic potential of the bacteriocinogenic strain Enterococcus lactis PMD74 isolated from ezine cheese. *Journal of Food Quality*, *2019*. https://doi.org/10.1155/2019/4693513

Vasconcelos, J. T., Elam, N. A., Brashears, M. M., and Galyean, M. L. (2008). Effects of increasing dose of live cultures of Lactobacillus acidophilus (Strain NP 51) combined with a single dose of Propionibacterium freudenreichii (Strain NP 24) on performance and carcass characteristics of finishing beef steers. *Journal of Animal Science*, *86*(3), 756-762. https://doi.org/10.2527/jas.2007-0526

Vlková, E., Grmanová, M., Rada, V., Homutová, I., and Dubná, S. (2009). Selection of probiotic bifidobacteria for lambs. *Czech Journal of Animal Science*, *54*(12), 552-565. https://cjas.agriculturejournals.cz/pdfs/cjs/2009/12/05.pdf

Wieërs, G., Belkhir, L., Enaud, R., Leclercq, S., Philippart de Foy, J. M., Dequenne, I., and Cani, P. D. (2020). How probiotics affect the microbiota. *Frontiers in cellular and infection microbiology*, *9*, 454. https://doi.org/10.3389/fcimb.2019.00454

Williams, N. T. (2010). Probiotics. *American Journal of Health-System Pharmacy*, *67*(6), 449-458. https://doi.org/10.2146/ajhp090168

Zhang, R., Zhou, M., Tu, Y., Zhang, N. F., Deng, K. D., Ma, T., and Diao, Q. Y. (2016). Effect of oral administration of probiotics on growth performance, apparent nutrient digestibility and stress-related indicators in Holstein calves. *Journal of Animal Physiology and Animal Nutrition*, *100*(1), 33-38. https://doi.org/10.1111/jpn.12338

Zulkhairi Amin, F. A., Sabri, S., Ismail, M., Chan, K. W., Ismail, N., Mohd Esa, N., and Zawawi, N. (2020). Probiotic properties of Bacillus strains isolated from stingless bee (Heterotrigona itama) honey collected across Malaysia. *International Journal of Environmental Research and Public Health*, *17*(1), 278. https://doi.org/10.3390/ijerph17010278

Chapter 10

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: 01-Jun-2024 Revised: 07-July-2024 Accepted: 22-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 (Boulianne et al., 2020). *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 10^2-10^4 colony-forming units (CFUs) per gram of small intestine digesta. Under disease-challenge conditions, the number increases to 10^7-10^9 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).

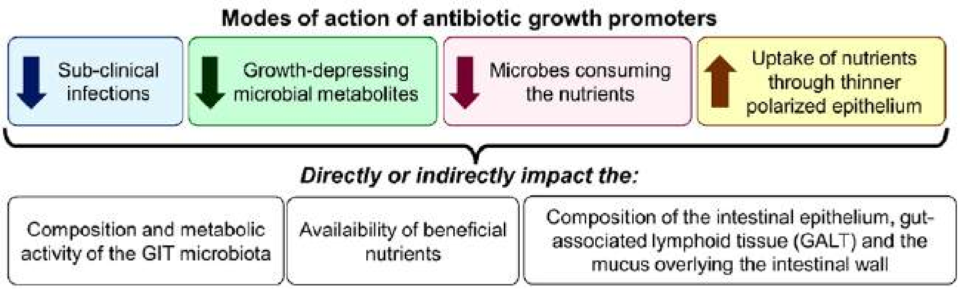

Fig. 1: Proposed mechanisms by which antibiotics function as growth promoters (Broom, 2017).

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).

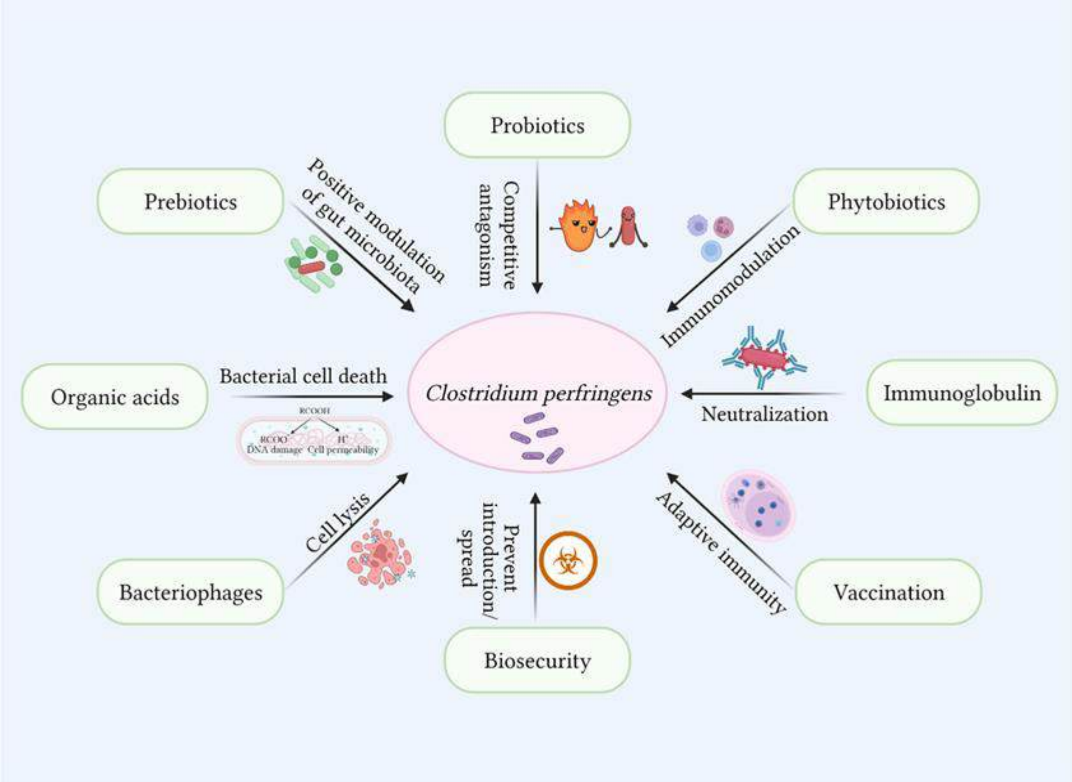

Fig. 2: Some of the proposed alternatives to AGPs for preventing and controlling NE {Source: Fathima et al. (2022)}.

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 (H_2O_2), 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 (Alagawany et al., 2021). The general mechanisms of action of probiotics against pathogens are depicted (Fig. 3).

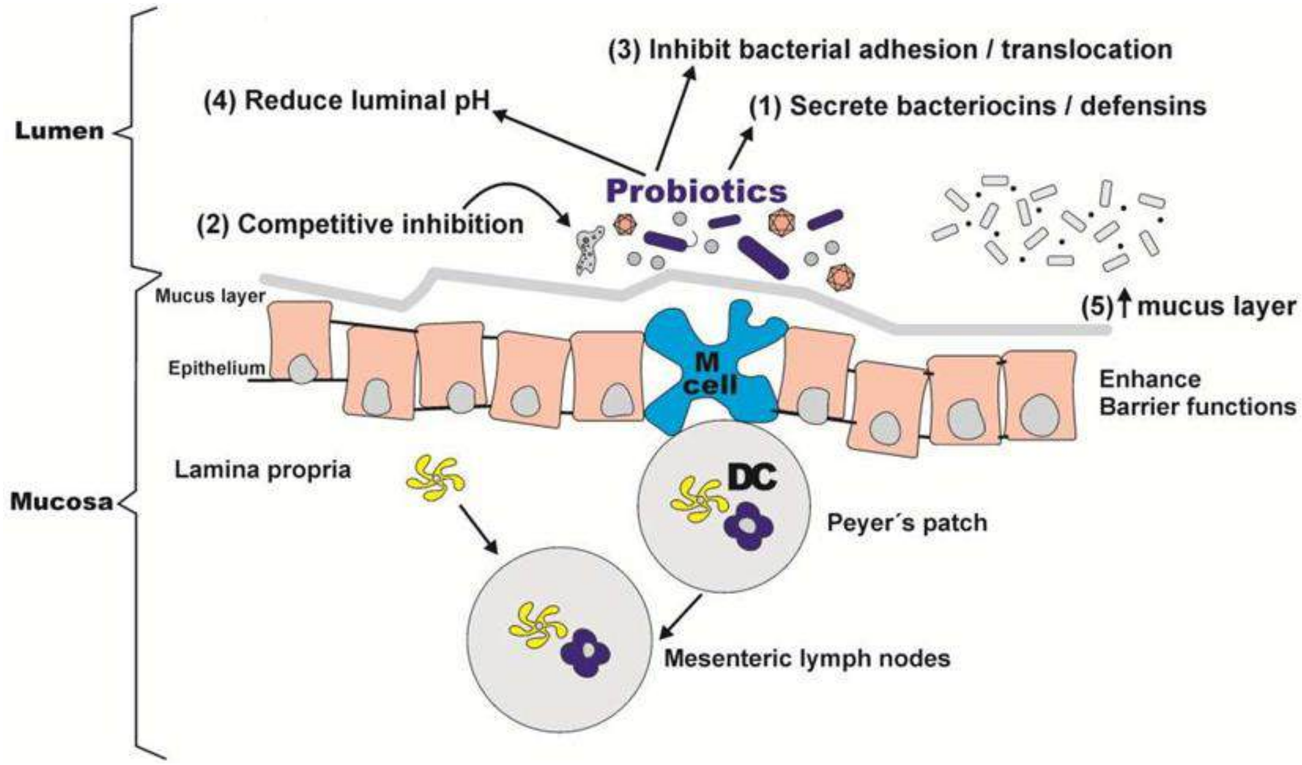

Fig. 3: Diagrammatic depiction of the interaction between gut mucosa and probiotic bacteria (Source: Ng et al. (2009)).

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*	*Bacillus licheniformis*
L. acidophilus	*B. infantis*	*Leuconstoc mesenteroides*	*B. subtilis*
L. paracasei	*B. bifidum*	*Lactococcus lactis*	*B. coagulans*
L. reuteri	*B. lactis*	*Streptococcus thermophilus*	*B. cereus*
L. plantarum	*B. longum*	*Pediococcus acidilactici*	*Saccharomyces cerevisiae*
L. casei	*B. breve*	*Enterococcus faecalis*	*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 x 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

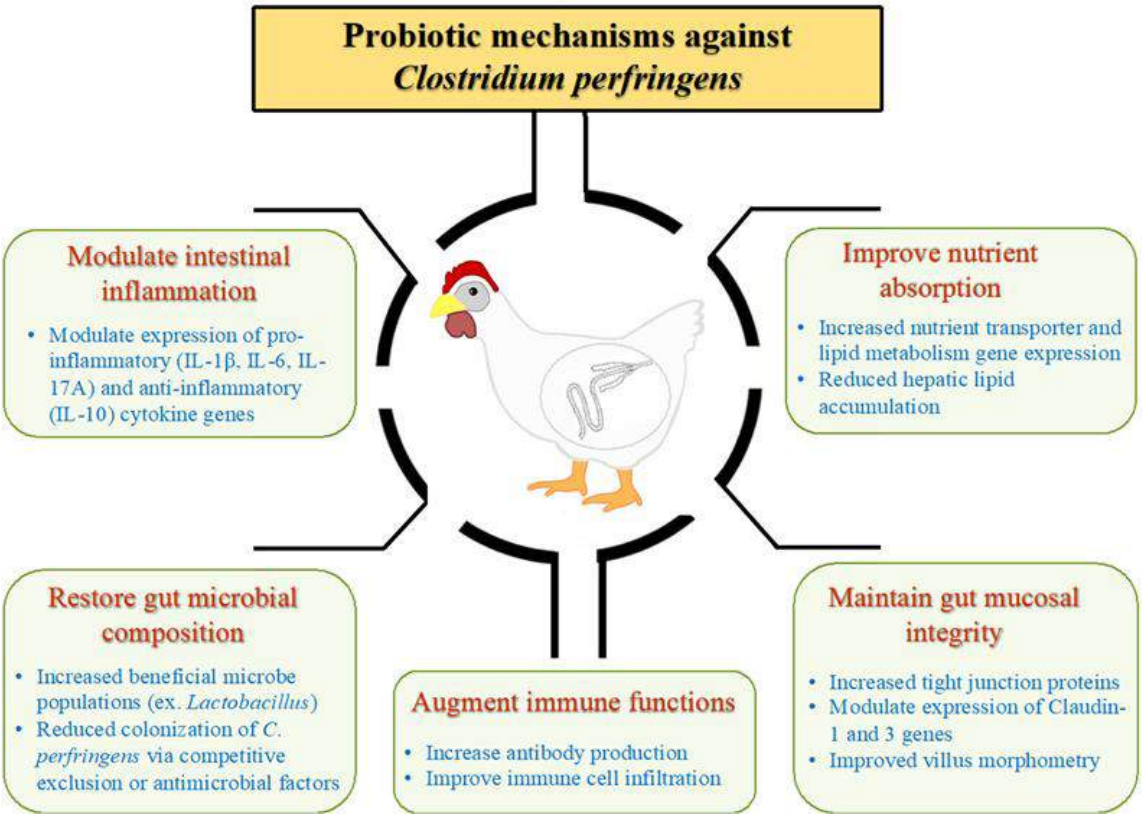

Fig. 4: Anti-*C. perfringens* mechanisms of probiotics (Kulkarni et al., 2022).

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 (Alayande et al., 2020). 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 been developed 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 probiotic bacteria, 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.

REFERENCES

Abd El-Ghany, W. A., Abdel-Latif, M. A., Hosny, F., Alatfeehy, N. M., Noreldin, A. E., Quesnell, R. R., Chapman, R., Sakai, L., and Elbestawy, A. R. (2022). Comparative efficacy of postbiotic, probiotic, and antibiotic against necrotic enteritis in broiler chickens. *Poultry Science*, *101*(8), 101988. https://doi.org/10.1016/j.psj.2022.101988

Abd El-Hack, M. E., El-Saadony, M. T., Elbestawy, A. R., Nahed, A., Saad, A. M., Salem, H. M., El-Tahan, A. M., Khafaga, A. F., Taha, A. E., and AbuQamar, S. F. (2022). Necrotic enteritis in broiler chickens: disease characteristics and prevention using organic antibiotic alternatives–a comprehensive review. *Poultry Science*, *101*(2), 101590. https://doi.org/10.1016/j.psj.2021.101590

Afshar Mazandaran, N., and Rajab, A. (2001). Probiotics and their use in animal and poultry diets. *Tehran: Noorbakhsh*.

Aggarwal, N., Breedon, A. M. E., Davis, C. M., Hwang, I. Y., and Chang, M. W. (2020). Engineering probiotics for therapeutic applications: recent examples and translational outlook. *Current Opinion in Biotechnology*, *65*, 171-179. https://doi.org/10.1016/j.copbio.2020.02.016

Angelin, J., and Kavitha, M. (2020). Exopolysaccharides from probiotic bacteria and their health potential. *International Journal of Biological Macromolecules*, *162*, 853-865. https://doi.org/10.1016/j.ijbiomac.2020.06.190

Bajagai, Y. S., Klieve, A. V., Dart, P. J., and Bryden, W. L. (2016). Probiotics in animal nutrition: production, impact and regulation. *FAO Animal Production and Health Paper (FAO) eng no. 179*. http://www.fao.org/3/a-i5933e.pdf

Broom, L. J. (2017). The sub-inhibitory theory for antibiotic growth promoters. *Poultry Science*, *96*(9), 3104-3108. https://doi.org/10.3382/ps/pex114

Brown, K., Uwiera, R. R., Kalmokoff, M. L., Brooks, S. P., and Inglis, G. D. (2017). Antimicrobial growth promoter use in livestock: a requirement to understand their modes of action to develop effective alternatives. *International Journal of Antimicrobial Agents*, *49*(1), 12-24. https://doi.org/10.1016/j.ijantimicag.2016.08.006

Caly, D. L., D'Inca, R., and Drider, D. (2015). Alternatives to antibiotics to prevent necrotic enteritis in broiler chickens: a microbiologist's perspective. *Frontiers in Microbiology*, *6*, 171469. https://doi.org/10.3389/fmicb.2015.01336

Cao, L., Wu, X., Bai, Y., Wu, X., and Gu, S. (2019). Anti-inflammatory and antioxidant activities of probiotic powder containing *Lactobacillus plantarum* 1.2567 in necrotic enteritis model of broiler chickens. *Livestock Science*, *223*, 157-163. https://doi.org/10.1016/j.livsci.2019.03.009

Chen, P., Lv, H., Du, M., Liu, W., Che, C., Zhao, J., and Liu, H. (2024). *Bacillus subtilis* HW2 enhances growth performance and alleviates gut injury via attenuation of endoplasmic reticulum stress and regulation of gut microbiota in broilers under necrotic enteritis challenge. *Poultry Science*, 103661. https://doi.org/10.1016/j.psj.2024.103661

Cheng, G., Hao, H., Xie, S., and Yuan, Z. (2014). Antibiotic alternatives: the substitution of antibiotics in animal husbandry? *Frontiers in Microbiology*, *5*, 87626. https://doi.org/10.3389/fmicb.2014.00217

Choi, T.-Y., Choi, Y. P., and Koo, J. W. (2020). Mental disorders linked to crosstalk between the gut microbiome and the brain. *Experimental Neurobiology*, *29*(6), 403. https://doi.org/10.5607/en20047

Cooper, K. K., Songer, J. G., and Uzal, F. A. (2013). Diagnosing clostridial enteric disease in poultry. *Journal of Veterinary Diagnostic Investigation*, *25*(3), 314-327. https://doi.org/10.1177/1040638713483468

Edens, F. (2003). An alternative for antibiotic se in poultry: probiotics. *Brazilian Journal of Poultry Science*, *5*, 75-97. https://doi.org/10.1590/S1516-635X2003000200001

Eeckhaut, V., Wang, J., Van Parys, A., Haesebrouck, F., Joossens, M., Ducatelle, R., and Van Immerseel, F. (2016). The probiotic *Butyricicoccus pullicaecorum* reduces feed conversion and protects from potentially harmful intestinal microorganisms and necrotic enteritis in broilers. *Frontiers in Microbiology*, *7*, 218034. https://doi.org/10.3389/fmicb.2016.01416

Emami, N. K., Calik, A., White, M. B., Kimminau, E. A., and Dalloul, R. A. (2020). Effect of probiotics and multi-component feed additives on microbiota, gut barrier and immune responses in broiler chickens during subclinical necrotic enteritis. *Frontiers in Veterinary Science*, *7*, 572142. https://doi.org/10.3389/fvets.2020.572142

Fathima, S., Hakeem, W. G. A., Shanmugasundaram, R., and Selvaraj, R. K. (2022). Necrotic enteritis in broiler chickens: A review on the pathogen, pathogenesis, and prevention. *Microorganisms*, *10*(10), 1958. https://doi.org/doi.org/10.3390/microorganisms10101958

Gharib-Naseri, K., Dorigam, J. C., Doranalli, K., Morgan, N., Swick, R. A., Choct, M., and Wu, S.-B. (2021). *Bacillus amyloliquefaciens* CECT 5940 improves performance and gut function in broilers fed different levels of protein and/or under necrotic enteritis challenge. *Animal Nutrition*, *7*(1), 185-197. https://doi.org/10.1016/j.aninu.2020.05.004

Hafez, H. M. (2011). Enteric diseases of poultry with special attention to *Clostridium Perfringens*. https://doi.org/10.17169/refubium-21098

Hazards, E. P. O. B., Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Girones, R., Herman, L., Koutsoumanis, K., and Lindqvist, R. (2017). Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. *EFSA Journal*, *15*(3), e04664. https://doi.org/10.2903/j.efsa.2017.4664

Hmidet, N., Ali, N. E.-H., Haddar, A., Kanoun, S., Alya, S.-K., and Nasri, M. (2009). Alkaline proteases and thermostable α-amylase co-produced by *Bacillus licheniformis* NH1: Characterization and potential application as detergent additive. *Biochemical Engineering Journal*, *47*(1-3), 71-79. https://doi.org/10.1016/j.bej.2009.07.005

Huang, Q., Liu, X., Zhao, G., Hu, T., and Wang, Y. (2018). Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. *Animal Nutrition*, *4*(2), 137-150. https://doi.org/10.1016/j.aninu.2017.09.004

Hussein, E. O., Ahmed, S. H., Abudabos, A. M., Suliman, G. M., Abd El-Hack, M. E., Swelum, A. A., and N. Alowaimer, A. (2020). Ameliorative effects of antibiotic-, probiotic-and phytobiotic-supplemented diets on the performance, intestinal health, carcass traits, and meat quality of *Clostridium perfringens*-infected broilers. *Animals*, *10*(4), 669. https://doi.org/10.3390/ani10040669

Joint, F. (2002). WHO Working Group: Guidelines for the evaluation of probiotics in food: report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. *London, ON, Canada*.

Joshi, D., Roy, S., and Banerjee, S. (2018). Prebiotics: A functional food in health and disease. In *Natural Products and Drug discovery* (pp. 507-523). Elsevier. https://doi.org/10.1016/B978-0-08-102081-4.00019-8

Keerqin, C., Rhayat, L., Zhang, Z.-H., Gharib-Naseri, K., Kheravii, S., Devillard, E., Crowley, T., and Wu, S.-B. (2021). Probiotic *Bacillus subtilis* 29,784 improved weight gain and enhanced gut health status of broilers under necrotic enteritis condition. *Poultry Science*, *100*(4), 100981. https://doi.org/10.1016/j.psj.2021.01.004

Krysiak, K., and Konkol, D. (2021). Korczy nski, M. *Overview of the use of probiotics in poultry production. Animals*, *11*, 1620.

Kulkarni, R. R., Gaghan, C., Gorrell, K., Sharif, S., and Taha-Abdelaziz, K. (2022). Probiotics as alternatives to antibiotics for the prevention and control of necrotic enteritis in chickens. *Pathogens*, *11*(6), 692. https://doi.org/10.3390/pathogens11060692

Kumar, H., Bhardwaj, K., Kaur, T., Nepovimova, E., Kuča, K., Kumar, V., Bhatia, S. K., Dhanjal, D. S., Chopra, C., and Singh, R. (2020). Detection of bacterial pathogens and antibiotic residues in chicken meat: a review. *Foods*, *9*(10), 1504. https://doi.org/10.3390/foods9101504

Li, P., Zheng, L., Qi, Y., Liu, Z., Du, E., Wei, J., Zhang, Z., Guo, S., and Ding, B. (2022). Dietary *Lactobacillus fermentum* and *Lactobacillus paracasei* improve the intestinal health of broilers challenged with coccidia and *Clostridium perfringens*. *Frontiers in Veterinary Science*, *9*, 1025677. https://doi.org/10.3389/fvets.2022.1025677

Liu, Y., Zhang, S., Luo, Z., and Liu, D. (2021). Supplemental *Bacillus subtilis* PB6 improves growth performance and gut health in broilers challenged with *Clostridium perfringens*. *Journal of Immunology Research*, *2021*. https://doi.org/10.1155/2021/2549541

Markowiak, P., and Śliżewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathogens*, *10*, 1-20. https://doi.org/10.1186/s13099-018-0250-0

Moore, R. J. (2016). Necrotic enteritis predisposing factors in broiler chickens. *Avian Pathology*, *45*(3), 275-281. https://doi.org/10.1080/03079457.2016.1150587

Mora, Z. V.-D. l., Macías-Rodríguez, M. E., Arratia-Quijada, J., Gonzalez-Torres, Y. S., Nuño, K., and Villarruel-López, A. (2020). *Clostridium perfringens* as foodborne pathogen in broiler production: pathophysiology and potential strategies for controlling necrotic enteritis. *Animals*, *10*(9), 1718. https://doi.org/10.3390/ani10091718

Musa, B. B., Duan, Y., Khawar, H., Sun, Q., Ren, Z., Elsiddig Mohamed, M. A., Abbasi, I. H. R., and Yang, X. (2019). *Bacillus subtilis* B21 and *Bacillus licheniformis* B26 improve intestinal health and performance of broiler chickens with *Clostridium perfringens*-induced necrotic enteritis. *Journal of Animal Physiology and Animal Nutrition*, *103*(4), 1039-1049. https://doi.org/10.1111/jpn.13082

Ng, S., Hart, A., Kamm, M., Stagg, A., and Knight, S. C. (2009). Mechanisms of action of probiotics: recent advances. *Inflammatory Bowel Diseases*, *15*(2), 300-310. https://doi.org/10.1002/ibd.20602

Niewold, T. (2007). The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. *Poultry Science*, *86*(4), 605-609. https://doi.org/10.1093/ps/86.4.605

Qing, X., Zeng, D., Wang, H., Ni, X., Liu, L., Lai, J., Khalique, A., Pan, K., and Jing, B. (2017). Preventing subclinical necrotic enteritis through *Lactobacillus johnsonii* BS15 by ameliorating lipid metabolism and intestinal microflora in broiler chickens. *Amb Express*, *7*, 1-12. https://doi.org/10.1186/s13568-017-0439-5

Rahman, M. R. T., Fliss, I., and Biron, E. (2022). Insights in the development and uses of alternatives to antibiotic growth promoters in poultry and swine production. *Antibiotics*, *11*(6), 766. https://doi.org/10.3390/antibiotics11060766

Ramlucken, U., Ramchuran, S. O., Moonsamy, G., Lalloo, R., Thantsha, M. S., and van Rensburg, C. J. (2020). A novel *Bacillus* based multi-strain probiotic improves growth performance and intestinal properties of *Clostridium perfringens* challenged broilers. *Poultry Science*, *99*(1), 331-341. https://doi.org/10.3382/ps/pez496

Rathnayaka, S. D., Selvanathan, S., and Selvanathan, E. (2021). Demand for animal-derived food in selected Asian countries: A system-wide analysis. *Agricultural Economics*, *52*(1), 97-122. https://doi.org/10.1111/agec.12609

Salim, H. M., Huque, K. S., Kamaruddin, K. M., and Haque Beg, A. (2018). Global restriction of using antibiotic growth promoters and alternative strategies in poultry production. *Science Progress*, *101*(1), 52-75. https://doi.org/10.3184/003685018X15173975498947

Sandvang, D., Skjoet-Rasmussen, L., Cantor, M. D., Mathis, G. F., Lumpkins, B. S., and Blanch, A. (2021). Effects of feed supplementation with 3 different probiotic *Bacillus s*trains and their combination on the performance of broiler chickens challenged with *Clostridium perfringens*. *Poultry Science*, *100*(4), 100982. https://doi.org/10.1016/j.psj.2021.01.005

Shojadoost, B., Alizadeh, M., Boodhoo, N., Astill, J., Karimi, S. H., Shoja Doost, J., Taha-Abdelaziz, K., Kulkarni, R., and Sharif, S. (2022). Effects of treatment with *lactobacilli* on necrotic enteritis in broiler chickens. *Probiotics and Antimicrobial Proteins*, *14*(6), 1110-1129. https://doi.org/10.1007/s12602-021-09901-5

Shojadoost, B., Vince, A. R., and Prescott, J. F. (2012). The successful experimental induction of necrotic enteritis in chickens by *Clostridium perfringens*: a critical review. *Veterinary Research*, *43*, 1-12. https://doi.org/10.1186/1297-9716-43-74

Sokale, A., Menconi, A., Mathis, G., Lumpkins, B., Sims, M., Whelan, R., and Doranalli, K. (2019). Effect of *Bacillus subtilis* DSM 32315 on the intestinal structural integrity and growth performance of broiler chickens under necrotic enteritis challenge. *Poultry Science*, *98*(11), 5392-5400. https://doi.org/10.3382/ps/pez368

Stęczny, K., and Kokoszyński, D. (2021). Effect of probiotic preparations (EM) on productive characteristics, carcass composition, and microbial contamination in a commercial broiler chicken farm. *Animal Biotechnology*, *32*(6), 758-765. https://doi.org/10.1080/10495398.2020.1754841

Sun, N., Xue, Y., Wei, S., Wu, B., Wang, H., Zeng, D., Zhao, Y., Khalique, A., Pan, K., and Zeng, Y. (2021). Compound probiotics improve body growth performance by enhancing intestinal development of broilers with subclinical necrotic enteritis. *Probiotics and Antimicrobial Proteins*, 1-15. https://doi.org/10.1007/s12602-021-09867-4

Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Padgett, C., Bailey, C., Gancel, F., and Drider, D. (2022). Protective effects of novel *Lactobacillaceae* strains isolated from chicken Caeca against necrotic enteritis infection: In vitro and in vivo evidences. *Microorganisms*, *10*(1), 152. https://doi.org/10.3390/microorganisms10010152

Wade, B., and Keyburn, A. (2015). The true cost of necrotic enteritis. *World Poultry*, *31*(7), 16-17.

Wang, B., Xie, K., and Lee, K. (2021). Veterinary drug residues in animal-derived foods: Sample preparation and analytical methods. *Foods*, *10*(3), 555. https://doi.org/10.3390/foods10030555

Wang, F., Mi, K., Ahmad, I., Xie, S., Hussain, H. I., Yuan, Z., Dai, M., and Huang, L. (2020). Antibacterial activity of cyadox against *Clostridium perfringens* in broilers and a dosage regimen design based on pharmacokinetic-pharmacodynamic modeling. *Microbial Pathogenesis*, *141*, 103981. https://doi.org/10.1016/j.micpath.2020.103981

Wang, H., Khalique, A., and Zeng, D. (2018). Probiotic *Lactobacillus johnsonii* BS15 improves blood parameters related to immunity in broilers experimentally infected with subclinical necrotic enteritis. *Frontiers in Microbiology*, *9*, 317472. https://doi.org/10.3389/fmicb.2018.00049

Wu, Y., Shao, Y., Song, B., Zhen, W., Wang, Z., Guo, Y., Shahid, M. S., and Nie, W. (2018). Effects of *Bacillus coagulans* supplementation on the growth performance and gut health of broiler chickens with *Clostridium perfringens*-induced necrotic enteritis. *Journal of Animal Science and Biotechnology*, *9*, 1-14. https://doi.org/10.1186/s40104-017-0220-2

Wu, Y., Zhen, W., Geng, Y., Wang, Z., and Guo, Y. (2019). Pretreatment with probiotic *Enterococcus faecium* NCIMB 11181 ameliorates necrotic enteritis-induced intestinal barrier injury in broiler chickens. *Scientific Reports*, *9*(1), 10256. https://doi.org/10.1038/s41598-019-46578-x

Xu, X., Yang, S., Olajide, J. S., Zhou, E., and Cai, J. (2021). *Clostridium butyricum* supplement can ameliorate the intestinal barrier roles in broiler chickens experimentally infected with *Clostridium perfringens*. *Frontiers in Physiology*, *12*, 737481. https://doi.org/10.3389/fphys.2021.737481

Zhang, H., Zhou, Y., Xu, H., Liang, C., and Zhai, Z. (2022). *Bacillus amyloliquefaciens* BLCC1-0238 alone or in combination with mannan-oligosaccharides alleviates subclinical necrotic enteritis in broilers. *Probiotics and Antimicrobial Proteins*, 1-11. https://doi.org/10.1007/s12602-021-09853-w

Zhao, Y., Zeng, D., Wang, H., Qing, X., Sun, N., Xin, J., Luo, M., Khalique, A., Pan, K., and Shu, G. (2020). Dietary probiotic *Bacillus licheniformis* H2 enhanced growth performance, morphology of small intestine and liver, and antioxidant capacity of broiler chickens against *Clostridium perfringens*–induced subclinical necrotic enteritis. *Probiotics and Antimicrobial Proteins*, *12*, 883-895. https://doi.org/10.1007/s12602-019-09597-8

Zhou, M., Zeng, D., Ni, X., Tu, T., Yin, Z., Pan, K., and Jing, B. (2016). Effects of *Bacillus licheniformis* on the growth performance and expression of lipid metabolism-related genes in broiler chickens challenged with *Clostridium perfringens*-induced necrotic enteritis. *Lipids in Health and Disease*, *15*, 1-10. https://doi.org/10.1186/s12944-016-0219-2

Chapter 11

Probiotics as an Alternative to Antibiotics in Poultry

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ABSTRACT

The poultry industry is crucial to the economy, providing essential protein sources through chicken meat and eggs. However, it encounters challenges such as stress, health issues, and harsh environmental conditions, leading to significant economic losses. Antibiotics are added in poultry feeds as growth promoters and as an effective approach to lessen the number of detrimental bacteria that harbor the gastrointestinal tract. The antibiotics used in feed can also eradicate beneficial bacteria and the unrestricted use can increase the chances of antibiotic resistance in pathogenic bacteria. So, to resolve this problem, researchers have considered a great interest in promoting remedies to antibiotics. The probiotics are used as a feed supplement to replace the feed antibiotics. The probiotics are commonly used in chicken production. It improves the quality of eggs, meat, bones, and growth performance of birds. The probiotics can alter the intestinal microbes, GIT microbes, and defense system stimulation. The main theme of this chapter is to emphasize on the advantages of feed supplements (probiotics) as an alternative to antibiotics in poultry.

KEYWORDS		
Probiotics, Antibiotics, Antibiotic resistance, Gut microbiota	Received: 21-May-2024 Revised: 12-Jul-2024 Accepted: 04-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

The poultry industry has a great role in the economy of any country (Shahbaz et al., 2024). Poultry rearing is extensively exposed to numerous problems such as stress, health-related problems, and harsh environmental conditions leading to huge economic losses to the chicken industry (Lutful Kabir, 2009). The chickens were domesticated around 5000 years ago, and people used to consume chicken meat and eggs as a source of protein (Rychlik, 2020). Since the 1940s, poultry feed has been supplemented with antibiotics (*Streptomyces aureofaciens*), which led to growth enhancement and feed additives (Eckert et al., 2010). However, there has been comparatively less emphasis on managing microbial infectious diseases caused by a variety of bacteria such as *Klebsiella, Yersinia, Enterococcus, Proteus, Pseudomonas*, *Salmonella*, *Bacillus*, *Clostridium*, *Mycobacterium*, *Campylobacter*, and *Escherichia Coli*. To mitigate this risk, broiler chicks are typically housed in enclosed facilities (Shurson et al., 2022). Many farmers administer antibiotics mixed in the feed to chicks (Mehdi et al., 2018), a practice that has been prevalent in traditional commercial chicken production for decades. The administration of antibiotics, mainly aims to boost feed utilization, growth rates, and chicken health, thus boosting production and profitability (Lourenco et al., 2019). Chickens serve as a principal food source globally, with antibiotics frequently employed to combat microbial threats. This results in the emergence of antibiotic-resistant genes, which may propagate to other bacteria (Żbikowska et al., 2020).

The essential natural or synthetic compounds known as antimicrobial agents can eliminate or hinder the growth of hazardous bacteria. The control of microorganisms that cause acute or chronic infection is extremely difficult despite antibiotics (Lewis, 2013). The pursuit of discovering novel antibiotics entered a significant phase following Alexander Fleming's discovery of penicillin, which effectively inhibited the growth of *Staphylococcus aureus*. Penicillin marked the advent of genuine antibiotic therapy (Khan, 2017). The tendency of these resistant germs to propagate, either directly or indirectly from one host to the other has resulted in antibiotic resistance (Zhao et al., 2020). The human and animal welfare are affected when antibiotics are used in larger doses or sub-therapeutic doses and it has a detrimental impact on both humans and animals (Grenni et al., 2018). The staphylococci associated with poultry farms have been discovered to be resistant to tetracycline and oxacillin. Distinct *Staphylococcus* species that frequently cause infection in chickens result in septicemia, and pododermatitis, together with evolving resistance to beta-lactam drugs (Pal et al., 2020).

Additionally, *E. coli* has become more resistant to most antibiotics which are frequently used in poultry including tetracycline (Varga et al., 2019). However, the discovery that antibiotic growth promoters contribute to the development of multi-drug-resistant microbes has sparked worries about the health of the world's populations. Antibiotic-resistant genes may have transferred from animals to humans as a result of an upsurge of antibiotic-resistant microbial communities in animals (Olusegun Oyebade et al., 2022). Many European countries have banned antibiotics in the feed of chickens since 2006 (Muhammad et al., 2020). Likewise, in 2015, the US Food and Drug Administration implemented veterinary feed directives, advising restricted use of antibiotics solely for animal treatment purposes (Yaqoob et al., 2022). Antimicrobial drugs for prophylaxis and as growth promoters were declared illicit in Sweden in 1986 and 1988, respectively (Neveling and Dicks, 2021). Similarly, in July 2011, the first Asian country, in which antibiotics growth boosters were banned was South Korea (Muhammad et al., 2019). The ban on the antibiotics used in feed increases the demand for substitutes to prevent loss in animal output. Over the last 20 years, nutritionists who specialized in poultry have observed a significant increase in the application of fatty acids, essential oils, prebiotics, symbiotics, and probiotics.

Additionally, probiotics have been demonstrated to enhance immunological response, GIT anatomy, and biological processes. Consequently, this improves the health and performance of chickens. Feed additives known as probiotics include useful fungi such as (*Aspergillus awamori, A. oryza, and A, noryi*), yeast (*Candida and Saccharomyces*), and beneficial bacteria such as *Bifidobcterium, lactobacillus,* and *streptococci*, all of which have the power to alter intestinal microflora and modulate the immune system (Abdel-Moneim et al., 2021).

Role of Probiotic in Growth Performance

Salmonella is the most common pathogen found in the lower GIT of poultry, especially broilers. Probiotics have evolved as the viable substitutes for growth advancement in most poultry farms after the antibiotic growth promoters were removed from poultry feed. Antibiotic growth promoters cause disturbance in gut microbiota by preventing the GIT pro-inflammatory cytokines from being produced and secreted (Adedokun and Olojede, 2018). The probiotics are non-pathogenic microorganisms in the GIT of broilers that compete with harmful bacteria for nutrition. Additionally, they colonize in the intestines, hindering the growth of hazardous bacteria and boosting the digestive enzymes (galactosidase and amylase) which enhances their growth performance by enhancing the absorption of nutrients (Al-Khalaifah, 2018). Employing a strain of *Lactiplantibacillus plantarum* (LT-113), the vaccinated chicks were found to be shielded against *Salmonella typhimurium* but minimizing intestinal cell production of tight junction genes and gastrointestinal invasion. Salmonella infection eroded the intestinal mucosal barrier in the control group (Wang et al., 2019). Alternatively, oral *Lactobacillus jhonsonii* treatment suppressed the bowel incursion of *Clostridium perfringens* and *Salmonella*. Furthermore, it has been proven that xylanase and multi-strain probiotics boost the bowel's absorption of food-derived energy and the liver's retention of that energy (Olnood et al., 2015).

The probiotics have been phased out for the ability to enhance the development of incorporated chicken outputs since the evaluation of antibiotic growth promoters but hinder the yield and secretion of metabolic regulators by intestinal cells which results in a reduction of intestinal microflora (Jha et al., 2020). Adversely, probiotics can increase growth by modifying the GIT premises and promoting GIT function through the fortification of useful microbes, defense system modification, and pathogenic competitive exclusion. Probiotics supplementation, beneficial microbes confront hazardous microbes for nutrition; and grow in the intestine, which inhibits detrimental microbes and secrete enzymes (beta-galactosidase and alpha-amylase), which accomplice in the assimilation of a nutritious diet and enhances the productivity of animals (Olnood et al., 2015).

Effects of Probiotics on Gut

The well-being and efficiency of the chickens are directly proportional to the GIT environment and microbes. The intestines of poultry are the main harbor of different beneficial microflora which disintegrate complex compounds into simple molecules that are easily digested (Olnood et al., 2015). Different techniques are utilized for the investigation of the beneficial effects of probiotics on GIT microbial activity, composition, and differences, which consist of culture-dependent techniques, genotyping, and *in-vivo* assays. In addition, *in-vivo* administration is the most fruitful and beneficial method for gaining better results. The most significant procedures for measuring the antagonistic potentials of probiotics include the low GIT pH, modification of the defense system, and secretion of organic acid. In addition, the supplementation of diet with probiotics has been researched to increase the GIT microbes by hindering pathogen multiplication and increasing the number of good microbes (Abdel-Moneim et al., 2021). The destruction of intestines by *Eimeria* not only damages epithelium, but also disrupts GIT microbial colonies, enhancing colonization and multiplication of pathogens *Clostridium perfringens*, increasing the chances of secondary diseases, and increasing mortality (Macdonald et al., 2019). The *Eimeria* invasion results in an imbalance in the gut microbial community known as dysbiosis (Ducatelle et al., 2015).

Antibiotic Resistance

Antibiotics have been used to treat infectious diseases in poultry. Since the discovery of antibiotics, poultry feed has been supplemented with antibiotics to enhance the growth of animals. Antibiotics have been crucial in the development of animal husbandry. Poultry farmers use antibiotics to raise chickens in better conditions and prevent different infections in poultry (Boamah and Agyare, 2016). It has been recognized that numerous zoonotic pathogens including *Salmonella, Clostridium sp.*, *Escherichia coli,* and *Campylobacter*, may be found in animal dung (Jones and Martin, 2003). Antibiotics are used in poultry farming however, they kill susceptible strains of bacteria and abandon or enhance variants with traits that are resistant to them. These resistant bacteria proliferate up to a million times per day, promoting resistance by mutation and plasmid mediation (Gould, 2008). Antibiotic resistance is the main outcome of antibiotics being excessively used in the poultry industry (Tiwari et al., 2014).

Probiotics as an Alternative to Antibiotics

The probiotics are defined as feed supplemented with live beneficial microbes (*Bifidobacterium, lactobacillus, and streptococci*), yeast cultures (*Saccharomyces and Candida*), fungi (*Aspergillus awamori, Aspergillus niger, and Aspergillus oryza*). It is crystal clear that probiotics lessen the risk of coccidiosis by enhancing the immune system, intestinal flora balance, and poultry performance (Ahmad et al., 2022). In another study, it was observed that infusing probiotics comprising *Lactobacillus salivarius*, *Enterococcus faecium,* and *Bacillus animalis* into broiler feed decreased the frequency of infections with *Eimeria tenella*, *Eimeria maxima,* and *Eimeria acervulina*. Lowering the lesion score and oocyst numbers in the duodenum, jejunum, and caeca were seen and also modulate the immune system and reduce the shedding oocysts from *E. acervulina*, and *E. tenella*, probiotics such as *Pediococcus* and *Saccharomyces* were added to the feed (Adhikari et al., 2020). In contrast, the administration of *Bacillus subtilis* (orally) significantly decreases the number of *Eimeria tenella* lesions in the caeca (Wang et al., 2019). The results revealed that while probiotic bacteria contend with *Eimeria* for attachment sites in the Gut, they may occupy similar receptors in the epithelium. This analogy prevents *Eimeria* from proliferation and releasing oocysts. However, severe coccidiosis can diminish the potency of probiotics or prebiotics, so more research needs to be evaluated (Adhikari et al., 2020). The biological efficacy of probiotics is becoming more and more verified through research, although employing these microbes for therapeutic purposes should be done with caution (de Melo Pereira et al., 2018).

Technique for Assessing Probiotic's Antibacterial Efficacy against Microbes

The antibacterial activity of probiotics can be assessed using a wide range of *in-vitro* techniques. However, it is viable to figure out whether probiotic cultures and pathogenic strains are directly antagonistic or whether probiotic extracts' antibacterial activity (Sabina, 2016). The microbial antagonist tests on solid media are most applicable when the main goal of the analysis is to identify microorganisms' antagonistic interaction (Balouiri et al., 2016). This technique entails identifying the indicator strain's growth suppression imposed by the test culture. This section provides a detailed examination of the primary techniques that are currently employed in probiotics' antibacterial activity *in-vitro*. Different other techniques are also reported in different research such as the agar well diffusion assay which describes, to figure out whether cell-free supernatants have antagonistic effects performed the agar well diffusion assay. Multiple nutrients are synthesized using selective or differential media. The indicator microbe is injected into the plates. Afterward, each plate with 6-7 mm wells was made in it. The probiotics microorganisms' supernatant is pipetted into the well following centrifugation and dilution in aliquots at different doses. The antibacterial activity is expressed as an inhibition zone or as arbitrary units (AU/ml) following incubation (Parente et al., 1995).

The probiotics that are utilized nowadays are *Lactobacillus acidophilus*, *lactobacillus casei*, *Lactobacillus plantarum*, *Shirota*, *Lactobacillus paracasei*, *Limosilactobacillus reuteri*, *Limosilactobacillus johnsonii*, *Limosilactobacillus. rhamnosus* (Sikorska and Smoragiewicz, 2013). The probiotic supplementation has been demonstrated to deliver advantages in recent years, with outcomes ranging from direct pathogen exclusion to strengthened host immune system performance (Rossoni et al., 2017). The purpose of probiotics is to enhance the host's health. Numerous probiotic-related studies have reported that most of these studies merely demonstrated how probiotics helped the host's intestinal health. Probiotics work by various techniques, including the generation of compounds that hinder gram-negative and gram-positive bacteria, such as hydrogen peroxide and bacteriocins, blocking of adhesion sites, and many more processes (MORAES et al., 2019). The probiotics modulate the immune response in numerous ways, including improving macrophage-mediated non-specific phagocytic activity (Jain et al., 2008). Numerous probiotics are used to alter the pro-anti-inflammatory cytokines (Plaza-Díaz et al., 2017) and have also been reported in different research (Villena et al., 2012). The benefits are antimutagenic (Yu and Li, 2016), anticarcinogenic, and anti-diarrheal (Devaraj et al., 2019). The probiotics are considered to enhance human health such as immunomodulatory effects or competition between bacteria (Piewngam et al., 2019). The antibiotics that are used in daily life possess adverse effects, are expensive, and face resistance (Vítor and Vale, 2011). The idea of a combination of probiotic microbes with traditional medication has been explored. This synergism has several advantages such as quicker healing, less dose of traditional medicine, lowering the side effects, and boosting the rate at which microbial diseases are eliminated.

Future Perspectives

As the poultry industry expands, the need for sustainable and effective antibiotic alternatives will become increasingly important. Future research should aim to optimize the use of probiotics to maximize their benefits in poultry farming. By understanding the specific roles and interactions of various probiotic strains, tailored formulations can be developed to address specific health issues or enhance aspects of poultry growth and productivity. Investigating the synergistic effects of combining probiotics with other natural feed additives, such as prebiotics, essential oils, and organic acids, could lead to more robust and comprehensive strategies for improving poultry health and performance. Raising consumer awareness about the advantages of probiotics in poultry farming and their role in reducing antibiotic resistance can drive demand for poultry products raised with probiotic supplements, encouraging a shift towards more sustainable farming practices. By focusing on these areas, the poultry industry can fully utilize the potential of probiotics to enhance animal health, boost productivity, and ensure food safety, thereby contributing to a more sustainable and resilient global food system.

Conclusion

The poultry industry is vital to the global economy, supplying a substantial portion of protein through chicken meat and eggs. Despite its importance, the sector faces significant challenges such as stress, health issues, and adverse environmental conditions, all of which can lead to considerable economic losses. Probiotics have emerged as a promising alternative to antibiotics in poultry farming. These beneficial microorganisms enhance gut health and the immune system in chickens, improve nutrient absorption, and outcompete harmful bacteria, leading to better overall growth performance. Probiotics such as *Lactobacillus, Bifidobacterium*, and *Saccharomyces* have demonstrated significant potential in reducing diseases like *Salmonella* and *Eimeria* infections in poultry. Furthermore, they help maintain a balanced gut microflora, which is crucial for the effective breakdown and absorption of nutrients. Probiotics can effectively replace antibiotic growth promoters, thereby mitigating the risks associated with antibiotic resistance. They promote the formation of fatty acids, boost the immune system, and improve gut morphology and function. Nonetheless, the effectiveness of probiotics can be influenced by several factors, including the specific strains used and the conditions under which they are administered.

REFERENCES

Abdel-Moneim, A.-M. E., Shehata, A. M., Khidr, R. E., Paswan, V. K., Ibrahim, N. S., El-Ghoul, A. A., and Ebeid, T. A. (2021). Nutritional manipulation to combat heat stress in poultry – A comprehensive review. *Journal of Thermal Biology*, *98*, 102915. https://doi.org/10.1016/j.jtherbio.2021.102915

Adedokun, S. A., and Olojede, O. C. (2018). Optimizing Gastrointestinal Integrity in Poultry: The Role of Nutrients and Feed Additives. *Frontier Veterinary Science*, *5*, 348. https://doi.org/10.3389/fvets.2018.00348

Adhikari, P., Kiess, A., Adhikari, R., and Jha, R. (2020). An approach to alternative strategies to control avian coccidiosis and necrotic enteritis. *Journal of Applied Poultry Research*, *29*(2), 515-534. https://doi.org/10.1016/j.japr.2019.11.005

Ahmad, R., Yu, Y. H., Hsiao, F. S., Su, C. H., Liu, H. C., Tobin, I., and Cheng, Y. H. (2022). Influence of Heat Stress on Poultry Growth Performance, Intestinal Inflammation, and Immune Function and Potential Mitigation by Probiotics. *Animals (Basel)*, *12*(17). https://doi.org/10.3390/ani12172297

Al-Khalaifah, H. S. (2018). Benefits of probiotics and/or prebiotics for antibiotic-reduced poultry. *Poult Science*, *97*(11), 3807-3815. https://doi.org/10.3382/ps/pey160

Balouiri, M., Sadiki, M., and Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal Pharmacy Analysis*, *6*(2), 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

Boamah, V., and Agyare, C. (2016). Antibiotic Practices and Factors Influencing the Use of Antibiotics in Selected Poultry Farms in Ghana. *Journal of Antimicrobial Agents*, *2*. https://doi.org/10.4172/2472-1212.1000120

de Melo Pereira, G. V., de Oliveira Coelho, B., Magalhães Júnior, A. I., Thomaz-Soccol, V., and Soccol, C. R. (2018). How to select a probiotic? A review and update of methods and criteria. *Biotechnology Advance*, *36*(8), 2060-2076. https://doi.org/10.1016/j.biotechadv.2018.09.003

Devaraj, N. K., Suppiah, S., Veettil, S. K., Ching, S. M., Lee, K. W., Menon, R. K., and Sivaratnam, D. (2019). The effects of probiotic supplementation on the incidence of diarrhea in cancer patients receiving radiation therapy: a systematic review with meta-analysis and trial sequential analysis of randomized controlled trials. *Nutrients*, *11*(12), 2886.

Ducatelle, R., Eeckhaut, V., Haesebrouck, F., and Van Immerseel, F. (2015). A review on prebiotics and probiotics for the control of dysbiosis: present status and future perspectives. *Animal*, *9*(1), 43-48. https://doi.org/10.1017/s1751731114002584

Eckert, N. H., Lee, J. T., Hyatt, D., Stevens, S. M., Anderson, S., Anderson, P. N., and Caldwell, D. J. (2010). Influence of probiotic administration by feed or water on growth parameters of broilers reared on medicated and nonmedicated diets. *Journal of Applied Poultry Research*, *19*(1), 59-67. https://doi.org/10.3382/japr.2009-00084

Gould, I. M. (2008). The epidemiology of antibiotic resistance. *International Journal Antimicrobiology Agents*, *32 Suppl 1*, S2-9. https://doi.org/10.1016/j.ijantimicag.2008.06.016

Grenni, P., Ancona, V., and Barra Caracciolo, A. (2018). Ecological effects of antibiotics on natural ecosystems: A review. *Microchemical Journal*, *136*, 25-39. https://doi.org/10.1016/j.microc.2017.02.006

Jain, S., Yadav, H., and Sinha, P. R. (2008). Stimulation of innate immunity by oral administration of dahi containing probiotic Lactobacillus casei in mice. *Journal Medicien Food*, *11*(4), 652-656. https://doi.org/10.1089/jmf.2006.0132

Jha, R., Das, R., Oak, S., and Mishra, P. (2020). Probiotics (Direct-Fed Microbials) in Poultry Nutrition and Their Effects on Nutrient Utilization, Growth and Laying Performance, and Gut Health: A Systematic Review. *Animals (Basel)*, *10*(10). https://doi.org/10.3390/ani10101863

Jones, P., and Martin, M. (2003). A Review of the Literature on the Occurrence and Survival of Pathogens of Animals and Humans in Garden Compost.

Khan, M. F. (2017). Brief history of Staphylococcus aureus: a focus to antibiotic resistance. *EC Microbiology*, *5*(2), 36-39.

Lewis, K. (2013). Platforms for antibiotic discovery. *National Review Drug Discovory*, *12*(5), 371-387. https://doi.org/10.1038/nrd3975

Lourenco, J. M., Rothrock Jr., M. J., Fluharty, F. L., and Callaway, T. R. (2019). The Successional Changes in the Gut Microbiome of Pasture-Raised Chickens Fed Soy-Containing and Soy-Free Diets [Original Research]. *Frontiers in Sustainable Food Systems*, *3*. https://doi.org/10.3389/fsufs.2019.00035

Lutful Kabir, S. M. (2009). The role of probiotics in the poultry industry. *International Journal Molecular Science*, *10*(8), 3531-3546. https://doi.org/10.3390/ijms10083531

Macdonald, S. E., van Diemen, P. M., Martineau, H., Stevens, M. P., Tomley, F. M., Stabler, R. A., and Blake, D. P. (2019). Impact of Eimeria tenella Coinfection on Campylobacter jejuni Colonization of the Chicken. *Infection Immunology*, *87*(2). https://doi.org/10.1128/iai.00772-18

Mehdi, Y., Létourneau-Montminy, M. P., Gaucher, M. L., Chorfi, Y., Suresh, G., Rouissi, T., and Godbout, S. (2018). Use of antibiotics in broiler production: Global impacts and alternatives. *Animal Nutrition*, *4*(2), 170-178. https://doi.org/10.1016/j.aninu.2018.03.002

Moraes, M. C. C., Costa, P. J. C., Segundo, A. S. G., and Peruzzo, D. C. (2019). Assessment of probiotic bacterial strains effect on S. aureus biofilm on titanium discs with treated surfaces. *Revista de Odontologia da UNESP*, *48*, e20190096.

Muhammad, J., Khan, S., Su, J. Q., Hesham, A. E.-L., Ditta, A., Nawab, J., and Ali, A. (2020). Antibiotics in poultry manure and their associated health issues: a systematic review. *Journal of Soils and Sediments*, *20*(1), 486-497. https://doi.org/10.1007/s11368-019-02360-0

Muhammad, J., Khan, S., Su, J. Q., Hesham, A. E.-L., Ditta, A., Nawab, J., and Gogoi, B. (2019). Antibiotics in poultry manure and their associated health issues: a systematic review. *Journal of Soils and Sediments*.

Neveling, D. P., and Dicks, L. M. T. (2021). Probiotics: an Antibiotic Replacement Strategy for Healthy Broilers and Productive Rearing. *Probiotics Antimicrob Proteins*, *13*(1), 1-11. https://doi.org/10.1007/s12602-020-09640-z

Olnood, C. G., Beski, S. S. M., Choct, M., and Iji, P. A. (2015). Novel probiotics: Their effects on growth performance, gut development, microbial community and activity of broiler chickens. *Animal Nutrition*, *1*(3), 184-191. https://doi.org/10.1016/j.aninu.2015.07.003

Olusegun Oyebade, I., Clyde, H., and Thando Conference, M. (2022). Alternatives to the Use of Antibiotics in Animal Production. In K. Asghar Ali (Ed.), *Antibiotics and Probiotics in Animal Food* (pp. Ch. 4). IntechOpen. https://doi.org/10.5772/intechopen.105922

Pal, M., Berhanu, G., Megersa, L., and Kandi, V. (2020). Epidemiology, Pathogenicity, Animal Infections, Antibiotic Resistance, Public Health Significance, and Economic Impact of Staphylococcus Aureus: A Comprehensive Review. *8*, 14-21. https://doi.org/10.12691/ajphr-8-1-3

Parente, E., Brienza, C., Moles, M., and Ricciardi, A. (1995). A comparison of methods for the measurement of bacteriocin activity. *Journal of Microbiological Methods*, *22*(1), 95-108. https://doi.org/https://doi.org/10.1016/0167-7012(94)00068-I

Piewngam, P., Quiñones, M., Thirakittiwatthana, W., Yungyuen, T., Otto, M., and Kiratisin, P. (2019). Composition of the intestinal microbiota in extended-spectrum β-lactamase-producing Enterobacteriaceae carriers and non-carriers in Thailand. *International Journal of Antimicrobial Agents*, *53*(4), 435-441.

Plaza-Díaz, J., Ruiz-Ojeda, F. J., Vilchez-Padial, L. M., and Gil, A. (2017). Evidence of the Anti-Inflammatory Effects of Probiotics and Synbiotics in Intestinal Chronic Diseases. *Nutrients*, *9*(6). https://doi.org/10.3390/nu9060555

Rossoni, R. D., Fuchs, B. B., de Barros, P. P., Velloso, M. D., Jorge, A. O., Junqueira, J. C., and Mylonakis, E. (2017). Lactobacillus paracasei modulates the immune system of Galleria mellonella and protects against Candida albicans infection. *PLoS One*, *12*(3), e0173332. https://doi.org/10.1371/journal.pone.0173332

Rychlik, I. (2020). Composition and Function of Chicken Gut Microbiota. *Animals (Basel)*, *10*(1). https://doi.org/10.3390/ani10010103

Sabina, F. (2016). Antimicrobial Effect of Probiotics against Common Pathogens. In R. Venketeshwer and G. R. Leticia (Eds.), *Probiotics and Prebiotics in Human Nutrition and Health* (pp. Ch. 10). IntechOpen. https://doi.org/10.5772/63141

Shahbaz, F., Muccee, F., Shahab, A., Safi, S. Z., Alomar, S. Y., and Qadeer, A. (2024). Isolation and in vitro assessment of chicken gut microbes for probiotic potential. *Frontier Microbiology*, *15*, 1278439. https://doi.org/10.3389/fmicb.2024.1278439

Shurson, G. C., Urriola, P. E., and van de Ligt, J. L. G. (2022). Can we effectively manage parasites, prions, and pathogens in the global feed industry to achieve One Health? *Transbound Emerg Disease*, *69*(1), 4-30. https://doi.org/10.1111/tbed.14205

Sikorska, H., and Smoragiewicz, W. (2013). Role of probiotics in the prevention and treatment of meticillin-resistant Staphylococcus aureus infections. *International Journal Antimicrobiology Agents*, *42*(6), 475-481. https://doi.org/10.1016/j.ijantimicag.2013.08.003

Tiwari, R., Chakraborty, S., M, S., Dhama, K., and Singh, S. (2014). Ashwagandha (Withania somnifera): Role in Safeguarding Health, Immunomodulatory Effects, Combating Infections and Therapeutic Applications: A Review. *Journal of Biological Sciences*, *14*, 77-94. https://doi.org/10.3923/jbs.2014.77.94

Varga, C., Guerin, M. T., Brash, M. L., Slavic, D., Boerlin, P., and Susta, L. (2019). Antimicrobial resistance in fecal Escherichia coli and Salmonella enterica isolates: a two-year prospective study of small poultry flocks in Ontario, Canada. *BMC Veterinary Research*, *15*(1), 464. https://doi.org/10.1186/s12917-019-2187-z

Villena, J., Chiba, E., Tomosada, Y., Salva, S., Marranzino, G., Kitazawa, H., and Alvarez, S. (2012). Orally administered Lactobacillus rhamnosus modulates the respiratory immune response triggered by the viral pathogen-associated molecular pattern poly(I:C). *BMC Immunology*, *13*, 53. https://doi.org/10.1186/1471-2172-13-53

Vítor, J. M., and Vale, F. F. (2011). Alternative therapies for Helicobacter pylori: probiotics and phytomedicine. *FEMS Immunology and Medical Microbiology*, *63*(2), 153-164.

Wang, X., Farnell, Y. Z., Kiess, A. S., Peebles, E. D., Wamsley, K. G. S., and Zhai, W. (2019). Effects of Bacillus subtilis and coccidial vaccination on cecal microbial diversity and composition of Eimeria-challenged male broilers. *Poultry Science*, *98*(9), 3839-3849. https://doi.org/10.3382/ps/pez096

Yaqoob, M. U., Wang, G., and Wang, M. (2022). An updated review on probiotics as an alternative of antibiotics in poultry - A review. *Animal Bioscience*, *35*(8), 1109-1120. https://doi.org/10.5713/ab.21.0485

Yu, A.-Q., and Li, L. (2016). The potential role of probiotics in cancer prevention and treatment. *Nutrition and Cancer*, *68*(4), 535-544.

Żbikowska, K., Michalczuk, M., and Dolka, B. (2020). The Use of Bacteriophages in the Poultry Industry. *Animals (Basel)*, *10*(5). https://doi.org/10.3390/ani10050872

Zhao, X., Yu, Z., and Ding, T. (2020). Quorum-sensing regulation of antimicrobial resistance in bacteria. *Microorganisms*, *8*(3), 425.

Chapter 12

Difference between Probiotics and Pre-biotics and its Best Time of use

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ABSTRACT

Probiotics and prebiotics are essential for enhancing gut health and overall wellness. Prebiotics are indigestible fibers that feed good bacteria on the gastrointestinal tract, whereas probiotics are live microorganisms that, when taken in adequate proportions, have been shown to have positive health effects. Prebiotics are plant-based foods that provide food for beneficial microbes in the gut. Examples of these foods include onions, garlic, bananas, and whole grain products. They improve mineral absorption, promote overall gut health, and aid in digestion by promoting the proliferation and activity of these microorganisms. Prebiotics can be ingested at any time of day as long as they are part of a balanced diet; however, they are best taken throughout the day. Probiotics, on the other hand, are live microorganisms that can be found in supplements and dairy products like yogurt, and cabbage. These good bacteria and yeasts colonize the gut, supporting a variety of biological processes, such as immunological response and digestion, and fostering a healthy microbial habitat. While the best time to take probiotics is dependent on the person and stress, some people find that taking them with meals helps them survive the acidic environment of the stomach, while others find that taking them on an empty stomach is beneficial. Maintaining a healthy gut microbiota and general well-being can be greatly improved by knowing the differences between these two entities and how best to use them.

KEYWORDS			
Prebiotics, probiotics, gut health, digestion, beneficial bacteria, fermented foods, microbial environment, dietary fibers, live microorganisms.	Received: 02-Jun-2024 Revised: 23-Jul-2024 Accepted: 02-Aug-2024		A Publication of Unique Scientific Publishers

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INTRODUCTION

In the past ten years, probiotic and prebiotic demand has increased globally. The ignorance of patients and medical professionals on the use of probiotics and prebiotics is a significant problem (Sanders et al., 2019). According to statistics examining hospitalized patients' perspectives, understanding, and usage of probiotics and prebiotics, patients take probiotics (56%) and prebiotics (33%) for health purposes. However, few know the item's proper usage (Betz 2011). The phrase "gut microbiota" describes the community of bacteria that may amount to 100 trillion in the gastrointestinal tract. Studies on the impact of chemicals that may positively modify gut microbiota, such as probiotics and prebiotics, are being carried out due to the possible benefits of the microbiota to host wellbeing. Probiotics and prebiotics may influence immunological response, gastrointestinal absorption and metabolic processes, and cholesterol metabolism, among other gut microbial activities (Gareau et al., 2010). The World Health Organization (WHO) states that probiotics are live bacteria that, when consumed in the right amounts, can help the host. Probiotic efficacy and usefulness are dependent on the specific strain utilized and its ability to survive and colonize the gastrointestinal tract (Cani, 2018). Probiotic use may aid in the treatment of several conditions, such as necrotizing enterocolitis, antibiotic-associated diarrhea, ulcerative colitis, and irritable bowel syndrome, according to new research (Shen et al., 2014).

A prebiotic is a material that has undergone specific fermentation, altering the gut microbiota's composition and/or activity to have the intended effects on the host's health. While there isn't as much data to support prebiotics' advantages for these conditions, those with gastrointestinal disorders may benefit from them the most (Scaldaferri et al., 2013). While some evidence does support the use of probiotics and prebiotics as dietary supplements for specific illnesses, not all studies support their use as supplements (Szajewska et al., 2012). The adaptability and efficiency of the gut microbiota are important characteristics. A healthy gut community assists in immunological control, homeostasis maintenance, and defense of the host against invasive microbes (Coyte et al., 2015). Atopy, metabolic syndrome, colon cancer, and inflammatory intestinal disorders are just a few of the inflammatory, pathogenic, and metabolic illnesses that can be supported by a gut microbiota that is disturbed by dietary changes, antibiotic use, aging, or infection (Walker and Lawley, 2013). The makeup and/or function of the gut microbiota can be altered by several techniques, including intestinal microbiota transplants, the use of probiotics and other live microorganisms, and the administration of non-digestible dietary supplements like prebiotics (Deehan et al., 2017).

Probiotics

Live bacteria known as probiotics enhance the health of the host when given in the right amounts. (Hill et al., 2014). Probiotics contain a wide variety of microorganisms. Therefore, it's critical to keep in mind that they are classified according to their genus, species, and strain names. Using *Lactobacillus rhamnosus,* a well-researched probiotic, as an example. The strain identifier is GG, the genus is *Lactobacillus,* and the species is *Rhamnosus*. It takes all three elements to recognize a probiotic. The reader can connect a particular strain to papers outlining safety evaluations and health advantages by using the complete name. Furthermore, even within the same species, the health benefits of one strain may not translate to another, even while shared processes between strains occasionally lead to comparable clinical results (Ritchie and Romanu, 2012).

Most species of Lactobacillus and Bifidobacterium can produce organic acids, such as lactate and acetate. The digestive system and other organs may gain from the organic acids that colon microorganisms create in several ways. By preventing the growth of dangerous bacteria and promoting the growth of advantageous gut microbes, they significantly contribute to the improvement of the gut environment. Butyrate is produced as a result, and intestinal epithelial cells are powered by it (Sanders et al., 2018).

Depending on the product category and region, probiotic product quality might vary greatly, including dependability and accuracy of the label. The regulatory frameworks controlling the manufacture of probiotics and the conditions for claim substantiation are not yet harmonized globally (O'Toole et al., 2017). A panel of probiotic specialists has been assembled by the US Pharmacopeia (Rockville, Maryland, US) to provide advice and guidelines on quality-related matters, including the recognition, counting, and standards for contaminating bacteria about probiotic dietary supplements (Flach et al., 2018). Probiotics have the potential to significantly impact the gut ecosystem's functioning to enhance health and nutritional condition (Versalovic, 2013).

Source of Probiotics

The principal sources may originate from human sources, including human breast milk or the large or small intestine. It could also come from animal sources or other dietary biotopes, such as fermented foods or raw milk. High levels of adherence to the human intestinal epithelial barrier set apart probiotic strains isolated from human microflora from other strains, perhaps increasing their safety. It has not been demonstrated that certain bacteria and germs found in probiotic-containing dietary goods and supplements are safe for ingestion by humans or other animals (Zommiti et al., 2020). Probiotic dietary supplements and foods contain bacterial strains that may be important participants in the following processes: cholesterol levels and metabolism; colonization in the gastrointestinal, respiratory, and urogenital tracts; lactose metabolism; calcium absorption and vitamin synthesis potential; the reductive potential of yeast and vaginal infection; alleviation of constipation and diarrheal disorders; reduction of gastritis and ulcers; alleviation of acne, rash faces, and skin issues; and the production of natural antimicrobials (Ricci et al., 2017).

Probiotics Action Mechanism

For the past 20 years, probiotic microbe research has progressed substantially, mostly in areas related to probiotic cultures' properties and selection criteria, potential uses, and direct and/or indirect benefits on human health (Carter et al., 2017). Probiotics are vital to the growth of the microflora that lives in the human gastrointestinal tract (GIT) because they maintain homeostasis or the optimum microbial equilibrium surrounded by pathogens and beneficial bacteria. These beneficial bacteria may aid in the native microbiota's recovery from antibiotic therapy by maintaining this equilibrium (Oelschlaeger, 2010). Probiotics also possess the extraordinary capacity to prevent harmful gut bacteria from doing their activities. Probiotics therefore have a great deal of potential to prevent food poisoning because they inhibit the growth of resistant pathogens such as *C. perfringens, Campylobacter jejuni, Salmonella enteritidis, Escherichia coli, multiple species of Shigella, Staphylococcus, Yersinia, Campylobacter coli, and Listeria* sp. (Saint-Cyr et al., 2017).

The research found that the synthesis of antimicrobial compounds, phagocytosis stimulation, resistance to colonization, anti-mutagenic effects, chemokine production, and impacts on enzyme activity and transport are some of the factors that influence the mechanism of action of probiotic bacteria (Zommiti et al., 2017). The basic theory underlying the health benefits of good bacteria, or "probiotics," has also been unraveled through extensive molecular, bioengineered, and genetic studies. These four mechanisms are closely linked to competition with pathogenic bacteria for nutrition and adherence to the epithelium; host immunomodulation; and inhibition of bacterial toxin synthesis. Microbial antagonism is achieved through the use of antimicrobial agents (Zommiti et al., 2018).

Probiotic Role in Gut Barrier Function

The mucus layer, the epithelium lining the mucosal tissues, and the immune cells at the sub-epithelial level make up the mucosal barrier. Therefore, by positively impacting barrier robustness, alteration at any of these levels can alter disease states. At the molecular level, epithelial cells play a major role in mediating the barrier effect (Hyland et al., 2014). They communicate with immune cells underneath and with the body as a whole using signaling chemicals. The gut lumen sends chemical signals to them as well. Many gastrointestinal disorders, including irritable bowel syndrome, viral enterocolitis, celiac disease, and inflammatory bowel disease (IBD), are significantly impacted by the gut barrier (Blaut and Klaus 2012). Therefore, choosing probiotic strains that can fortify the gut barrier seems like a pertinent approach with a wide range of effects on many illnesses. *L. rhamnosus* GG or the probiotic blend may interact directly with intestinal epithelial cells and preserve the integrity of the epithelial barrier, according to several experiments conducted on Caco-2 intestinal cells and animals. The ability of LGG to stay in the GIT has been associated with the in vivo creation of pili with a mucus-binding domain (van Hemert et al., 2013).

If these outcomes are repeatable in vivo, they might aid in maintaining homeostasis and excluding pathogens. Additionally, it shows the different ways that different probiotic strains affect the same tissue, in this case, the epithelium all of which help to maintain the barrier effect. In a therapeutic environment, lactobacillus plantarum is administered to the small intestines of healthy persons. (Lebeer et al., 2012. As a result, there are structural alterations in epithelial tight junctions as well as an increase in the tight junction-specific proteins occludin and zonula occludens-1. The *L. plantarum* strain yielded results that are pertinent to an intervention in the corresponding subjects because several diseases, including IBD, IBS, and celiac disease, are linked to increased intestinal permeability to macromolecules and a loss of tight junction integrity (Liu et al., 2011).

Treatments with different strains and species of *Lactobacillus,* such as *L. plantarum, L. acidophilus, L. casei*, and *L. rhamnosus*, result in different gene-regulatory networks and pathways in the human mucosa. One of these pathways is the up-regulation of IL-1b, an activator of the NF-kB signaling cascade, which may stimulate the transcription of genes linked to B-cell maturation and lymphogenesis, ultimately improving the function of the barrier (Van Baarlen et al., 2011). Differential expression of genes involved in wound healing and repair, angiogenesis, the IFN response, calcium signaling, and ion homeostasis affect the vascularization and feeding of epithelial cells. Furthermore, the changes in transcriptional networks that have been discovered bear resemblance to the responses that bioactive substances and drugs evoke, indicating a novel use of probiotics in conjunction with therapeutic and/or preventive nutritional regimes (Wang et al., 2014).

Security of Probiotics

Probiotic bacteria are generally recognized for their safety, having been granted the World Health Organization's GRAS (Generally Regarded as Safe) designation (WHO). The primary factor in the choosing of probiotics is safety for human health. According to Snydman, probiotic strains should be identified by their low level of antibiotic resistance and absence of virulent character (Hanchi et al., 2018). An update on safety concerns and the probiotic potential of the genus *Enterococcus.* The usage of Lactobacilli and Bifidobacteria strains has historically resulted in an excellent safety record for these helpful germs. There is little field research and experience with other bacteria species that are utilized as probiotics. From a conventional frame of view, host susceptibility is never completely safe. While looking for new potential probiotic microorganisms, it might be challenging to identify novel bacterial strains and even new genera with more specialized traits and/or greater potential for good health. A thorough examination, risk-benefit analysis, and safety assessment are required when introducing novel bacteria. In general, probiotics are safe (Landete, 2017). Care should be taken to prevent any potentially harmful effects, while there have been sporadic instances of bad outcomes. Probiotics may theoretically result in four different kinds of side effects, according to a 2002 report jointly released by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO): (i) systemic infections; (ii) detrimental metabolic activities; (iii) excessive immune stimulation in susceptible individuals; and (vi) potential gene transfer (Bull et al., 2013).

Probiotics are becoming more and more popular, and this growth is due to both the diversity of probiotic products available and the introduction of new strains of probiotics. Future studies and research should give a more thorough description of the probiotic microbe under investigation, including its genus, species, and strain level, as well as the daily dosage and duration of treatment (Zoumpopoulou et al., 2018). The three key stakeholders that need to get over the challenges surrounding probiotics are the general public, healthcare professionals, and probiotic manufacturers. They should focus on worldwide regulations and standards and provide recommendations for strain-specific evidence-based therapy (de Melo Pereira et al., 2018).

Pre-biotics

The prebiotics theory was first put forth by Glenn Gibson and Marcel Roberfroid in 1995. "A non-digestible food ingredient that selectively stimulates the growth and/or activity of one or a limited number of bacteria in the colon, thereby improving host health," according to the definition of prebiotics. For more than fifteen years, this definition stayed mostly unchanged (Trompette et al., 2014). A restricted set of carbohydrate group molecules, including lactulose, GOS, and short- and long-chain β-fructans [FOS and inulin], can be categorized as prebiotics according to this criterion. "A selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" is the definition given to "dietary prebiotics" at the 2008 International Scientific Association of Probiotics and Prebiotics (ISAPP) 6th Meeting (Gibson et al., 2010).

While not all prebiotics originate from carbs, the two following characteristics can be used to differentiate between prebiotics that do and those that don't: Fibers are defined as carbohydrates that have three or more degrees of polymerization (DP), and they can't be hydrolyzed by the small intestine's natural enzymes. Remember that it's not required for the fiber to be soluble or fermentable (Slavin, 2013). Cross-feeding, or when one species eats the products of another, was found to enhance the prebiotic effect in 2013. This suggests that the term "selectivity" used in the definition of prebiotics may not be entirely accurate. Although there is ongoing debate on the precise definition of prebiotics (Hutkins et al., 2016).

Sources of Pre-biotics

Prebiotics are vital for human health. Some dietary food products in which they naturally occur are asparagus, sugar beet, garlic, chicory, onion, Jerusalem artichokes, wheat, honey, bananas, barley, tomatoes, rye, soybeans, human and cow's milk, peas, beans, and, more recently, seaweeds and microalgae (Al-Sheraji et al., 2013). Because of their poor food content, they are made industrially. The main components utilized to create prebiotics include lactose, sucrose, and starch. The industrial synthesis of prebiotics has been the subject of numerous pertinent studies, the majority of which fall into the GOS and FOS categories (Varzakas et al., 2018).

Mechanism of Pre-biotics:

Prebiotics alter the makeup and activity of the gut microbiota by giving these microbes sources of energy. Phylogeny indicates that distant bacterial species can frequently ingest a certain prebiotic. Additionally, a functional metagenomics technique was recently published on it. This technique identifies genes from a human microbiota metagenomic collection that break down various forms of prebiotics using a heterologous host, such as *Escherichia coli* (Cecchini et al., 2013). Several species' clones are capable of fermenting FOS, GOS, and xylooligosaccharides (XOS). Among these organisms are Firmicutes, Bacteroidetes, and Actinobacteria. However, some research indicates that a certain prebiotic might be broken down by a particular species.

Two examples of this include the fermentation of fructans and starch by *Bifidobacterium* spp. (Ze et al., 2012). Chain length is another key characteristic that helps toidentify species that can ferment a certain prebiotic. Cross-feeding occurs when complex prebiotic ferments and produces a byproduct that serves as a substrate for another microbe. Resistance starches can be broken down by *Ruminococcus bromii*, and the fermentation products that are produced can be utilized by several species (Scott et al., 2013). Conversely, certain items might negatively impact other species. Prebiotics also can change the environment in the stomach. As mentioned previously, the main products of prebiotic fermentation are acids, which cause the stomach's pH to decrease. Research has demonstrated that a single unit shift in the gut pH from 6.5 to 5.5 can alter the quantity and makeup of the gut microbiota. Changes in pH can affect the populations of species that are sensitive to acid, like Bacteroids, and can also encourage Firmicutes to produce butyrate. The "butyrogenic effect" is the name given to this mechanism (Scott et al., 2014).

Safety of Pre-biotics

Prebiotics are thought to have no negative or potentially lethal side effects. Both polysaccharides and oligosaccharides are indigestible to intestinal enzymes. The gut bacteria carry them to the colon, where fermentation takes place. Thus, the primary reason why prebiotics have adverse consequences is because of their osmotic actions. In this instance, prebiotic users may experience bloating, cramps, gas, and osmotic diarrhea as adverse effects (Svensson and Håkansson 2014). One factor that affects how their detrimental effects appear is how long the prebiotic chain is. It's noteworthy to notice that shorter chain-length prebiotics can be more detrimental. One reason for this phenomenon could be that longer-chain inulin molecules ferment later and more slowly in the distal colon, while shorter-chain inulin molecules are mainly digested and ferment more quickly in the proximal colon (Davani-Davari et al., 2019).

Its safety profile can be influenced by the prebiotic dose in addition to chain length. For instance, osmotic diarrhea and flatulence may result from large dosages of prebiotics (40–50 g/day) and low dosages (2.5–10 g/day), respectively. Note that for prebiotics to be helpful to human health, a daily consumption of 2.5–10 g is necessary. This implies that prebiotics may have mild to moderate adverse effects if used as directed. The majority of prebiotic products on the market contain 1.5–5 g of probiotics per serving (Garg et al., 2018). As potential replacements or additional therapies (synbiotics), prebiotics may provide similar safety concerns as probiotics. Particularly in those with severe malnutrition, a compromised intestinal epithelial barrier (such as severe diarrhea or NEC), or a marked immunodeficiency (e.g., HIV, cancer, transplant). Prebiotics provides a high safety risk of bacteremia, sepsis, or endocarditis. Surprisingly, relevant clinical trials that have solely examined prebiotics have not considered or at least not recorded these potential adverse effects (Tsai et al., 2019).

Difference between Prebiotics and Probiotics

Probiotics are live bacteria or yeast that are consumed in large enough amounts to provide health benefits. These microorganisms naturally resemble the beneficial bacteria present in the human stomach. Probiotics boost immunity, facilitate better digestion, enhance nutritional absorption, colonize the stomach maintain a balance of healthy bacteria, and even promote mental health. Probiotics are frequently found in foods including several yogurt varieties, kefir, sauerkraut, kimchi, miso, tempeh, and other dietary supplements (Quigley, 2019).

Prebiotics are indigestible fibers or substances that provide nourishment for probiotics and other advantageous microorganisms in the gastrointestinal tract. Unlike probiotics, which are real living organisms, prebiotics are substances that promote the growth and activity of helpful bacteria in the stomach. Prebiotics are transported undigested down the digestive tract to the colon, where the local bacteria ferment them. During this fermentation process, short-chain fatty acids are created, including butyrate, acetate, and propionate, which provide energy to the lining cells of the colon and maintain the health of the gut environment. Along with other fruits and vegetables, whole grains, legumes, apples, bananas, onions, garlic, and various nutritional supplements are common sources of prebiotics. Galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), inulin, and other prebiotic fibers (Brüssow, 2019).

Best Time of use:

By protecting the bacteria from stomach acid and bile salts, probiotics eaten with food increase the likelihood that the bacteria will enter the intestines alive and begin to function. Oftentimes, timing consistency matters more than the precise time of day. Choose a time that works for your schedule and stick to it each time if you want to get the most out of it (Gu and Roberts, 2019).

By taking prebiotics before meals, you can encourage the growth of beneficial bacteria in your stomach and enhance their fermentation process during digestion. Many different foods, such as whole grains, fruits, and vegetables, contain prebiotics (Cunningham et al., 2021).

Conclusion

In conclusion, probiotics and prebiotics are equally important for maintaining gut health, yet they function differently and offer distinct advantages. Live bacteria known as probiotics can improve gut microbiota balance and replenishment when taken in sufficient quantities. They can strengthen the immune system, promote better digestion, increase nutritional absorption, and enhance general well-being. Probiotics work best when taken with meals, right before bed, or right after taking antibiotics. Indigestible fibers or compounds known as prebiotics nourish the beneficial bacteria in the stomach. By promoting the development and activity of probiotics and other beneficial bacteria, they maintain a healthy gut environment. Prebiotics can be consumed throughout the day by eating foods high in prebiotics, or they can be taken before meals. Probiotics and prebiotics can both offer complete support for gut health and general well-being when added to your routine. Timing and consumption must be consistent, but the ideal strategy may vary depending on personal tastes and lifestyle choices. Speaking with a healthcare provider can provide tailored advice on the best ways to use probiotics and prebiotics, especially for those with certain health issues or illnesses.

REFERENCES

Al-Sheraji, S. H., Ismail, A., Manap, M. Y., Mustafa, S., Yusof, R. M., and Hassan, F. A. (2013). Prebiotics as functional foods: A review. *Journal of Functional Foods*, *5*(4), 1542-1553.

Betz, M., (2011). Knowledge, Use, and Perceptions of Probiotics and Prebiotics in Rush University Medical Center Patients [MS thesis]. Chicago, IL: Rush Medical Center; 2011

Blaut, M., and Klaus, S. (2012). Intestinal microbiota and obesity. *Appetite Control*, 251-273.

Brüssow, H. (2019). Probiotics and prebiotics in clinical tests: an update. *F1000Research*, *8*.

Bull, M., Plummer, S., Marchesi, J., and Mahenthiralingam, E. (2013). The life history of Lactobacillus acidophilus as a probiotic: a tale of revisionary taxonomy, misidentification and commercial success. *FEMS Microbiology Letters*, *349*(2), 77-87.

Cani, P. D. (2018). Human gut microbiome: hopes, threats and promises. *Gut*, *67*(9), 1716-1725.Cani, P. D. (2018). Human gut microbiome: hopes, threats and promises. *Gut*, *67*(9), 1716-1725.

Carter, A., Adams, M., La Ragione, R. M., and Woodward, M. J. (2017). Colonisation of poultry by Salmonella Enteritidis S1400 is reduced by combined administration of Lactobacillus salivarius 59 and Enterococcus faecium PXN-33. *Veterinary Microbiology*, *199*, 100-107.

Cecchini, D. A., Laville, E., Laguerre, S., Robe, P., Leclerc, M., Dore, J., and Potocki-Veronese, G. (2013). Functional metagenomics reveals novel pathways of prebiotic breakdown by human gut bacteria. *PloS one*, *8*(9), e72766.

Coyte, K. Z., Schluter, J., and Foster, K. R. (2015). The ecology of the microbiome: networks, competition, and stability. *Science*, *350*(6261), 663-666.

Cunningham, M., Azcarate-Peril, M. A., Barnard, A., Benoit, V., Grimaldi, R., Guyonnet, D., and Gibson, G. R. (2021). Shaping the future of probiotics and prebiotics. *Trends in Microbiology*, *29*(8), 667-685.

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J., and Ghasemi, Y. (2019). Prebiotics: definition, types, sources, mechanisms, and clinical applications. *Foods*, *8*(3), 92.

de Melo Pereira, G. V., de Oliveira Coelho, B., Júnior, A. I. M., Thomaz-Soccol, V., and Soccol, C. R. (2018). How to select a probiotic? A review and update of methods and criteria. *Biotechnology Advances*, *36*(8), 2060-2076.

Deehan, E. C., Duar, R. M., Armet, A. M., Perez-Muñoz, M. E., Jin, M., and Walter, J. (2017). Modulation of the gastrointestinal microbiome with nondigestible fermentable carbohydrates to improve human health. *Microbiology Spectrum*, *5*(5), 10-1128.

Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., and Fernández Escámez, P. S. (2017). EFSA Panel on Biological Hazards (BIOHAZ), Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. *EFSA Journal*, *15*(3), e04664.

Flach, J., van der Waal, M. B., van den Nieuwboer, M., Claassen, E., and Larsen, O. F. (2018). The underexposed role of food matrices in probiotic products: Reviewing the relationship between carrier matrices and product parameters. *Critical Reviews in Food Science and Nutrition*, *58*(15), 2570-2584.

Gareau, M. G., Sherman, P. M., and Walker, W. A. (2010). Probiotics and the gut microbiota in intestinal health and disease. *Nature Reviews Gastroenterology and Hepatology*, *7*(9), 503-514.

Garg, B. D., Balasubramanian, H., and Kabra, N. S. (2018). Physiological effects of prebiotics and its role in prevention of necrotizing enterocolitis in preterm neonates. *The Journal of Maternal-Fetal and Neonatal Medicine*, *31*(15), 2071-2078.

Gibson, G. R., Scott, K. P., Rastall, R. A., Tuohy, K. M., Hotchkiss, A., Dubert-Ferrandon, A., and Buddington, R. (2010). Dietary prebiotics: current status and new definition. *Food Science Technology Bull. Function Foods*, *7*(1), 1-19.

Gu, J., and Roberts, K. (2019). Probiotics and prebiotics. In *Adult Short Bowel Syndrome* (pp. 67-80). Academic Press.

Hanchi, H., Mottawea, W., Sebei, K., and Hammami, R. (2018). The genus Enterococcus: between probiotic potential and safety concerns—an update. *Frontiers in Microbiology*, *9*, 346489.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., and Sanders, M. E. (2014). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature reviews Gastroenterology and Hepatology*

Hutkins, R. W., Krumbeck, J. A., Bindels, L. B., Cani, P. D., Fahey Jr, G., Goh, Y. J., and Sanders, M. E. (2016). Prebiotics: why definitions matter. *Current Opinion in Biotechnology*, *37*, 1-7.

Hyland, N. P., Quigley, E. M., and Brint, E. (2014). Microbiota-host interactions in irritable bowel syndrome: epithelial barrier, immune regulation and brain-gut interactions. *World Journal of Gastroenterology: WJG*, *20*(27), 8859.

Landete, J. M. (2017). A review of food-grade vectors in lactic acid bacteria: from the laboratory to their application. *Critical Reviews in Biotechnology*, *37*(3), 296-308.

Lebeer, S., Claes, I., Tytgat, H. L., Verhoeven, T. L., Marien, E., von Ossowski, I., and Vanderleyden, J. (2012). Functional analysis of Lactobacillus rhamnosus GG pili in relation to adhesion and immunomodulatory interactions with intestinal epithelial cells. *Applied and Environmental Microbiology*, *78*(1), 185-193.

Liu, Z., Qin, H., Yang, Z., Xia, Y., Liu, W., Yang, J., and Zheng, Q. (2011). Randomised clinical trial: the effects of perioperative probiotic treatment on barrier function and post-operative infectious complications in colorectal cancer surgery–a double-blind study. *Alimentary Pharmacology and Therapeutics*, *33*(1), 50-63.

O'Toole, P. W., Marchesi, J. R., and Hill, C. (2017). Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nature Microbiology*, *2*(5), 1-6.

Oelschlaeger, T. A. (2010). Mechanisms of probiotic actions–a review. *International Journal of Medical Microbiology*, *300*(1), 57-62.

Quigley, E. M. (2019). Prebiotics and probiotics in digestive health. *Clinical Gastroenterology and Hepatology*, *17*(2), 333-344.

Ritchie, M. L., and Romanuk, T. N. (2012). A meta-analysis of probiotic efficacy for gastrointestinal diseases. *PloS one*, *7*(4), e34938.

Saint-Cyr, M. J., Haddad, N., Taminiau, B., Poezevara, T., Quesne, S., Amelot, M., and Guyard-Nicodème, M. (2017). Use of the potential probiotic strain Lactobacillus salivarius SMXD51 to control Campylobacter jejuni in broilers. *International Journal of Food Microbiology*, *247*, 9-17.

Sanders, M. E., Benson, A., Lebeer, S., Merenstein, D. J., and Klaenhammer, T. R. (2018). Shared mechanisms among probiotic taxa: implications for general probiotic claims. *Current Opinion in Biotechnology*, *49*, 207-216.

Sanders, M. E., Merenstein, D. J., Reid, G., Gibson, G. R., and Rastall, R. A. (2019). Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. *Nature Reviews Gastroenterology and Hepatology*, *16*(10), 605-616.

Scaldaferri, F., Gerardi, V., Lopetuso, L. R., Del Zompo, F., Mangiola, F., Boškoski, I., and Gasbarrini, A. (2013). Gut microbial flora, prebiotics, and probiotics in IBD: their current usage and utility. *BioMed Research International*, *2013*.

Scott, K. P., Gratz, S. W., Sheridan, P. O., Flint, H. J., and Duncan, S. H. (2013). The influence of diet on the gut microbiota. *Pharmacological Research*, *69*(1), 52-60.

Scott, K. P., Martin, J. C., Duncan, S. H., and Flint, H. J. (2014). Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, in vitro. *FEMS Microbiology Ecology*, *87*(1), 30-40.

Shen, J., Zuo, Z. X., and Mao, A. P. (2014). Effect of probiotics on inducing remission and maintaining therapy in ulcerative colitis, Crohn's disease, and pouchitis: meta-analysis of randomized controlled trials. *Inflammatory Bowel Diseases*, *20*(1), 21-35.

Slavin, J. (2013). Fiber and prebiotics: mechanisms and health benefits. *Nutrients*, *5*(4), 1417-1435.

Svensson, U. K., and Håkansson, J. (2014). Safety of food and beverages: safety of probiotics and prebiotics.

Szajewska, H., Weizman, Z., Abu-Zekry, M., Kekez, A. J., Braegger, C. P., Kolacek, S., and Vukavic, T. (2012). Inulin and fructo-oligosaccharides for the prevention of antibiotic-associated diarrhea in children: report by the ESPGHAN Working Group on Probiotics and Prebiotics. *Journal of Pediatric Gastroenterology and Nutrition*, *54*(6), 828-829.

Trompette, A., Gollwitzer, E. S., Yadava, K., Sichelstiel, A. K., Sprenger, N., Ngom-Bru, C., and Marsland, B. J. (2014). Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nature Medicine*, *20*(2), 159-166.

Tsai, Y. L., Lin, T. L., Chang, C. J., Wu, T. R., Lai, W. F., Lu, C. C., and Lai, H. C. (2019). Probiotics, prebiotics and amelioration of diseases. *Journal of Biomedical Science*, *26*, 1-8.

van Baarlen, P., Troost, F., van der Meer, C., Hooiveld, G., Boekschoten, M., Brummer, R. J., and Kleerebezem, M. (2011). Human mucosal in vivo transcriptome responses to three lactobacilli indicate how probiotics may modulate human cellular pathways. *Proceedings of the National Academy of Sciences*, *108*(supplement_1), 4562-4569.

van Hemert, S., Verwer, J., and Schütz, B. (2013). Clinical studies evaluating effects of probiotics on parameters of intestinal barrier function.

Varzakas, T., Kandylis, P., Dimitrellou, D., Salamoura, C., Zakynthinos, G., andProestos, C. (2018). Innovative and fortified food: Probiotics, prebiotics, GMOs, and superfood. In *Preparation and processing of religious and cultural foods* (pp. 67-129). Woodhead Publishing.

Versalovic, J. (2013). The human microbiome and probiotics: implications for pediatrics. *Annals of Nutrition and Metabolism*, *63*(Suppl. 2), 42-52.

Walker, A. W., and Lawley, T. D. (2013). Therapeutic modulation of intestinal dysbiosis. *Pharmacological Research*, *69*(1), 75-86.

Wang, H., Gong, J., Wang, W., Long, Y., Fu, X., Fu, Y., and Hou, X. (2014). Are there any different effects of Bifidobacterium, Lactobacillus and Streptococcus on intestinal sensation, barrier function and intestinal immunity in PI-IBS mouse model?. *PLoS One*, *9*(3), e90153.

Ze, X., Duncan, S. H., Louis, P., and Flint, H. J. (2012). Ruminococcusbromii is a keystone species for the degradation of resistant starch in the human colon. *The ISME Journal*, *6*(8), 1535-1543.

Zommiti, M., Bouffartigues, E., Maillot, O., Barreau, M., Szunerits, S., Feuilloley, M., and Ferchichi, M. (2018). In vitro assessment of the probiotic properties and bacteriocinogenic potential of Pediococcuspentosaceus MZF16 isolated from artisanal Tunisian meat "Dried Ossban". *Frontiers in Microbiology*, *9*, 416531.

Zommiti, M., Connil, N., Hamida, J. B., and Ferchichi, M. (2017). Probiotic characteristics of Lactobacillus curvatus DN317, a strain isolated from chicken ceca. *Probiotics and Antimicrobial Proteins*, *9*, 415-424.

Zommiti, M., Feuilloley, M. G., and Connil, N. (2020). Update of probiotics in human world: a nonstop source of benefactions till the end of time. *Microorganisms*, *8*(12), 1907.

Zoumpopoulou, G., Kazou, M., Alexandraki, V., Angelopoulou, A., Papadimitriou, K., Pot, B., and Tsakalidou, E. (2018). Probiotics and prebiotics: an overview on recent trends. *Probiotics and Prebiotics in Animal Health and Food Safety*, 1-34

Chapter 13

Gut Health in Avian Coccidiosis and its Prevention using Probiotics

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ABSTRACT

The poultry industry as a key player in food industry saw tremendous growth since its establishment. However, this growth was not free from various types of type's challenges including diseases and management of birds. The challenge of controlling and eradicating diseases mainly focused on bacterial and viral diseases for most of the time. Later on, several other types of ailments were also observed among poultry birds. The most prominent out these was the problem of coccidiosis among birds. Coccidiosis is a disease marked by bloody diarrhea, loose droppings and is caused by protozoan species of *Eimeria*. Coccidiosis markedly affected the production of birds led to severe economic losses as it directly affected the GIT tract. Various types of drugs were introduced for treating coccidiosis. At later stages these drugs became ineffective as resistance against them developed in the pathogens. This trend soon led rise of consideration among researchers to find medicinal alternatives against coccidiosis beyond traditional drugs.

KEYWORDS		
Eimeria, Coccidiosis, Poultry industry, Pathogens, Protozoan, Economics losses	Received: 14-May-2024 Revised: 17-July-2024 Accepted: 08-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

One of the key protozoan ailments in poultry is coccidiosis. Coccidiosis is reported to occur due to the infection of the *Eimeria* genus including a number of notable species ranging up to seven (Blake and Tomley, 2014; Abbas et al., 2019; Khater et al., 2020). In terms of pathology the infection severity depends on the site of infection in the alimentary tract and the impact of infection on the efficiency of the host's immune response. The effect of infection is predominantly seen locally on the immune response state of the intestine (Alnassan et al., 2014; Ali et al., 2015). Parasitic infestation can be seen in the lumen of the intestine of the host. Their presence in the intestine leads to sloughing of intestinal epithelial cells. Sloughing of epithelial cells in turn results in impairment of nutrient absorption from the intestinal lumen. Lack of nutrients reaching body cells can consequently induce diarrhea, weight loss and weakness (Bozkurt et al., 2013; Bachaya et al., 2015; Abbas et al., 2017a; Abdel-Saeed and Salem, 2019). Studies conducted by some researchers in the past estimated (Peek and Landman, 2011; Seddiek, 2015), that USD 3 billion were lost due to coccidial infections in the poultry industry all across the globe. The utilization of drugs used against coccidiosis as feed additives or feed supplements in drinking water became a regular habit for the farmers to treat and save their birds from avian coccidiosis (Lillehoj et al., 2008; Arczewska-Włosek and Świątkiewicz, 2013). This strategy that was useful for some time turned out to be even more damaging than the disease itself as the frequent use of coccidiostats lead to the rise of resistant strains against anticoccidial drugs among *Eimeria* species. This problem was global as these drugs were available all over the world and were being commonly used by farmers (Abbas et al., 2019; Zhang et al., 2020). A few steps were taken to replace the commonly used anticoccidial drugs, most effective of them being use of vaccines (Khater et al., 2020), utilizing extracts drive from plants and essential oils (Idris et al., 2017; Abbas et al., 2017b), antioxidant species (Markowiak and Śliżewska, 2017), probiotic chemicals (Ritzi et al., 2014) and prebiotic compounds (Hutsko et al., 2016). Vaccination takes the central stage in terms of controlling a disease like coccidiosis effectively controlled (Dalloul et al., 2005). Vaccines also enhance the health status of birds by improving their overall immunity strength against parasites like the *Eimeria*. Although, there is also a chance that if a flock is poorly managed the administration of live vaccines may lead to severe reactions that can ultimately lead to coccidiosis becoming an outbreak (Chapman, 2000). On the other hand, the high production costs may make the attenuated vaccines seem like a less attractive alternative but they also have lower chances of starting a reaction outbreak among birds (Sharman et al., 2010). Essential oils are another useful alternative that can have a serious impact on the viability of coccidiosis in poultry flocks, hence effectively controlling the disease (Christaki et al., 2012; Idris et al., 2017). The catch with use of essential oils is that they may induce cytotoxic effects of the cells leading to destruction of cell membrane of the birds (Christaki et al., 2004). Another approach considered best for control of enteric problems is the use of probiotics that can help in treating various maladies including coccidial infections (Christaki et al., 2004; Eckert et al., 2010; Ritzi et al., 2014). Probiotics contain live microorganisms, that are useful for gut health and help in maintaining the populations of intestinal microbes that are essential for proper functioning of intestines (Ohimain and Ofongo, 2012; Abdou et al., 2019; Mousa and Marwan, 2019; Sarwar et al., 2019). Probiotics can be utilized effectively in various ways against coccidiosis infection among poultry birds. Some of the effect aspects for use of probiotics include immunomodulation (Ritzi, 2015), antioxidant effect (Wang et al., 2017), reduced shedding of oocyst, lowered number of lesion (Ritzi et al., 2014) and enhanced intestinal health (Sen et al., 2012).

Probiotics

The term prebiotic were first used by two scientists named Gibson and Roberfroid in year 1995 (Kechagia et al., 2013). For the production of prebiotic, several species of bacteria are used, for example *Lactobacillus* (Fioramonti et al., 2003; Markowiak and Śliżewska, 2017). The mechanism of action of prebiotic has not been explained completely, how they work as immunity stimulating agents and how they help to enhance the phagocytic activity (McNaught and MacFie, 2001; Guarner and Malagelada, 2003). The mode of action of probiotics has been divided into three classes:

1. Stimulating the immune response of the host hence enabling the skill of survival against diseases.
2. Actively in reducing the infection level through creation of competition against the pathogenic agents in epithelium cells of the intestine while also participating in the activities that help in restoring balance of beneficial microbes in intestinal tract.
3. Detoxification of the toxic substances residing in the GIT lumen, improving the overall metabolism in body metabolism through microbial action (Oelschlaeger, 2010).

There are several possibilities through which probiotics can approach and act against pathogenic agents whose infection produces disease. The mode of actions for resisting against pathogens include:

1. Activating biological molecule production through probiotics like bacteriocins, oxygen peroxide, antibiotics, free fatty acids having antibacterial properties,
2. Regulation of the environment in intestine (nutrients, pH, enhancing state of beneficial intestinal microbiota, simulating immunity, reducing inflammation activity, increased presence of epithelial cell receptors,
3. Regulating the immune system by maturation of dendritic cells to Th1 and Th2 or Tregs (Regulatory T cells) lymphocytes, consequently cytokines induction and activation of immune response of humoral nature via production of IgA, IgG and IgM,
4. Working as antioxidation agent by reducing the number of reactive oxygen species produced by enzymes, chelating metal ions, stimulation of production of antioxidases. These include catalase and superoxide dismutase, improving formation of antioxidation metabolic agent like folate and GSH (glutathione), increasing productive capacity for other probiotics while also having a positive impact on the physicochemical environment of the host's intestine. A key role can be played by probiotic substances for the regulation of cell signaling pathways such as Protein Kinase C, which consequently lead to the significant role of probiotics as antioxidant agents (Wang et al., 2017; Azad et al., 2018).

Therapeutic Effect

Probiotics containing live microbes help in maintaining the number and performance of the intestinal microflora. The use of antibacterial drugs comes with various adverse effects such as diarrhea is usually cause when antibacterial drugs are used against pathogenic bacteria. The probiotics have very excellent effects when used against diarrhea which usually caused by pathogenic agents like bacteria as these probiotics know to exhibit therapeutic effects and produced bacteriocins to fight against bacteria (Moslehi-Jenabian et al., 2011; Carter et al., 2016). Scientists have been working to find the true nature of probiotics and they revealed that probiotics have curative and therapeutic characteristics to treat coccidiosis in poultry. The probiotics give better results as an alternative of synthetic drugs used to treat coccidial infections. Probiotics containing multiple bacterial species for example PoultryStar® have been used by (Ritzi et al., 2014) in chickens feed at a dose rate of (1 g/kg) and obtain best results. The best outcomes of PoultryStar® includes decrease in number of oocysts and less lesions in birds having mixed *Eimeria* species infections. Another probiotic supplementation containing multiple bacterial species named ProLive® has been used against *E. tenella* in an experimental study. In this study, water infected with 1.1 × 10 11 (CFU) live microbes was administered to the chickens. The impact of probiotics was checked in terms of feed conversion ratio and the health of the gut. The result of the study reveals that probiotics have beneficial effects on the FCR and health of the gut as compared to the synthetic antibacterial drugs such as salinomycin (ERDOĞMUŞ et al., 2019). Another evaluation study has been done to find out the therapeutic effects of Primalac®

(probiotic) and Fermacto® (prebiotic) at dose rate (1g/kg) against coccidial infections in broiler chickens. The evaluation was based on lesion score and number of oocysts in the fecal samples. The results show that the birds treated with probiotics has less lesion score and oocysts count in comparison with salinomycin. So it is concluded that the probiotic supplementation improves the performance of the bird against mixed infection of *Eimeria* in poultry birds (Behnamifar et al., 2019). The characteristic immune-regulatory and therapeutic effects of *Lactobacillus* and *Saccharomyces* based probiotics were used experimentally against *Eimeria* species. These probiotics were used for their actions in enhancing immunity responses by increasing antibody titers and proliferation of lymphocytes to enhance their responses. These probiotics done many other functions including lowering the lesion score, decreasing the fatality rate and increased number of oocysts in fecal samples in the chickens infected with *Eimeria* especially in broiler chickens (Awais et al., 2019). Another probiotics supplementation named Mitomax® has been used in poultry birds experimentally against infections of *Eimeria* which results in reduction of oocysts number and increased antibody count (Lee et al., 2007a). Another probiotic product called as MitoGrow® also gives similar results of increased antibody production level against *Eimeria* infections in broiler chickens (Lee et al., 2007b). The continous used of PoultryStar® probiotic and vaccine (Immucox) against coccidiosis in poultry birds help in improving the health and performance of the bird also protect the birds from leading infections of different *Eimeria* species (Ritzi et al., 2016). In a recent research study, a probiotic containing four different strains of lactic acid bacteria were used in broiler chickens which helps in decreasing intestinal ulcers caused by *E. tenella* (Chen et al., 2016).

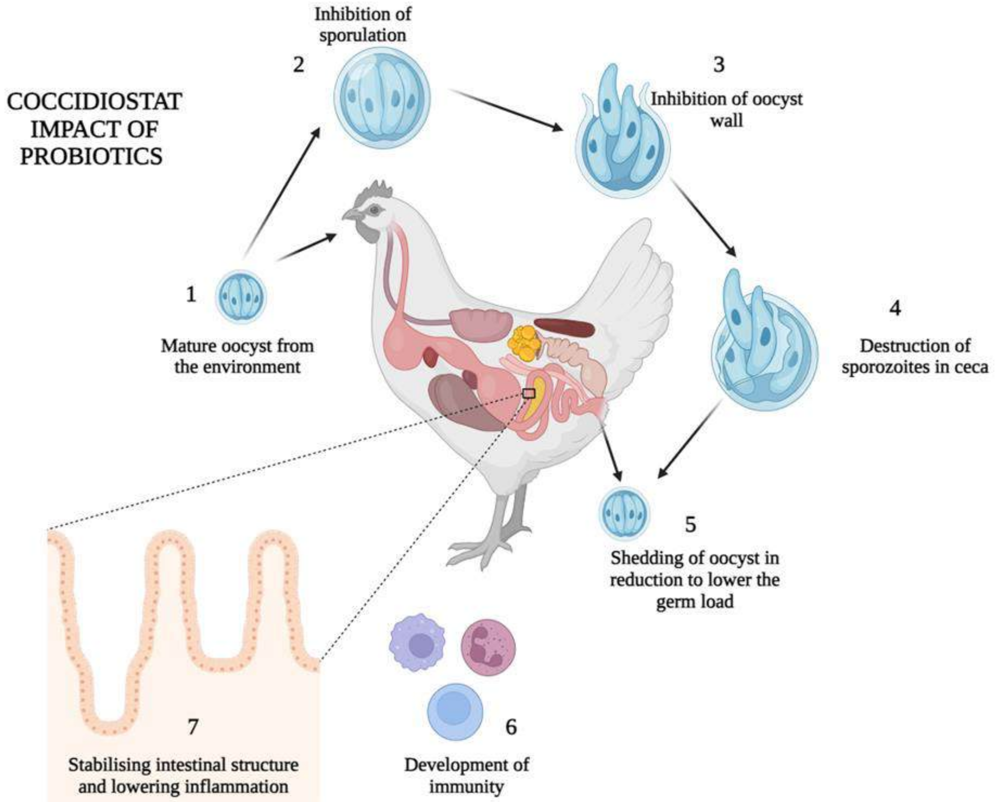

Fig. 1: Coccidiostatic impact of probiotics.

Antioxidant Effect

In the mechanism of poultry coccidiosis, free radical formation results in the necrosis of the enteric tissue. In the chicken infected with the coccidiosis, the oxygen species of reactive nature (ROS) causes cytotoxicity and alterations in the enteric route (Georgieva et al., 2011). The unregulated production of oxygen as a result of oxidative stress causes serious damage to the de-oxy ribonucleic acids, fat and amino acid products. A number of different ROS production for example radical of hydroxyl, hydrogen peroxide and superoxide anion radicals damage the host body at the level going deepest to the individual cells (Abbas et al., 2013). ROS are of two types, exogenous and endogenous. Exogenous ROS are produced due to some external factors for example ultraviolet light, ionizing radiation, cytokines and pathogens but endogenous ROS are produced inside the body of organisms including Cytochrome p450 and NADPH (Wang et al., 2017). Probiotics play a major role in treating poultry coccidiosis as they act as antioxidants and decrease the oxidative streak by triggering the antioxidant system of the host body (Wang et al., 2017). Antioxidant nature of probiotics can be observed in many ways which include formulation of various enzymes with antioxidant properties. Examples of such enzymes include antioxidant enzymes (superoxide dismutase (SOD)), and through metabolism products. The role of probiotic chemicals as antioxidants has a vital part in treating several intestinal infections such as poultry coccidiosis as probiotics also possess therapeutic action (Georgieva et al., 2011). Acting as antioxidants, probiotic compounds have a very desirable effect on the performance of the poultry birds as they help in fighting against coccidiosis and maintain the gut environment of the birds (Azad et al., 2018). By improving the antioxidants status of the host body, probiotics help in improving the healthy state of the poultry birds. The mechanism of activity of probiotics is explained as improvement of intestinal health by enhancing the antioxidants capacity, decreasing the pH level of the gut, triggering the immune responses associated with intestinal tract and stimulation of intraepithelial lymphocytes. Many research studies proved the role of probiotics as antioxidants, inhibition of excessive production of ROS to stop destruction of cell (Wang et al., 2017).

Immunomodulator Effect

The immunity mechanism of birds is classified into two types: innate and adaptive immune systems. In the innate immunity system, the immune response happens by the action of the immune system immediately after an invading body or cell gets entry in the body of the bird, without any specific mechanism of action, immediate protection is provided to the host. The innate immune responses occurred before the adaptive immune responses and the innate immunity reaction have a crucial part in protection of chickens through different mechanics including complement component system, gastric secretions, and phagocytosis (by engulfing the foreign body of cell or bacteria). The heterophils in chickens play the same role as neutrophils in the mammalian body. The heterophils provided the first line of defense (innate immunity) through the mechanism of degranulation and oxidative burst. It has been observed that the chicken feeding with probiotics feed supplementation shows heterophilic activity for example chain of oxidation reactions or granule removal process (Stringfellow et al., 2011). During another research it has been proved that the birds supplemented with Poultrystar® (probiotics supplementation) show oxidative bursts of heterophils (Stringfellow et al., 2011). As soon as the bird gets infected with coccidia infection, it triggers the adaptive immune responses which decrease the number of parasites in the body through the production of antibodies. It has been proved that the birds with probiotics addition to feed have increased the number of antibodies produced including IgG, Ig A, IgM against several infections and health problems (Ritzi, 2015). The probiotic supplementation enhances the number of immunoglobulin against *E. acervulina* (Lee et al., 2007a; Lee et al., 2007b). The mode of action of antibodies in protecting the host against microorganisms is still not clear but humoral immune responses helps in decreasing the number of pathogens not removing from the body (Dalloul et al., 2005; Ali et al., 2015). Not only the humoral immune responses, probiotics also helps in enhancing the cellular immune responses by improving the rate of proliferation of lymphocytes at the site of infections at the end protecting the body of host from coccidiosis (Dalloul et al., 2005). Probiotics help in enhancing the number of lymphocytes at the intestinal epithelium cells and play an important role in improving cellular immune responses. Poultry birds having *E. acervulina* infections, have different levels of cytokines due to the different rates of doses of probiotics and strains utilized (Dalloul et al., 2005).

Anticoccidials Effect

The mechanism of action against poultry coccidiosis is difficult to explain as there are a number of biological actions involved. These biological actions are controlled by genes, in some cases many genes producing small effects or fewer genes exhibiting greatest (Lee et al., 2007a). To control the tissue damage and improve the health conditions of the chickens, genes and biological pathways play a significant role and also provide protection against *E. maxima* infection. Probiotics helps in enhancing the performance of antibody and act as antioxidants, which results in decreased number of oocysts in poultry droppings while the bird is infected with *E. acervulina* and *E. tenella* (Dalloul et al., 2005; Lee et al., 2007a; Lee et al., 2007b). The use of probiotics with Bacillus species helps in decreasing the rate of infection of *E. maxima* in chickens. To diagnose the degree of infection, lesion scoring is the best parameter as less number of lesions indicates the higher possibility of recovering in infected chickens (Ritzi et al., 2014). Chickens supplemented with Bacillus species containing probiotics show fewer lesion scores in case of *E. maxima* infection (Lee et al., 2007b).

Gut Health and Performance

The major symptom of *Eimeria* is stunted growth which results in less feed utilization and lowered rate in weight gain which causes major economic losses (Dalloul et al., 2005). The obvious signs of coccidial infection in poultry is decreased body weight and weight gain due to the huge damage to the intestinal epithelial which causes malabsorption of nutrients ultimately resulting in poor performance. Use of probiotics helps in improving the intestinal health by controlling the damage due to disease through the stimulation of local immune responses (Dalloul et al., 2005). Use of probiotics containing Bacillus subtilis species helps in enhancing the growth and size of intestinal villi and cells of gut in chickens (Markowiak and Śliżewska, 2017). Increased size of villi result in increased absorption of nutrients and intestinal crypts helps in generation and replacement of cells as a result of any intestinal infection (Awais et al., 2019). The consumption of *Lactobacillus*-based probiotics have been observed to enhance the number of intraepithelial lymphocytes in the intestine which helps in protecting the birds against coccidiosis. Chickens infected with *Eimeria acervulina* were fed with probiotics which afterwards observed that improve the local immune responses by the alterations of lymphocytes subpopulations which helps in reducing the number of oocysts in droppings (Dalloul et al., 2005). Many studies proved that the probiotics help in enhancing the health aspects of chickens which includes balancing the intestinal microflora, increased weight gain, carcass yield and feed conversion. There are some evidences that shows that probiotics have no significant effect on birds infected with *Eimeria* parasites (Ritzi et al., 2016). This difference may be due to different strains of bacteria present in probiotics, formulation protocols, and the dose rate of probiotics (Peek and Landman, 2011).

Conclusion

With its progress the poultry industry became exposed to various kinds of challenges. One of such problems was the spread of protozoan caused GIT ailment coccidiosis. Coccidiosis is caused by *Eimeria spp*. This disease soon became a matter of grave concern for researchers and farmers alike as it resulted in grave economic losses in the poultry industry. Soon, various drugs found their use as effective weapons against this disease. However, this victory was short lived because the *Eimeria spp.* Soon started gaining resistance against these drugs. This problem was soon observed as a threat to poultry industry and was met by alternative solutions like use of essential oil, vaccines and prebiotics. The solution like prebiotics were seen as effective measures as they not only countered coccidiosis but also other problematic issues like oxidation and toxicity. However, the proper application of prebiotics still requires thorough research and comprehension.

REFERENCES

Abbas, A., Abbas, R. Z., Khan, M. K., Raza, M. A., Mahmood, M. S., Saleemi, M. K., and Sindhu, Z. (2019). Anticoccidial effects of Trachyspermum ammi (Ajwain) in broiler chickens. *Pakistan Veterinary Journal*, *39*(2), 301-304.

Abbas, A., Iqbal, Z., Abbas, R. Z., Khan, M. K., and Khan, J. A. (2017a). Immunomodulatory activity of Pinus radiata extract against coccidiosis in broiler chicken. *Pakistan Veterinary Journal*, *37*, 145-149.

Abbas, A., Iqbal, Z., Abbas, R. Z., Khan, M. K., Khan, J. A., Mahmood, M. S., and Saleemi, M. K. (2017b). In vivo anticoccidial effects of Beta vulgaris (sugar beet) in broiler chickens. *Microbial Pathogenesis*, *111*, 139-144.

Abbas, R., Iqbal, Z., Mansoor, M., Sindhu, Z., Zia, M., and Khan, J. (2013). Role of natural antioxidants for the control of coccidiosis in poultry. *Pakistan Veterinary Journal*, *33*(4), 401-407.

Abdel-Saeed, H., and Salem, N. (2019). Clinical, hematologic, sero-biochemical and IgE response in lambs with diarrhea caused by Eimeria. *International Journal of Veterinary Science*, *8*(1), 10-12.

Abdou, A. M., Fouad, E. A., Alam, S. S., and Hakim, A. S. (2019). Isolation and identification of probiotic lactobacilli from non-ruminant animals. *International Journal of Veterinary Science*, *8*(4), 349-354.

Ali, A. M., Khater, H. F., Seddeik, S. A., and Nada, M. O. (2015). Comparative efficacy of synbiotic and diclazuril on broilers experimentally infected with Eimeria acervulina. *Assiut Veterinary Medical Journal*, *61*(146), 24-33.

Alnassan, A., Kotsch, M., Shehata, A., Krüger, M., Daugschies, A., and Bangoura, B. (2014). Necrotic enteritis in chickens: development of a straightforward disease model system. *Veterinary Record*, *174*(22), 555-555.

Arczewska-Włosek, A., and Świątkiewicz, S. (2013). Improved performance due to dietary supplementation with selected herbal extracts of broiler chickens infected with Eimeria spp. *Journal of Animal and Feed Science*, *22*(3), 257-263.

Awais, M. M., Jamal, M. A., Akhtar, M., Hameed, M. R., Anwar, M. I., and Ullah, M. I. (2019). Immunomodulatory and ameliorative effects of Lactobacillus and Saccharomyces based probiotics on pathological effects of eimeriasis in broilers. *Microbial Pathogenesis*, *126*, 101-108.

Azad, M. A. K., Sarker, M., and Wan, D. (2018). Immunomodulatory effects of probiotics on cytokine profiles. *BioMed Research International*, *2018*(1), 8063647.

Bachaya, H. A., Abbas, R. Z., Raza, M. A., Iqbal, Z., Rehman, T. U., Baber, W., and Hussain, R. (2015). Existence of coccidiosis and associated risk factors in broiler chickens in Southern Punjab, Pakistan. *Pakistan Veterinary Journal*, *35*(1), 81-84.

Behnamifar, A., Rahimi, S., Kiaei, M., and Fayazi, H. (2019). Comparison of the effect of probiotic, prebiotic, salinomycin and vaccine in control of coccidiosis in broiler chickens. *Iranian Journal of Veterinary Research*, *20*(1), 51.

Blake, D. P., and Tomley, F. M. (2014). Securing poultry production from the ever-present Eimeria challenge. *Trends in Parasitology*, *30*(1), 12-19.

Bozkurt, M., Giannenas, I., Küçükyilmaz, K., Christaki, E., and Florou-Paneri, P. (2013). An update on approaches to controlling coccidia in poultry using botanical extracts. *British Poultry Science*, *54*(6), 713-727.

Carter, G. M., Esmaeili, A., Shah, H., Indyk, D., Johnson, M., Andreae, M., and Sacks, H. S. (2016). Probiotics in human immunodeficiency virus infection: a systematic review and evidence synthesis of benefits and risks. *Open Forum Infectious Diseases*, *3*(4), ofw164.

Chapman, H. (2000). Practical use of vaccines for the control of coccidiosis in the chicken. *World's Poultry Science Journal*, *56*(1), 7-20.

Chen, C., Chuang, L., Chiang, Y., Lin, C. L., Lien, Y., and Tsen, H. (2016). Use of a probiotic to ameliorate the growth rate and the inflammation of broiler chickens caused by Eimeria tenella infection. *Journal of Animal Research and Nutrition*, *1*(2), 1-7.

Christaki, E., Bonos, E., Giannenas, I., and Florou-Paneri, P. (2012). Aromatic plants as a source of bioactive compounds. *Agriculture*, *2*(3), 228-243.

Christaki, E., Florou-Paneri, P., Giannenas, I., Papazahariadou, M., Botsoglou, N. A., and Spais, A. B. (2004). Effect of a mixture of herbal extracts on broiler chickens infected with Eimeria tenella. *Animal Research*, *53*(2), 137-144.

Dalloul, R. A., Lillehoj, H. S., Tamim, N. M., Shellem, T. A., and Doerr, J. A. (2005). Induction of local protective immunity to Eimeria acervulina by a Lactobacillus-based probiotic. *Comparative Immunology, Microbiology and Infectious Diseases*, *28*(5-6), 351-361.

Eckert, N., Lee, J., Hyatt, D., Stevens, S., Anderson, S., Anderson, P., and Caldwell, D. (2010). Influence of probiotic administration by feed or water on growth parameters of broilers reared on medicated and nonmedicated diets.

Journal of Applied Poultry Research, *19*(1), 59-67.

Erdoğmuş, S. Z., Gülmez, N., Findik, A., Hüseyin, Ş., and Gülmez, M. (2019). Efficacy of probiotics on health status and growth performance of Eimeria tenella infected broiler chickens. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, *25*(3).

Fioramonti, J., Theodorou, V., and Bueno, L. (2003). Probiotics: what are they? What are their effects on gut physiology? *Best Practice and Research Clinical Gastroenterology*, *17*(5), 711-724.

Georgieva, N., Gabrashanska, M., Koinarski, V., and Ermidou-Pollet, S. (2011). Antioxidant status in Eimeria acervulina infected chickens after dietary selenium treatment. *Trace Elements and Electrolytes*, *28*(1), 42.

Guarner, F., and Malagelada, J.-R. (2003). Gut flora in health and disease. *The Lancet*, *361*(9356), 512-519.

Hutsko, S., Meizlisch, K., Wick, M., and Lilburn, M. (2016). Early intestinal development and mucin transcription in the young poult with probiotic and mannan oligosaccharide prebiotic supplementation. *Poultry Science*, *95*(5), 1173-1178.

Idris, M., Abbas, R., Masood, S., Rehman, T., Farooq, U., Babar, W., and Riaz, U. (2017). The potential of antioxidant rich essential oils against avian coccidiosis. *World's Poultry Science Journal*, *73*(1), 89-104.

Kechagia, M., Basoulis, D., Konstantopoulou, S., Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N., and Fakiri, E. M. (2013). Health benefits of probiotics: a review. *International Scholarly Research Notices*, *2013*(1), 481651.

Khater, H. F., Ziam, H., Abbas, A., Abbas, R. Z., Raza, M. A., Hussain, K., and Selim, A. (2020). Avian coccidiosis: Recent advances in alternative control strategies and vaccine development. *Agrobiological Records*, *1*, 11-25.

Lee, S., Lillehoj, H., Dalloul, R., Park, D., Hong, Y., and Lin, J. (2007a). Influence of Pediococcus-based probiotic on coccidiosis in broiler chickens. *Poultry Science*, *86*(1), 63-66.

Lee, S., Lillehoj, H. S., Park, D. W., Hong, Y. H., and Lin, J. (2007b). Effects of Pediococcus-and Saccharomyces-based probiotic (MitoMax®) on coccidiosis in broiler chickens. *Comparative Immunology, Microbiology and Infectious Diseases*, *30*(4), 261-268.

Lillehoj, H., Hong, Y., and Kim, C. (2008). Quantitative genetic and functional genomics approaches to investigating parasite disease resistance and protective immune mechanisms in avian coccidiosis. In *Animal Genomics for Animal Health* (Vol. 132, pp. 67-75). Karger Publishers.

Markowiak, P., and Śliżewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients*, *9*(9), 1021.

McNaught, C., and MacFie, J. (2001). Probiotics in clinical practice: a critical review of the evidence. *Nutrition Research*, *21*(1-2), 343-353.

Moslehi-Jenabian, S., Vogensen, F. K., and Jespersen, L. (2011). The quorum sensing luxS gene is induced in Lactobacillus acidophilus NCFM in response to Listeria monocytogenes. *International Journal of Food Microbiology*, *149*(3), 269-273.

Mousa, S., and Marwan, A. (2019). Growth performance, rumen fermentation and selected biochemical indices in buffalo calves fed on Basillis subtilus supplemented diet. *International Journal of Veterinary Science*, *8*(3), 151-156.

Oelschlaeger, T. A. (2010). Mechanisms of probiotic actions–a review. *International Journal of Medical Microbiology*, *300*(1), 57-62.

Ohimain, E. I., and Ofongo, R. T. (2012). The effect of probiotic and prebiotic feed supplementation on chicken health and gut microflora: a review. *International Journal of Animal and Veterinary Advances*, *4*(2), 135-143.

Peek, H., and Landman, W. (2011). Coccidiosis in poultry: anticoccidial products, vaccines and other prevention strategies. *Veterinary Quarterly*, *31*(3), 143-161.

Ritzi, M. M. (2015). *The effects of probiotics on performance and immune response of broiler chickens during coccidiosis* Virginia Tech].

Ritzi, M. M., Abdelrahman, W., Mohnl, M., and Dalloul, R. A. (2014). Effects of probiotics and application methods on performance and response of broiler chickens to an Eimeria challenge. *Poultry Science*, *93*(11), 2772-2778.

Ritzi, M. M., Abdelrahman, W., Van-Heerden, K., Mohnl, M., Barrett, N. W., and Dalloul, R. A. (2016). Combination of probiotics and coccidiosis vaccine enhances protection against an Eimeria challenge. *Veterinary Research*, *47*, 1-8.

Sarwar, N., Mehmood, A., Sheraz, A., and Noman, M. (2019). Protective Effect of Probiotics in Combination with Vaccination on Antibody Response, Biochemical and Hematological Indices in Broilers. *Pakistan Veterinary Journal*, *39*(3).

Seddiek, K. (2015). Protozoal incidence in balady chicken flocks after viral vaccinations. *Benha Veterinary Medical Journal*, *29*(1), 108-111.

Sen, S., Ingale, S., Kim, Y., Kim, J., Kim, K., Lohakare, J., and Kwon, I. (2012). Effect of supplementation of Bacillus subtilis LS 1-2 to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology. *Research in Veterinary Science*, *93*(1), 264-268.

Sharman, P. A., Smith, N. C., Wallach, M. G., and Katrib, M. (2010). Chasing the golden egg: vaccination against poultry coccidiosis. *Parasite Immunology*, *32*(8), 590-598.

Stringfellow, K., Caldwell, D., Lee, J., Mohnl, M., Beltran, R., Schatzmayr, G., and Farnell, M. (2011). Evaluation of probiotic administration on the immune response of coccidiosis-vaccinated broilers. *Poultry Science*, *90*(8), 1652-1658.

Wang, Y., Wu, Y., Wang, Y., Xu, H., Mei, X., Yu, D., and Li, W. (2017). Antioxidant properties of probiotic bacteria. *Nutrients*, *9*(5), 521.

Zhang, K.-x., Li, X.-l., Na, C., Abbas, A., Abbas, R. Z., and Zaman, M. A. (2020). Anticoccidial effects of Camellia sinensis (green tea) extract and its effect on blood and serum chemistry of broiler chickens. *Pakistan Veterinary Journal*, *40*(1), 77-80.

Chapter 14

The Beneficial Role of Probiotics and Prebiotics for Control of Zoonotic Parasitic Diseases

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ABSTRACT

Zoonoses are infections that humans can contract from animals in a reversible manner. The zoonotic helminths are a significant health hazard, infecting one-third of the world's population. There is currently no reliable human vaccine available to prevent helminth infections. As a result, in the past few years, probiotics and prebiotics have gained attention due to their possible uses as a preventative or treatment strategy towards parasites. During the previous decade, probiotics have been reported to be effective in controlling parasitic infections, which were described as involving primarily gastrointestinal disorders as well as certain non-gut infections, all of which are crucial for both humans and animals. The probiotic strains have been shown to have anti parasitic effects on parasites in the gastrointestinal tract at both the egg and larval stages of development. In the majority of case studies, the animal models provided the majority of data for beneficial effects. The *Lactobacillus, Bifidobacterium* and *Enterococcus* are most frequently used microbes. Still, these beneficial microbes' effects on helminth infections are mostly understudied. There is a full discussion of the most recent research on the beneficial effects of bacteria against helminth infections, as well as the suggested mechanism of action in this chapter.

KEYWORDS
Parasitic zoonosis, Probiotics, Prebiotics, Immunomodulation, Control

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INTRODUCTION

When a parasitic organism spends a particular phase of its life cycle living on or inside another host in tight biological and environmental circumstances, this is termed as parasitism. The zoonotic parasites are specific parasites to which animals are the primary hosts, although they can also invade and inflict illnesses in individuals. Globally, animal and human parasites represent a major risk to the production and health (Omeragic et al., 2022).

Nearly 60% of newly discovered infections affecting humans are considered to be zoonotic. Animals are the source of the majority of illnesses that harm people. Companion animals including sheep, goats, cattle, horses, cats and dogs are susceptible to zoonotic infections and transfer them to people. Examples of few most frequent zoonotic parasitic diseases that are present in the world are Toxocariasis, Schistosomiasis, and Trichinellosis (Rahman et al., 2020).

These zoonotic diseases can be transmitted from diseased animals to individuals through several ways such as eating of raw feed, direct contact with feces and touching of companion animals. The helminth infections caused by parasites have become major frequent zoonotic illnesses, infecting roughly one third of people internationally. The helminths often have complicated life cycles with several phases and hosts. Throughout their entire life cycle, the majority of parasitic helminth species belong to many niches in their living hosts, and the majority of them lead to persistent infections. It is acceptable to avoid the outbreaks of zoonotic parasitic diseases with routine deworming and anti-parasitic drugs as well as basic sanitation and good hygiene practice (Reda, 2018).

Still, the elimination of parasitic infestation remains an issue, requiring the development of novel potential strategies because there are no appropriate immunizations as well as anthelmintic resistance of different parasites to medications has emerged. Hence, in the past few years, there has been a noticeable increase in the interest in using probiotics as a medicine supplement (Saracino et al., 2021).

As a matter of fact, the scientific community's keen interest in analyzing the connections between parasites like cestodes, trematodes and nematodes and the microbiota of gastrointestinal tract has increased recently, primarily due to the desire to gain more knowledge of how host malnutrition is impacted by variations in the microbial population composition brought about by parasites. Several studies have investigated the immunomodulatory characteristics of commensal bacteria and gastrointestinal parasites to better understand the function of helminth-induced variations in the gut microbiota in parasite-driven inflammation reduction (Duarte et al., 2016).

The human and animal digestive tract helminths represent some of the most common infections worldwide, contributing extensively to mortality as well as morbidity, especially in developing countries. According to data from the WHO, gastrointestinal parasitic infections, such as *Trichuris trichiura*, *Ascaris lumibricoides*, and *Necatar americanus*, ultimately affect 24% of the world's population (Rooney et al., 2023).

The probiotics have been defined in a number of ways in the scientific literature. Therefore, ten years ago, probiotics were defined as live microbes that improve the intestinal microbiota balance of the host and had a positive impact on the host's health. The probiotics that are currently most frequently utilized include *Bifidobacterium, Lactobacillus* and *Saccharomyces cerevisiae, Boulardii* (Yeast). The prebiotics, as an idea, have also changed dramatically in the recent years. A prebiotic was initially described as a non-digestible food component that improves health of the host by specifically promoting the growth or activity of one or a small number of microbes in the colon. Insulin, fructooligosaccharides, galactooligosachharides are the most commonly utilized prebiotics (Figueroa-González et al., 2011).

Still, the role of probiotics and prebiotics in avoiding the spread of zoonotic parasitic infections is neglected. In this chapter, we are going to address the most recent research on the beneficial usage of probiotics and prebiotics towards specific zoonotic parasites.

Use of Probiotics against Zoonotic Parasites

The zoonotic infections caused by parasites continue to be a global concern, affecting health of the public, food security, and agribusiness (Torgerson and Macpherson, 2011). The resistance frequencies to anthelmintic medications are increasing day by day, indicating the need for novel therapeutic techniques. For that reason, probiotics are becoming more prevalent as a prophylaxis and medicinal strategy for a variety of diseases. The current studies on the use of beneficial live microbes, their impact on parasites and immune response in the GI tract have produced positive findings. For further information on investigating the mechanism of action and positive effect of probiotics against parasites, it is necessary to acknowledge the current developments in helminth research.

Role of Probiotics to Control Giardiasis

According to WHO, giardiasis is one of the most prevalent zoonotic parasitic infection globally is caused by *Giardia doudenalis*, also referred to as *Giardia lamblia* and *Giardia intestinalis*. It affects approximately 280 million individuals annually (Ankarklev et al., 2010). This single cell aquatic parasite is capable of infecting a variety of species. Almost, ten environmentally resistant cysts must infect individuals for clinical infection to begin. During GIT transportation, cysts release trophozoites, which are both replicative and motile. The presence of trophozoites in the digestive tract can cause symptoms such as epigastric pain, discomfort, abdominal cramping, watery diarrhea, vomiting, and reduced appetite within 6-15 days of cyst intake. However, 50% of the infections may remain undiagnosed. The medications include metronidazole and nitroimidazole, but infections can frequently cure on their own (Darwesh and El Sayed, 2022).

Broadly, introducing probiotic strains like *Lactobacillus* and *Saccharomyces* proved to decrease the severity of GIT symptoms and repair the damage, particularly in people with giardiasis. The probiotics have the capacity to regulate the balance and composition of intestinal microbiota, which has a positive medical impact. Many probiotic strains have the ability to boost antioxidant levels, eliminate oxidative products, control chronic inflammation, trigger mucosal immune system responses, and shorten the duration of gastrointestinal symptoms. These actions help to prevent harm to the gut triggered by parasites. Furthermore, they may decrease the *G. duodenalis* percentage burden by directly attacking the parasite itself (Dashti and Zarebavani, 2021). The *in-vitro* and *in-vivo* efficacy of *L. acidophilus* and *L. palantrum* bacteriocin trophozites of *Giardia lamblia* has been proved (Amer et al., 2014).

Role of Probiotics to Control Toxocariasis

Toxocara canis is the common cause of toxocariasis, which is defined by the movement and infestation of parasitic larvae in men. This zoonotic parasite is prevalent in the intestine of dogs (Avila et al., 2013). The *Toxocara* species accidentally infect people when they consume infectious eggs or raw meat or viscera from hosts that are infected (Ruiz-Manzano et al., 2019). The researchers also looked into how *Lactobacillus rhamnosus* (ATCC 7469) and *Lactobacillus acidophilus* (ATCC 4356) affected the *Toxocara canis* infection in a single trial. Prior a parasitic trial using an embryonated *Toxocara canis* egg, the probiotic therapy was started. The use of these probiotics effectively decreased the overall quantity of migratory larvae seen in the hepatocytes at forty-eight hours post-infection (58% decrease for *L. acidophilus* and 52% for *L. rhamnosus* (Cadore et al., 2021; Walcher et al., 2018).

Role of Probiotics to Control Cryptosporidiosis

The *Cryptosporidium parvum* is a highly prevalent zoonotic parasite of veterinary and medical importance, affecting the health of both humans and animals. It is known that around 15 different types of *Cryptosporidium* species can infect humans. The transmission occurs via the oro-fecal route (Ali et al., 2024). One study investigated the treatment of *C. parvum* infection in immunosuppressed mice by administration of *E. faecalis* (CECT 7121) as an oral probiotic strain. The impact of *Cryptosporidium parvum* infection on the intestinal mucosa was evaluated at each site of the intestine. The results revealed that when both *C. parvum* and *E. faecalis* were found in the same intestinal area, they competed with each other. The effects of *Cryptosporidium parvum* infection on the intestinal mucosa were assessed in each part of the gut. The findings demonstrated that when *C. parvum* and *E. faecalis* were identified in the same intestinal location, they competed with one another. Moreover, supplementation with *E. faecalis* can reduce the negative effects of *C. parvum* infection (Del coco et al., 2016).

Role of Probiotics to Control Scabies

Scabies is an infection of the skin that poses a serious threat to the human health around the world. The symptoms of scabies in people are hives, vesicles, and papules. Rubbing can cause excoriation and crusting of the skin. Regularly used topical scabies lotion can cause itching and dermatitis, as well as secondary infections by bacteria *Streptococcus pyoderma*, thus an adequate plan should be developed to address this issue. The probiotics in goat milk soaps may work as an antiseptic on the surface of the skin, due to the inclusion of lactic acid bacteria, which can kill harmful microbes. The *Pediococcus pentosaceus* is a probiotic that has been known to suppress both pathogenic and spoilage microbes. It can also restrict contamination of pathogenic microbes and a toxin generator because of its capacity to make lactic acid and reduce the pH level of the substrate (Mawarti et al., 2014).

Role of Probiotics to Control Trichinellosis

The trichinellosis is a zoonotic illness triggered by nematodes of the genus *Trichinella,* which belong to the most common parasites class of domestic and wild omnivores. The probiotic strains may protect from zoonotic *Trichinella spiralis* infection, as part of a new treatment approach for controlling parasitic zoonoses. Eating raw meat might lead to infection of the hosts. The anthelmintics do not efficiently treat all developmental stages of human trichinellosis, only targeting adult worms. The probiotics are now being utilized in experimental models to treat parasitic infections, as the research community is still in its early stages. The probiotics impact on *Trichinella* species beyond *T. spiralis* remains unknown. The beneficial effects of probiotic bacteria decrease parasitic burden and pathogenic modifications in the experimental trichinellosis by stimulating local as well as systemic immune responses has been observed in several studies (Boros et al., 2022; Ortega-Pierres et al.,2015).

Worms mature in the intestine of pig. Then, enter the bloodstream and lymphatic system, eventually ending up in striated muscles. The larvae movement can harm host tissue and trigger inflammatory reactions, perhaps leading to mortality. Treatment with albendazole and mebendazole has varying degrees of effectiveness. The oral administration of *Lactobacillus casei* ATCC7469 to mice showed a considerable reduction in both adult worms (58% and 44%, respectively) and larvae per gram of muscle (up to70%), showing an immune response. Treatment with *Lactobacillus casei* culture supernatant had a significant impact (32% decreased adult worms), although being less effective (Travers et al., 2011).

Role of Probiotics to Control Schistosomiasis

Schistosomiasis is a zoonotic parasitic disease triggered by infected trematode worms of the genus *Schistosoma*, affecting 240 million individuals globally (Inobaya et al., 2014). *Schistosoma mansoni* and *Schistosoma japonicum* are the most common causes of intestinal schistosomiasis, while *Schistosoma haematobium* causes urogenital schistosomiasis. *S. mansoni* infection causes fibrosis and impaired function of gastrointestinal tract systems. The intensity of symptoms depends on the parasitic load and the response of the host's immune system (Dejon-Agobé et al., 2022). The recommended drug for treating schistosomiasis is praziquantel (da Paixão Siqueira et al., 2017).

Probiotics protect the intestinal mucosa against dysbiosis from opportunistic infections such intestinal parasites. Biovicerin®, a probiotic containing *Bacillus cereus* GM, modulates the immunological response of *S. mansoni* infected hosts. The decrease in parasite load, the quantity of eggs within the liver, and the morphology of schistosomal granuloma are indicators of infection progression. Mice administered with *Bacillus cereus* had significantly lower worms compared to the control group (Dos Santos et al., 2024).

Two *Lactobacillus* strains named as *Lactobacillus bulgaricus* DSM 20080 and *Lactobacillus acidophilus* ATCC 4356 have reduced the worms load in animals treated for a week prior to *S. mansoni* infection by 67.8% and 59.8%, respectively (El-khadragy et al., 2019). The mice treated with *Bacillus clausii* (Enterogermina®) prior *S. mansoni* infection showed a 41.4% reduction in overall worms load. Following 38 days of infection, the treated group showed a 30.1% decline, according to the same study (Cruz et al., 2022).

Mechanism of Action of Probiotics against Parasites

1) Modulation of Intestinal Microbiota

The probiotics can alter the intestinal environment by inhibiting the growth of bacteria or competing for a common biotope (Gupta and Garg, 2009). The probiotics can compete for iron, a limiting nutrient required by the majority of microbes. The *Lactobacillus* can make iron un-accessible for pathogens. The Microbes can bind ferric hydroxide on their surfaces (Elli et al., 2000).

Table 1: Effects of probiotics against parasites

Parasites	Probiotic used	Studied Model	Mechanism of Action	Effects	References
Trichinella spiralis	*L. plantrum*	Mice	Increase serum IFN-γ	Increase larval count	(El Temsahy et al., 2015)
Trichinella spiralis	*L. casei*	Mice	Increase IgA and IgG	Increase protection	(Martínez-Gómez et al., 2011)
Toxocara canis	*S. boulardii*	Mice	increase IL12andIFN-γ	Increase protection	(de Avila et al., 2016)
Schistosoma mansoni	*L. plantrum* *L. acidophilus* *L. reuteri*	Mice	Increase IgM, Decrease AST,LDH and gGT	Decrease weight of spleen and liver Decrease parasitic complications	(Ghanem et al., 2005)

The imbalance of the gut microbiota is a major contributor to a variety of diseases. The probiotics help human health since they are live microorganisms that have the potential to significantly control the microbial composition of the GI tract. The VSL#3, a probiotic mixture made up of eight live bacterial strains, is crucial for preventing and curing gastrointestinal disorders in humans as well as animals. It can increase tight junction protein activity, alter the makeup of microbiota in the gut as well as modulate immune-related cytokine release (Cheng et al., 2020).

2) Production of Active Substances

The bacteriocins, free fatty acids, antibiotics, and hydrogen peroxide (H_2O_2) can regulate the development and survival of bacteria. The bacteriocins are released as protein or peptide molecules that typically kill closely associated microbes by permeabilizing their cell membranes. The probiotics also produce lactic acid bacteria that lower gut pH (Wohlgemuth et al., 2010).

3) Immune Modulating Effects Towards Helminths

Effects on the immune response are caused by reactions among microbes and the cell receptors. The dendritic cells, which exude cytoplasmic processes into the GI tract, are located in the epithelial and immune cells and play a crucial role in regulating the effects of probiotics (Sánchez et al., 2017). The probiotics alter the gut microbiota and immune receptor targets, influencing the immune system's innate as well as adaptive responses. These modulations occur at both systemic and local levels (Sivan et al., 2015). The interleukins (IL)-4, IL-5, IL-9, IL-10, and IL-13 production are indicative of type 2 immunity response, and this is the usual immunological response to helminth infections (Allen and Maizels, 2011). The innate lymphoid cells (ILCs), innate immune cells, including mast cells, basophils, and eosinophils, and adaptive immunity cells like CD^{4+} Th^2 cells and B-cells are both significant effector cells and providers of type 2 cytokines (Gause et al., 2020).

Use of Prebiotics and Immunity against Helminths

The prebiotics have a long history of safe use and have been shown to improve human health by increasing mineral bioavailability, modulating the immune system, preventing gastrointestinal infections, modifying inflammatory conditions, and regulating metabolic disorders. The prebiotic substances can influence microbial composition and activity on the luminal and mucosal surfaces, promoting positive host-microbe interactions (Roberfroid et al., 2010). Although all prebiotics are fibers, not all fibers are prebiotics. The prebiotics must have demonstrated health benefits for animals. The prebiotics for animal health and disease prevention are equally important as for people (Gibson et al., 2017).

Innovations in knowing about the prebiotic effects of dietary chemicals and GM-dependent modifications to mucosal immune system function highlight an issue regarding how prebiotic dietary components, such as dietary fiber, can influence anti-helminth immunity. The host GM undergoes significant alterations during parasitic infection; however a consistent set of taxa has yet to be identified (Walk et al., 2010). Transferring GM from helminth-infected mice to mice without germs replicated immune function, including the Treg response, indicating that helminth-induced alterations in the GM may contribute to the immune-mediated effects resulting from infection (Su et al., 2018).

Healthy mice administered inulin had an enriched gut microbiota with *Actinobacteria* and *Akkermansia muciniphila*, as well as higher short-chain fatty concentrations, indicating the beneficial effects of prebiotic carbohydrates. Regarding this, serological tests revealed that administration of insulin during *Trichuris muris* infection had significantly reduced the type-2 immune response, showing that the prebiotic fiber inhibited instead of increasing the immune response against infection. However, in one study, mice infected with the parasite *Trichinella spiralis* and fed β-glucans were observed to be protected by β-glucan-mediated proliferation of *Akkermansia muciniphila* within the GM, activating the TLR2-dependent immune response to promote worm expulsion (Jin et al., 2022).

One study found that giving malnourished mice prebiotic insulin before or during *Giardia* infection decreased the degree of severity of giardiasis, increased the mass of the body and small intestine, and raised the number of lactobacilli in the feces in comparison to mice that were not infected with *Giardia*. More precisely, compared to starved Giardia-infected mice, administration of prebiotics markedly raised anti-giardial IgA along with IgG antibodies, anti-inflammatory cytokines IL-6 and IL-10, and decreased the pro-inflammatory cytokine TNF-α in both the intestinal fluid and serum. The nitric oxide levels were also higher. This study is the sole effort to show that prebiotic therapy improves immunological function and gut morphology in malnourished Giardia-infected mice (Shukla et al., 2016).

Future Perspectives of Probiotics and Prebiotics

The scientific and methodological advancements offer great opportunities for the probiotic and prebiotic studies as well as applications. The real-time investigations in humans, monitoring microbes as they integrate into the microbiota, and measuring health levels will advance this field of study. Monitoring the microorganisms, how they interact with the host, and factors related to the environment (e.g., medications, nutrition) will become standard for the future physical examination. The innovative techniques for sampling will reveal how probiotics and prebiotics affect the immune system, metabolism, and the gut microbiome. As early career scientists, we aim to contribute to a society that utilizes helpful microbes to address global issues such as disease prevention and toxin removal from food and the environment. These will prove to be dynamic moments, with various job options. The probiotics and prebiotics can be used in various fields, including science (Spacova et al., 2020).

Conclusion

This chapter highlighted the favorable effects of probiotics and prebiotics on the immunity and digestive tract health of humans as well as animals. The probiotics are commonly utilized in human aquaculture, livestock, and poultry to improve health and prevent intestinal diseases. To address the financial impact of zoonotic helminth infections and anthelmintic treatment resistance, novel control measures, such as probiotics and prebiotics, are urgently needed. The probiotics and other treatments are crucial for reducing parasitic infections. The analysis suggests that probiotics may be a more effective therapy option for gastrointestinal parasitic infections, as current care options are inadequate. The probiotics as a therapy for helminth infections is a significant, newly investigated field. The probiotic strains from *Lactobacillus*, *Bacillus clausii*, and *Enterococcus* have been extensively studied for their effectiveness in treating giardiasis, cryptosporidiosis, schistosomiasis, trichinellosis, and toxocariasis. The probiotics can benefit hosts through several modes of action, including immunomodulation, if administered properly. To fully understand the benefits and drawbacks of probiotics, high-throughput confirmation methodologies and solid clinical, *in-vivo*, and *in-vitro* investigations are necessary.

REFERENCES

Amer, E. I., Mossallam, S. F., and Mahrous, H. (2014). Therapeutic enhancement of newly derived bacteriocins against *Giardia lamblia*. *Experimental Parasitology* 146: 52-63.

Allen, J. E., and Maizels, R. M. (2011). Diversity and dialogue in immunity to helminths. *Nature Reviews Immunology*, 11(6): 375-388.

Ali, M., Xu, C., Nawaz, S., Ahmed, A. E., Hina, Q., and Li, K. (2024). Anti-Cryptosporidial Drug-Discovery Challenges and Existing Therapeutic Avenues: A "One-Health" Concern. *Life,* 14(1): 80.

Ankarklev, J., Jerlström-Hultqvist, J., Ringqvist, E., Troell, K., and Svärd, S. G. (2010). Behind the smile: cell biology and disease mechanisms of *Giardia* species. *Nature Reviews Microbiology* 8(6): 413-422.

Avila, L. F. D. C. D., Telmo, P. D. L., Martins, L. H. R., Glaeser, T. A., Conceição, F. R., Leite, F. P. L., and Scaini, C. J. (2013). Protective effect of the probiotic *Saccharomyces boulardii* in *Toxocara canis* infection is not due to direct action on the larvae. *Revista do Instituto de Medicina Tropical de São Paulo,* 55: 363-365.

Boros, Z., Băieș, M. H., Vodnar, D. C., Gherman, C. M., Borșan, S. D., Cozma-Petruț, A., and Cozma, V. (2022). Antiparasitic action of *Lactobacillus casei* ATCC 393 and *Lactobacillus paracasei* CNCM strains in CD-1 mice experimentally infected with *Trichinella britovi Pathogens,* 11(3): 296.

Cadore, P. S., Walcher, D. L., Sousa, N. F. G. C. D., Martins, L. H. R., Hora, V. P. D., Groll, A. V., and Scaini, C. J. (2021). Protective effect of the probiotic *Lactobacillus acidophilus* ATCC 4356 in BALB/c mice infected with *Toxocara canis*. *Revista do Instituto de Medicina Tropical de São Paulo,* 63.

Cheng, F. S., Pan, D., Chang, B., Jiang, M., and Sang, L. X. (2020). Probiotic mixture VSL# 3: An overview of basic and clinical studies in chronic diseases. *World Journal of Clinical Cases,* 8(8): 1361.

Cruz, C. S., França, W. W. M., de Arújo, H. D. A., Ximenes, E. C. P. A., de Souza, V. M., Albuquerque, M. C. P. A., and Costa, V. M. A. (2022). *In vitro* and *in vivo* evaluation of *Bacillus clausii* against *Schistosoma mansoni*. *Acta Tropica,* 235: 106669.

da Paixão Siqueira, L., Fontes, D. A. F., Aguilera, C. S. B., Timóteo, T. R. R., Ângelos, M. A., Silva, L. C. P. B. B., and Neto, P. J. R. (2017). Schistosomiasis: Drugs used and treatment strategies. *Acta Tropica,* 176: 179-187.

Dashti, N., and Zarebavani, M. (2021). Probiotics in the management of Giardia duodenalis: an update on potential mechanisms and outcomes. *Naunyn-Schmiedeberg's Archives of Pharmacology,* 394: 1869-1878.

Darwesh, O. M., and El-Sayed, H. S. (2022). Perspective chapter: application of probiotics to inactivate helminth parasitic zoonosis. In Parasitic Helminths and Zoonoses-From Basic to Applied Research. IntechOpen.

Del Coco, V. F., Sparo, M. D., Sidoti, A., Santín, M., Basualdo, J. A., and Córdoba, M. A. (2016). Effects of *Enterococcus faecalis* CECT 7121 on *Cryptosporidium parvum* infection in mice. *Parasitology Research,* 115: 3239-3244.

de Avila, L. D. C., De Leon, P. M. M., De Moura, M. Q., Berne, M. E. A., Scaini, C. J., and Leivas Leite, F. P. (2016). Modulation of IL-12 and IFN γ by probiotic supplementation promotes protection against *Toxocara canis* infection in mice. *Parasite Immunology,* 38(5): 326-330.

Dejon-Agobé, J. C., Edoa, J. R., Adegnika, A. A., and Grobusch, M. P. (2022). Schistosomiasis in Gabon from 2000 to 2021-A review. *Acta Tropica,* 228: 106317.

Dos Santos, V. H. B., de Azevedo Ximenes, E. C. P., de Souza, R. A. F., da Silva, R. P. C., da Conceição Silva, M., de Andrade, L. V. M., and de Azevedo Albuquerque, M. C. P. (2024). Effects of the probiotic *Bacillus cereus* GM on experimental *schistosomiasis mansoni*. *Parasitology Research,* 123(1):1-13.

Duarte, A. M., Jenkins, T. P., Latrofa, M. S., Giannelli, A., Papadopoulos, E., de Carvalho, L. M., and Cantacessi, C. (2016). Helminth infections and gut microbiota–a feline perspective. *Parasites and Vectors,* 9(1): 1-9.

El-Khadragy, M. F., Al-Olayan, E. M., Elmallah, M. I., Alharbi, A. M., Yehia, H. M., and Abdel Moneim, A. E. (2019). Probiotics and yogurt modulate oxidative stress and fibrosis in livers of *Schistosoma mansoni*-infected mice. *BMC Complementary and Alternative Medicine* 19: 1-13.

El Temsahy, M. M., Ibrahim, I. R., Mossallam, S. F., Mahrous, H., Bary, A. A., and Salam, S. A. A. (2015). Evaluation of newly isolated probiotics in the protection against experimental intestinal trichinellosis. *Veterinary Parasitology*, 214(3-4): 303-314.

Elli, M., Zink, R., Rytz, A., Reniero, R., and Morelli, L. (2000). Iron requirement of *Lactobacillus* spp. in completely chemically defined growth media. *Journal of Applied Microbiology,* 88(4): 695-703.

Figueroa-González, I., Quijano, G., Ramírez, G., and Cruz-Guerrero, A. (2011). Probiotics and prebiotics—perspectives and challenges. *Journal of the Science of Food and Agriculture,* 91(8): 1341-1348.

Gause, W. C., Rothlin, C., and Loke, P. N. (2020). Heterogeneity in the initiation, development and function of type 2 immunity. *Nature Reviews Immunology,* 20(10): 603-614.

Ghanem, K. Z., Abdel-Salam, A. M., and Magharby, A. S. (2005). Immunoprophylactic effect of probiotic yoghurt feeding on *Schistosoma mansoni*-infected mice. *Pol Journal Food Nutrition Science,* 14(2): 123-126.

Gibson, G. R., Hutkins, R. W., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., and Reid, G. (2017). The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics.

Gupta, V., and Garg, R. (2009). Probiotics. *Indian Journal of Medical Microbiology,* 27(3): 202-209.

Inobaya, M. T., Olveda, R. M., Chau, T. N., Olveda, D. U., and Ross, A. G. (2014). Prevention and control of schistosomiasis: a current perspective. *Research and Reports in Tropical Medicine,* 65-75.

Jin, X., Liu, Y., Wang, J., Wang, X., Tang, B., Liu, M., and Liu, X. (2022). β-Glucan-triggered *Akkermansia muciniphila* expansion facilitates the expulsion of intestinal helminth via TLR2 in mice. *Carbohydrate Polymers,* 275: 118719.

Martínez-Gómez, F., Fuentes-Castro, B. E., and Bautista-Garfias, C. R. (2011). The intraperitoneal inoculation of Lactobacillus casei in mice induces total protection against *Trichinella spiralis* infection at low challenge doses. *Parasitology Research,* 109: 1609-1617.

Mawarti, H., Rajin, M., Zakaria, A., and Purwati, D. E. (2014). 1Bachelor of Nursing Program, Faculty of Health Sciences, Pesantren Tinggi Darul Ulum University, Indonesia 2Laboratory of Animal Products Technology, Faculty of Animal Husbandry, Andalas University, Indonesia.

Omeragic, J., Seric-Haracic, S., and Kapo, N. (2022). Zoonotic Parasites and Vector-Borne Parasitoses. In Zoonosis of Public Health Interest. IntechOpen.

Ortega-Pierres, G., Vaquero-Vera, A., Fonseca-Linan, R., Bermudez-Cruz, R. M., and Argüello-García, R. (2015). Induction of protection in murine experimental models against *Trichinella spiralis*: an up-to-date review. *Journal of Helminthology,* 89(5): 526-539.

Rahman, M. T., Sobur, M. A., Islam, M. S., Ievy, S., Hossain, M. J., El Zowalaty, M. E., and Ashour, H. M. (2020). Zoonotic diseases: etiology, impact, and control. *Microorganisms*, 8(9): 1405.

Reda, A. A. (2018). Probiotics for the control of helminth zoonosis. *Journal of Veterinary Medicine,* 2018.

Roberfroid, M., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., and Meheust, A. (2010). Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition,* 104(S2): S1-S63.

Rooney, J., Cantacessi, C., Sotillo, J., and Cortés, A. (2023). Gastrointestinal worms and bacteria: From association to intervention. *Parasite Immunology,* 45(4): e12955.

Ruiz-Manzano, R. A., Hernández-Cervantes, R., Del Río-Araiza, V. H., Palacios-Arreola, M. I., Nava-Castro, K. E., and Morales-Montor, J. (2019). Immune response to chronic *Toxocara canis* infection in a mice model. *Parasite Immunology,* 41(12): e12672.

Sánchez, B., Delgado, S., Blanco-Míguez, A., Lourenço, A., Gueimonde, M., and Margolles, A. (2017). Probiotics, gut microbiota, and their influence on host health and disease. *Molecular Nutrition and Food Research,* 61(1): 1600240.

Saracino, M. P., Vila, C. C., Baldi, P. C., and Gonzalez Maglio, D. H. (2021). Searching for the one (s): Using probiotics as anthelmintic treatments. *Frontiers in Pharmacology,* 12: 714198.

Shukla, G., Bhatia, R., and Sharma, A. (2016). Prebiotic inulin supplementation modulates the immune response and restores gut morphology in *Giardia duodenalis*-infected malnourished mice. *Parasitology Research,* 115: 4189-4198.

Sivan, A., Corrales, L., Hubert, N., Williams, J. B., Aquino-Michaels, K., Earley, Z. M., and Gajewski, T. F. (2015). Commensal Bifidobacterium promotes antitumor immunity and facilitates anti–PD-L1 efficacy. *Science,* 350(6264): 1084-1089.

Su, C., Su, L., Li, Y., Long, S. R., Chang, J., Zhang, W., and Shi, H. N. (2018). Helminth-induced alterations of the gut microbiota exacerbate bacterial colitis. *Mucosal immunology,* 11(1): 144-157.

Spacova, I., Dodiya, H. B., Happel, A. U., Strain, C., Vandenheuvel, D., Wang, X., and Reid, G. (2020). Future of probiotics and prebiotics and the implications for early career researchers. *Frontiers in Microbiology,* 11: 1400.

Torgerson, P. R., and Macpherson, C. N. (2011). The socioeconomic burden of parasitic zoonoses: global trends. *Veterinary Parasitology* 182(1):79-95.

Travers, M. A., Florent, I., Kohl, L., and Grellier, P. (2011). Probiotics for the control of parasites: an overview. *Journal of Parasitology Research,* 2011.

Walcher, D. L., Cruz, L. A. X., de Lima Telmo, P., Martins, L. H. R., da Costa de Avila, L. F., Berne, M. E. A., and Scaini, C. J. (2018). *Lactobacillus rhamnosus* reduces parasite load on *Toxocara canis* experimental infection in mice, but has no effect on the parasite in vitro. *Parasitology Research,* 117: 597-602.

Walk, S. T., Blum, A. M., Ewing, S. A. S., Weinstock, J. V., and Young, V. B. (2010). Alteration of the murine gut microbiota during infection with the parasitic helminth *Heligmosomoides polygyrus*. *Inflammatory Bowel Diseases* 16(11): 1841-1849.

Wohlgemuth, S., Loh, G., and Blaut, M. (2010). Recent developments and perspectives in the investigation of probiotic effects. *International Journal of Medical Microbiology,* 300(1): 3-10.

Chapter 15

Exploring the Impact of Prebiotics on Gut Microbiota in the context of Atopic Diseases

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ABSTRACT

The gut microbiota, consisting of hundreds of defined bacterial species, plays a crucial role in modulating the immune system and maintaining gut health. However, alterations in the gut microbiota, known as this dysbiosis, increases susceptibility to allergic reactions and have a link with atopic diseases. Prebiotics which would promote the growth or activity of the beneficial bacteria in the gut have been studied for their role in modulating gut microbiota and reducing their risk of atopic diseases. Prebiotics can be obtained from various dietary sources and added as supplements to infant formulas and dietary products. Evidence suggests that prebiotic supplementation in infants and adults may positively influence the gut microbiota composition and activity, potentially reducing the risks of allergic disorders. However, further research is needed to establish definitive conclusions regarding the long-term benefits of prebiotics in reducing the incidence of atopic diseases. This aims to explore the relationship between gut microbiota, dysbiosis and atopic diseases, highlighting the potential role of prebiotic in preventing and managing these conditions. By understanding the complex interplay between the gut microbiota and the immune system, we can develop targeted interventions to restore gut microbiota balance and alleviate the burden of atopic diseases.

KEYWORDS		
Prebiotics; Gut Microbiota; Dysbiosis; Atopic Diseases; Atopic Dermatitis	Received: 21-May-2024 Revised: 12-Jul-2024 Accepted: 04-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Atopic Disease

"Atopy" is from Greek word 'Atopia' means 'out of place'. Atopic disease refers to a hereditary tendency to bring forth immunoglobulin E (IgE) antibodies in result to small amounts of environmental protein e.g. pollen, house dust mite and food allergens. Atopic diseases are prevalent worldwide, affecting individuals of all ages and ethnic backgrounds and reached an epidemic proportions during past industrializing era (Thomsen, 2015). At least 171 million individual were affected with atopic dermatitis (Faye et al., 2023). The prevalence varies across regions and is influenced by genetic, environment, climate, exposure to allergens and lifestyle factors. In 2019, 418 million cases were reported per year (Logoń et al., 2023).

The atopic diseases like atopic dermatitis often begin in infancy and early childhood. Asthma and allergic rhinitis may manifest at any age, with different patterns observed in childhood, adolescence and adulthood (Gray et al., 2017). Family history, genetic predisposition, environment, obesity, stress, allergic sensitization, paracetamol use, tobacco smoke, cesarean section, respiratory virus infection, occupational exposures, diet, obesity, air pollution and breastfeeding and mental health are risk factors of atopic diseases. Factors during pregnancy, modes of birth and early childhood, such as maternal age, maternal smoking, exposure to tobacco smoke, and early introduction of certain foods, may progress towards atopic diseases (Lin et al., 2022). Limited exposure to microbes and infections during early childhood, often associated with increased hygiene and reduced family size, may contribute to a higher risk of atopic diseases (Faye et al., 2023).

Gut Microbiota

The gut microbiota, consisting of hundreds of defined bacterial species, plays a crucial role in modulating the immune system and maintaining gut health. However, alterations in the gut microbiota, known as this dysbiosis, increases susceptibility to allergic reactions and have a link with atopic diseases.

Prebiotics which would promote the growth or activity of the beneficial bacteria in the gut have been studied for their role in modulating gut microbiota and reducing their risk of atopic diseases. Prebiotics can be obtained from various dietary sources and added as supplements to infant formulas and dietary products. Evidence suggests that prebiotic supplementation in infants and adults may positively influence the gut microbiota composition and activity, potentially reducing the risks of allergic disorders. However, further research is needed to establish definitive conclusions regarding the long-term benefits of prebiotics in reducing the incidence of atopic diseases.

This aims to explore the relationship between gut microbiota, dysbiosis and atopic diseases, highlighting the potential role of prebiotic in preventing and managing these conditions. By understanding the complex interplay between the gut microbiota and the immune system, we can develop targeted interventions to restore gut microbiota balance and alleviate the burden of atopic diseases.

Beneficial Effects of Prebiotics

Atopic diseases are complex conditions characterized by immune dysfunction and inflammation. Recent studies have shed light on the significant influence of the gut microbiota on the pathogenesis and severity of atopic diseases, including atopic dermatitis, asthma, and allergic diseases (Muir et al., 2016). The gut microbiota plays a key role in grooming and modulating the immune system, and exposure to a diverse range of microbes during early life contributes to the development of immune tolerance (Kunst et al., 2023). Dysbiosis, or the dis-regulation of the microbiome, potentially leads to an increased susceptibility to various health problems (Dahiya and Nigam, 2023). Inadequate microbial stimulus causes an imbalance in the gut microbiota, resulting in a persistent Th2-dominant immune response and atopy (Rø et al., 2017). Dysbiosis has been observed in individuals with atopic dermatitis, and may influence the development of respiratory allergies and asthma through complex interactions with the immune system (Pantazi et al., 2023). Atopic dermatitis in infants progresses due to a lack of immune system modulation in the gut microbiota (Cukrowska, 2018). Patients with atopic disease have reduced gut microbiota diversity (Candela et al., 2012). The intestinal microbiota of atopic children has increased Clostridium and reduced Bifidobacterium compared to non-atopic children (Kalliomäki et al., 2001). An appropriate gut microbiome and its intestinal metabolites act as a protective factor for patients (Stec et al., 2023). Understanding the interplay between atopic diseases and gut microbiota is crucial for developing targeted therapeutic interventions (Donald and Finlay, 2023).

Prebiotics

Prebiotics promote the growth of beneficial gut microbiota by improving gut barrier function, enhancing immune response, regulating host metabolism, and reducing the risk of allergies (Markowiak-Kopeć and Śliżewska, 2020). They are abundant in human milk, superior meal for good species, and prevent the adhesiveness of pathogens. Long-term use of prebiotics improves immune function, lowers inflammatory cytokines, improves digestion, and produces SCFAs (Alderete et al., 2015). By boosting the growth of beneficial bacteria, prebiotics can create an environment difficult for the maturation of pathogenic bacteria, lowering the risk of infections and imbalances in the gut (Zhou et al., 2024). Diet plays an important role in shaping gut microbiota, and a lack of essential nutrients and diet diversity leads to dysbiosis and other health issues (Piccioni et al., 2023). Diet can modify intestinal microbial diversity and improve its function (Li et al., 2014). Diet supplemented with prebiotics can be used to balance gut microbiota (Scott et al., 2013). Prebiotics can be obtained from dietary sources as shown in table 1 (Khan et al., 2023).

Table 1: Prebiotics Dietary sources

Fruits	Mango, orange, green banana (resistant starch), strawberries, blue berries and raspberries, papaya pulp, apples (pectin), kiwi
Vegetables	Garlic (inulin), onions (inulin, FOS), asparagus (inulin), leeks, bamboo shoots, gourd (5 families), leafy green vegetables, mushrooms
Whole grains	Oatmeal, whole oats (beta-glucan), barley (beta-glucans and soluble fiber), whole wheat bread and whole wheat pasta, quinoa (fiber), corn
Legumes	Chick peas (fiber, resistant startch), lentils green beans (soluble fiber), kidney beans (red), lima beans, cow beans, soy beans,
Nuts and seeds	Flaxseeds (soluble fiber, alpha-linolenic acid), chia seeds (soluble fiber) , fenogreek seeds, almonds (soluble and insoluble fiber), walnuts , cashew apple, chest nut and defatted coconut residues
Root vegetables	Sweet potatoes (fiber, resistant starch), carrots (soluble fiber), chicory roots.
Miscellaneous	Jerusalem artichokes (inulin), dandelion greens (inulin), seaweed like nori and kombu (prebiotic fibers), olive oil,
Dairy Food	Yogurt (inulin), kefir,
Spices	Cinnamon, cayenne pepper, black pepper, turmeric, rosemary, editerranean oregano
Honey	Oligosaccharides

Prebiotics Impact on Gut Microbiota Modulation

Prebiotics and certain dietary interventions may have prophylactic or therapeutic effect (Logoń et al., 2023). Prebiotics helps in manufacturing of short-chain fatty acids, lowering pH in colon, stimulation of mucus production, selective growth of beneficial bacteria, enhancing nutrient absorption, anti-inflammatory effects and modulation of immune responses (Roberfroid et al., 2010). The gut microbiota assists in the development of immune tolerance, healthy bacteria maintain gut health, immunity, integrity and homeostasis (Mishra et al., 2023) allowing immune system to differentiate between a-toxic substances and possible threats. This is crucial for preventing unnecessary allergic for autoimmune responses. Gut bacteria produces different metabolites, such as short-chain fatty acids (SCFAs), that have anti-inflammatory attributes (Maslowski et al., 2009). These metabolites can modulate immune cell activity and contribute to a balanced immune response. The SCFAs anti-inflammatory effects and maintain epithelial function (Maslowski and Mackay, 2011).

Prebiotics helps stimulating in microflora activity, gut microbiota diversity and maintaining normal gut health (Shirsath and Zawar, 2024). Prebiotics promotes beneficial bacteria by selective fermentation, microbial fermentation and produce SCFAs that act as nutrient source for beneficial bacteria. They selectively enhance development of certain probiotic bacteria in colon, esp. *Bifidobacteria* species (Liu et al., 2024).

Inulin helps in promoting the growth of gut bacteria, improves digestion, reduce inflammation, reduce blood cholesterol level, and increases blood sugar level (Slavin, 2013). The FOS improves gut health, reduces risk of colon cancer, improves mineral absorption and enhances immunity (Sabater-Molina et al., 2009), beta-Glucan Improve gut health (Davani-Davari et al., 2019), Pectin Improve digestion, reduce inflammation (Jackson et al., 2007), and XOS reduce inflammation, improve mineral absorption, enhance immune function (Aachary et al., 2015).

Once in the colon, prebiotics like inulin and oligosaccharides are fermented by the enzymes of gut bacteria, specifically by healthful bacteria like *Bifidobacteria* and *Lactobacilli*. The fermentation process of prebiotics produces short-chain fatty acids such as acetate, propionate and butyrate along with gases like hydrogen, methane and carbon dioxide. They help to maintain a somewhat acidic environment of colon, which suppress the growth of noxious bacteria and pathogens while promoting the growth and activity of beneficial bacteria (Zhou et al., 2024).

The SCFAs improves nutrient absorption and are energy sources for colonocytes in particular butyrate (Mishra et al., 2023), serves as a favored energy source for colonocytes (colonic epithelial cells), promoting their health, integrity and enhance the production of mucins (the proteins that make the protective mucus layer in the gut) (Song et al., 2023). The mucins play crucial role in keeping the integrity of mucosal barrier, providing protection against pathogens and prevent inflammation. This strengthen the gut barrier function, permeability reduction and prevents the translocation of harmful substances from the gut into the bloodstream (Ney et al., 2023). Prebiotics in the colon promotes the proliferation of beneficial bacteria, which can have various positive effects on health, including enhanced nutrient absorption, improved gut barrier function, immune system modulation, and potential reduction in inflammation (Peredo-Lovillo et al., 2020).

Upon prebiotics fermentation by gut bacteria produces short-chain fatty acids including butyrate, propionate and acetate. The SCFAs have immunomodulatory effects and maintains immune system balance (Kim, 2023).

Evidence of Prebiotics Supporting in Atopic Disease

Prebiotics and certain dietary interventions may have a prophylactic or therapeutic effect (Logoń et al., 2023). Prebiotics help manufacture short-chain fatty acids, lower pH in the colon, stimulate mucus production, selectively grow beneficial bacteria, enhance nutrient absorption, and have anti-inflammatory effects and modulate immune responses (Roberfroid et al., 2010). The gut microbiota assists in the development of immune tolerance, and healthy bacteria maintain gut health, immunity, integrity, and homeostasis (Mishra et al., 2023), allowing the immune system to differentiate between harmless substances and possible threats.

Gut bacteria produce different metabolites, such as short-chain fatty acids (SCFAs), which have anti-inflammatory attributes (Maslowski et al., 2009). These metabolites can modulate immune cell activity and contribute to a balanced immune response. SCFAs have anti-inflammatory effects and maintain epithelial function (Maslowski and Mackay, 2011).

Prebiotics stimulate microflora activity, gut microbiota diversity, and maintain normal gut health (Shirsath and Zawar, 2024). Prebiotics promote beneficial bacteria by selective fermentation, microbial fermentation, and produce SCFAs that act as a nutrient source for beneficial bacteria (Liu et al., 2024).

Inulin helps promote the growth of gut bacteria, improves digestion, reduces inflammation, reduces blood cholesterol level, and increases blood sugar level (Slavin, 2013). FOS improves gut health, reduces the risk of colon cancer, improves mineral absorption, and enhances immunity (Sabater-Molina et al., 2009). Beta-Glucan improves gut health (Davani-Davari et al., 2019), while Pectin improves digestion and reduces inflammation (Jackson et al., 2007).

Once in the colon, prebiotics like inulin and oligosaccharides are fermented by the enzymes of gut bacteria, specifically by healthful bacteria like bifidobacteria and lactobacilli. The fermentation process produces short-chain fatty acids such as acetate, propionate, butyrate, along with gases like hydrogen, methane, and carbon dioxide. These help maintain a somewhat acidic environment in the colon, which suppresses the growth of noxious bacteria and pathogens while promoting the growth and activity of beneficial bacteria (Zhou et al., 2024; Peredo-Lovillo et al., 2020).

SCFAs improve nutrient absorption and are energy sources for colonocytes, particularly butyrate (Mishra et al., 2023), which serves as a favored energy source for colonocytes, promoting their health, integrity, and enhancing the production of mucins (Song et al., 2023). Mucins play a crucial role in keeping the integrity of the mucosal barrier, providing protection against pathogens and preventing inflammation. This strengthens the gut barrier function, reduces permeability, and prevents the translocation of harmful substances from the gut into the bloodstream (Ney et al., 2023).

Prebiotics in the colon promote the proliferation of beneficial bacteria, which can have various positive effects on health, including enhanced nutrient absorption, improved gut barrier function, immune system modulation, and potential reduction in inflammation (Peredo-Lovillo et al., 2020). Upon prebiotics fermentation by gut bacteria, short-chain fatty acids, including butyrate, propionate, and acetate, are produced (Kim, 2023). SCFAs have immunomodulatory effects and maintain immune system balance.

Research Indicating Therapeutic Effect of Prebiotic on Atopic Diseases

Moderate evidence is available for prebiotic supplementation to reduce the risk of eczema in high-risk children (Sestito et al., 2020). A shift from healthy gut microbiota to gut microbiota dysbiosis can lead to conditions like atopic dermatitis and allergic diseases (Pantazi et al., 2023). Prebiotics, probiotics, and synbiotics can be used to restore gut microbiota balance and manipulate it like healthy gut microbiota (Sestito et al., 2020).

Human milk contains 200 Human Milk Oligosaccharids (HMO), which induce tolerance and stimulate gut microbiota (Oozeer et al., 2013). Infants who are breastfed have a reduced risk of atopic dermatitis (Lodge et al., 2015). Studies have shown that short-chain galactooligosaccharides (ScGOS) and long-chain fructooligosaccharides (lcFOS) mixtures have prebiotic activities and create a similar gut microbiota to breastfed infants (Knol et al., 2005; Rinne et al., 2005).

Randomized Controlled Trials (RCTs) analyzing prebiotics in children have shown long-term benefits for the prevention of atopic eczema and common infections in healthy infants (Thomas, Greer, Bhatia, et al., 2010). Prebiotics like XOS and Red ginseng Extract (RGE) have been shown to improve the Firmicutes/Bacteroidetes ratio, increase beneficial bacteria, and lower harmful bacteria, helping to restore gut microbiota health distorted by antibiotics (Ibáñez et al., 2018).

Studies Exploring Therapeutic Effect of Prebiotic on Atopic Diseases

However, a comprehensive review of studies shed light on the potential benefits of prebiotic supplementation in infants' reveals varying positive outcomes. Boehm et al. (2002) found that adding oligosaccharides to preterm infants formula increased *Bifidobacteria* levels significantly similar to breast fed infants. Moro et al. (2002), conducted a study on term infants at high risk of atopy and founded that prebiotic supplementation significantly reduced the incidence of atopic dermatitis at 6 months of age. Schmelzle et al. (2003), reported good tolerance and no adverse effects when prebiotics were added to infant formula in a multi-center trial. Scholtens et al. (2006) found that adding prebiotics to solid food had a bifidogenic effect. The GOS was given to healthy term infants resulted in high *Bifidobacteria* (Sierra et al., 2015). Ziegler et al. (2007) observed that infants fed formula with a pre-biotic mixture had growth and stool characteristics similar to breastfed infants, with a lower incidence of eczema. Gruber et al. (2010) demonstrated that a formula containing a specific mixture of oligosaccharides (ScGOS/lcFOS) reduced the risk of atopic dermatitis in low atopy risk infants. Pontes et al. (2016) showed that a cow's milk based beverage with prebiotics reduced allergic manifestations (atopic dermatitis, wheezing, allergic rhinitis) compared to controls. Partially hydrolyzed whey formula containing oligosaccharides pHF-OS showed immune modulatory effects and increased T cells in infants at high risk of allergic diseases stating that may protect against later allergic diseases (Boyle et al., 2016). Study discovered that prebiotic supplementation reduced the commutative incidences of allergic manifestations (allergic wheezing and allergic urticaria) at 2 years of age and atopic dermatitis were significantly reduced at 5 years of age. Concluding prebiotic supplementation in early infancy reduced the risk of atopic diseases at high risk infants (Nisticò and Conti, 2013).

Ranucci et al. (2018) found that prebiotic enriched formula reduced the risk of atopic dermatitis by 35% conferred to standard formula. Wopereis et al. (2018) observed infants fed with prebiotic (partially hydrolyzed formula) showed differences in gut microbiota composition and altered levels of certain compounds, potentially influencing eczema development. Prebiotics have also been shown to increase beneficial gut bacteria and short-chain fatty acids, while decreasing harmful bacteria in a study by Francavilla et al. (2012). Additionally, prebiotics have been found to reduce the concentration of Ig-Free light chain, which is associated with atopic diseases (Schouten et al., 2011).

In adults, prebiotics have been found to have numerous benefits as well. They can help promote a healthy gut microbiome, boost the immune system, and even reduce symptoms of certain diseases (Biagioli et al., 2024).

Prebiotics may be a complementary approach to reducing atopic diseases, particularly when combined with human milk or supplemented feed in early infancy and dietary or supplementary prebiotics in adulthood (Biagioli et al., 2024). The European Commission's Scientific Committee on food has no objections to adding oligosaccharides to infant formula, and the FAO of UNWHO supports prebiotic products as infant formula for infants over 5 months old (Leuschner et al., 2010).

Potential Benefits and Limitations

Regardless of prebiotics studies showing its benefits in improving gut microbiota, increasing healthy bacteria, decreasing harmful bacteria, enhancing selective growth to produce SCFAs, and reducing the severity of atopic conditions like eczema, asthma allergies. Still there are some limitations in therapeutic effect of prebiotics.

The relationship between atopic diseases and gut microbiota is quite complex making it even more challenging (Donald and Finlay, 2023), and individual responses to prebviotics vary due to unique microbes and factors (Li et al., 2014). Improper dosage may also lead to gastrointestinal issues (Ballan et al., 2020). Moreover, due to limited clinical evidence (Kang et al., 2023) and varied prebiotic studies, this makes it difficult to draw a concrete conclusion about its therapeutic results in atopic diseases management. Prebiotics may interact with other treatments or other diets, which may not produce desirable results. Therefore, more research is needed to fully understand its method and their results.

Combination of prebiotics and probiotics can produce more productive result than prebiotic and probiotics alone for atopic conditions. A study showed positive results in reducing atopic dermatitis severity with oral administration of synbiotic supplementation in children. An improved SCORAD index regardless of underlying treatments (Ibáñez et al., 2018).

Conclusion

In conclusion atopic diseases, including allergies and asthma, have become increasingly prevent globally. Factors like genetics, environment, lifestyle and microbial imbalances contribute to their development. Dysbiosis, or disruption of the good microbiota, is linked to atopic diseases. Prebiotic, non-digestible food components that promote beneficial gut bacteria, show promise in modulating the gut microbiota and reducing the risk of atopic diseases. They promote short chain fatty acids production, immune modulation, and gut barrier function, reducing inflammation and allergic responses. While studies have shown varying outcomes, there is growing evidence supporting prebiotics role, especially in high risk infants. Dietary sources rich in prebiotics foods and considering supplementation, especially during infancy and early childhood, may contribute to better overall health and reduce the burden of atopic diseases. Further research is needed to fully understand prebiotic supplementation's mechanism and long-term effects.

REFERENCES

Aachary, A. A., Gobinath, D., Srinivasan, K., and Prapulla, S. G. (2015). Protective effect of xylooligosaccharides from corncob on 1, 2-dimethylhydrazine induced colon cancer in rats. *Bioactive Carbohydrates and Dietary Fibre*, *5*(2), 146–152.

Alderete, T. L., Autran, C., Brekke, B. E., Knight, R., Bode, L., Goran, M. I., and Fields, D. A. (2015). Associations between human milk oligosaccharides and infant body composition in the first 6 mo of life. *The American Journal of Clinical Nutrition*, *102*(6), 1381–1388.

Araya, M., Morelli, L., Reid, G., Sanders, M., Stanton, C., Pineiro, M., and Ben Embarek, P. (2002). Joint FAO/WHO Working Group report on drafting guidelines for the evaluation of probiotics in food. *London, Canada: World Health Organization, Food and Agriculture Organization of the United Nations*.

Arslanoglu, S., Moro, G. E., Schmitt, J., Tandoi, L., Rizzardi, S., and Boehm, G. (2008). Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. *Journal of Nutrition*, *138*(6), 1091–1095. https://doi.org/10.1093/jn/138.6.1091

Ballan, R., Battistini, C., Xavier-Santos, D., and Saad, S. M. I. (2020). Interactions of probiotics and prebiotics with the gut microbiota. *Progress in Molecular Biology and Translational Science*, *171*, 265–300.

Biagioli, V., Volpedo, G., Riva, A., Mainardi, P., and Striano, P. (2024). From birth to weaning: a window of opportunity for microbiota. *Nutrients*, *16*(2), 272.

Björkstén, B. (1999). The intrauterine and postnatal environments. *Journal of Allergy and Clinical Immunology*, *104*(6), 1119–1127.

Boehm, G., Lidestri, M., Casetta, P., Jelinek, J., Negretti, F., Stahl, B., and Marini, A. (2002). Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of faecal bifidobacteria in preterm infants. *Archives of Disease in Childhood-Fetal and Neonatal Edition*, *86*(3), F178–F181.

Boyle, R. J., Tang, M. L. K., Chiang, W. C., Chua, M. C., Ismail, I., Nauta, A., Hourihane, J. O. B., Smith, P., Gold, M., Ziegler, J., Peake, J., Quinn, P., Rao, R., Brown, N., Rijnierse, A., Garssen, J., Warner, J. O., Axelrad, C., Jeffries, S., and Knipping, K. (2016). Prebiotic-supplemented partially hydrolysed cow's milk formula for the prevention of eczema in high-risk infants: A randomized controlled trial. *Allergy: European Journal of Allergy and Clinical Immunology*, *71*(5), 701–710. https://doi.org/10.1111/all.12848

Candela, M., Rampelli, S., Turroni, S., Severgnini, M., Consolandi, C., De Bellis, G., Masetti, R., Ricci, G., Pession, A., and Brigidi, P. (2012). Unbalance of intestinal microbiota in atopic children. *BMC Microbiology*, *12*, 1–9.

Cukrowska, B. (2018). Microbial and nutritional programming—the importance of the microbiome and early exposure to potential food allergens in the development of allergies. *Nutrients*, *10*(10), 1541.

Dahiya, D., and Nigam, P. S. (2023). Biotherapy Using Probiotics as Therapeutic Agents to Restore the Gut Microbiota to Relieve Gastrointestinal Tract Inflammation, IBD, IBS and Prevent Induction of Cancer. *International Journal of Molecular Sciences*, *24*(6). https://doi.org/10.3390/ijms24065748

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J., Berenjian, A., and Ghasemi, Y. (2019). Prebiotics: Definition, types, sources, mechanisms, and clinical applications. *Foods*, *8*(3). https://doi.org/10.3390/foods8030092

Donald, K., and Finlay, B. B. (2023). Early-life interactions between the microbiota and immune system: impact on immune system development and atopic disease. *Nature Reviews Immunology*, *23*(11), 735–748.

Fassio, F., and Guagnini, F. (2018). House dust mite-related respiratory allergies and probiotics: a narrative review. *Clinical and Molecular Allergy*, *16*(1), 15.

Faye, O., Flohr, C., Kabashima, K., Ma, L., Paller, A. S., Rapelanoro, F. R., Steinhoff, M., Su, J. C., Takaoka, R., Wollenberg, A., Yew, Y. W., Postigo, J. A. R., Schmid-Grendelmeier, P., and Taïeb, A. (2023). Atopic dermatitis: A global health perspective. *Journal of the European Academy of Dermatology and Venereology*, *November*, 1–11. https://doi.org/10.1111/jdv.19723

Francavilla, R., Calasso, M., Calace, L., Siragusa, S., Ndagijimana, M., Vernocchi, P., Brunetti, L., Mancino, G., Tedeschi, G., and Guerzoni, E. (2012). Effect of lactose on gut microbiota and metabolome of infants with cow's milk allergy. *Pediatric*

Allergy and Immunology, *23*(5), 420–427.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., and Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews*, *17*(2), 259–275.

Gray, L. E. K., O'Hely, M., Ranganathan, S., Sly, P. D., and Vuillermin, P. (2017). The maternal diet, gut bacteria, and bacterial metabolites during pregnancy influence offspring asthma. *Frontiers in Immunology*, *8*(MAR), 1–13. https://doi.org/10.3389/fimmu.2017.00365

Grüber, C., van Stuijvenberg, M., Mosca, F., Moro, G., Chirico, G., Braegger, C. P., Riedler, J., Boehm, G., Wahn, U., and Group, M. 1 W. (2010). Reduced occurrence of early atopic dermatitis because of immunoactive prebiotics among low-atopy-risk infants. *Journal of Allergy and Clinical Immunology*, *126*(4), 791–797.

Hotel, A. C. P., and Cordoba, A. (2001). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. *Prevention*, *5*(1), 1–10.

Ibáñez, M. D., Rodríguez del Río, P., González-Segura Alsina, D., and Villegas Iglesias, V. (2018). Effect of synbiotic supplementation on children with atopic dermatitis: an observational prospective study. *European Journal of Pediatrics*, *177*, 1851–1858.

Jackson, C. L., Dreaden, T. M., Theobald, L. K., Tran, N. M., Beal, T. L., Eid, M., Gao, M. Y., Shirley, R. B., Stoffel, M. T., and Kumar, M. V. (2007). Pectin induces apoptosis in human prostate cancer cells: correlation of apoptotic function with pectin structure. *Glycobiology*, *17*(8), 805–819.

Kalliomäki, M., Kirjavainen, P., Eerola, E., Kero, P., Salminen, S., and Isolauri, E. (2001). Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *Journal of Allergy and Clinical Immunology*, *107*(1), 129–134.

Kang, M., Jung, J.-H., Kim, J.-Y., Hong, S.-H., and Her, Y. (2023). Therapeutic and preventive effect of orally administered prebiotics on atopic dermatitis in a mouse model. *Allergy, Asthma and Immunology Research*, *15*(3), 303.

Khan, F. F., Sohail, A., Ghazanfar, S., Ahmad, A., Riaz, A., Abbasi, K. S., Ibrahim, M. S., Uzair, M., and Arshad, M. (2023). Recent innovations in non-dairy prebiotics and probiotics: Physiological potential, applications, and characterization. *Probiotics and Antimicrobial Proteins*, *15*(2), 239–263.

Kim, C. H. (2023). Complex regulatory effects of gut microbial short-chain fatty acids on immune tolerance and autoimmunity. *Cellular and Molecular Immunology*, *20*(4), 341–350. https://doi.org/10.1038/s41423-023-00987-1

Knol, J., Scholtens, P., Kafka, C., Steenbakkers, J., Gro, S., Helm, K., Klarczyk, M., Schöpfer, H., Böckler, H.-M., and Wells, J. (2005). Colon microflora in infants fed formula with galacto-and fructo-oligosaccharides: more like breast-fed infants. *Journal of Pediatric Gastroenterology and Nutrition*, *40*(1), 36–42.

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., and Bäckhed, F. (2016). From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell*, *165*(6), 1332–1345.

Kunst, C., Schmid, S., Michalski, M., Tümen, D., Buttenschön, J., Müller, M., and Gülow, K. (2023). The influence of gut microbiota on oxidative stress and the immune system. *Biomedicines*, *11*(5), 1388.

Leuschner, R. G. K., Robinson, T. P., Hugas, M., Cocconcelli, P. S., Richard-Forget, F., Klein, G., Licht, T. R., Nguyen-The, C., Querol, A., and Richardson, M. (2010). Qualified presumption of safety (QPS): a generic risk assessment approach for biological agents notified to the European Food Safety Authority (EFSA). *Trends in Food Science and Technology*, *21*(9), 425–435.

Li, M., Wang, M., and Donovan, S. M. (2014). Early development of the gut microbiome and immune-mediated childhood disorders. *Seminars in Reproductive Medicine*, *32*(01), 74–86.

Lin, T. L., Fan, Y. H., Chang, Y. L., Ho, H. J., Wu, C. Y., and Chen, Y. J. (2022). Early-life infections in association with the development of atopic dermatitis in infancy and early childhood: a nationwide nested case–control study. *Journal of the European Academy of Dermatology and Venereology*, *36*(4), 615–622. https://doi.org/10.1111/jdv.17908

Liu, J., Lu, X., Fang, F., Wu, K., Wu, J., and Gao, J. (2024). Comparison of the in-vitro effect of five prebiotics with different structure on gut microbiome and metabolome. *Food Bioscience*, 103810. https://doi.org/10.1016/j.fbio.2024.103810

Lodge, C. J., Tan, D. J., Lau, M. X. Z., Dai, X., Tham, R., Lowe, A. J., Bowatte, G., Allen, K. J., and Dharmage, S. C. (2015). Breastfeeding and asthma and allergies: a systematic review and meta-analysis. *Acta Paediatrica*, *104*, 38–53

Logoń, K., Świrkosz, G., Nowak, M., Wrześniewska, M., Szczygieł, A., and Gomułka, K. (2023). The Role of the Microbiome in the Pathogenesis and Treatment of Asthma. *Biomedicines*, *11*(6). https://doi.org/10.3390/biomedicines11061618

Markowiak-Kopeć, P., and Śliżewska, K. (2020). The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. *Nutrients*, *12*(4), 1107.

Maslowski, K. M., and Mackay, C. R. (2011). Diet, gut microbiota and immune responses. *Nature Immunology*, *12*(1), 5–9.

Maslowski, K. M., Vieira, A. T., Ng, A., Kranich, J., Sierro, F., Yu, D., Schilter, H. C., Rolph, M. S., Mackay, F., and Artis, D. (2009). Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature*, *461*(7268), 1282–1286.

Mishra, P., Badiyani, V. M., Jain, S., Subramanian, S., Maharaj, S. V., Kumar, A., and Singh, B. N. (2023). Prebiotics: Ignored player in the fight against cancer. *Cancer Reports*, *6*(11), 1–12. https://doi.org/10.1002/cnr2.1870

Moro, G., Minoli, I., Mosca, M., Fanaro, S., Jelinek, J., Stahl, B., and Boehm, G. (2002). Dosage-related bifidogenic effects of galacto-and fructooligosaccharides in formula-fed term infants. *Journal of Pediatric Gastroenterology and Nutrition*, *34*(3), 291–295.

Muir, A. B., Benitez, A. J., Dods, K., Spergel, J. M., and Fillon, S. A. (2016). Microbiome and its impact on gastrointestinal atopy.

Allergy: European Journal of Allergy and Clinical Immunology, *71*(9), 1256–1263. https://doi.org/10.1111/all.12943

Neaville, W. A., Tisler, C., Bhattacharya, A., Anklam, K., Gilbertson-White, S., Hamilton, R., Adler, K., DaSilva, D. F., Roberg, K. A., and Carlson-Dakes, K. T. (2003). Developmental cytokine response profiles and the clinical and immunologic expression of atopy during the first year of life. *Journal of Allergy and Clinical Immunology*, *112*(4), 740–746.

Ney, L. M., Wipplinger, M., Grossmann, M., Engert, N., Wegner, V. D., and Mosig, A. S. (2023). Short chain fatty acids: key regulators of the local and systemic immune response in inflammatory diseases and infections. *Open Biology*, *13*(3). https://doi.org/10.1098/rsob.230014

Nisticò, G., and Conti, P. (2013). Journal of Biological Regulators and Homeostatic Agents: Introduction. *Journal of Biological Regulators and Homeostatic Agents*, *27*(2 SUPPL.).

Oozeer, R., Van Limpt, K., Ludwig, T., Amor, K. Ben, Martin, R., Wind, R. D., Boehm, G., and Knol, J. (2013). Intestinal microbiology in early life: specific prebiotics can have similar functionalities as human-milk oligosaccharides. *The American Journal of Clinical Nutrition*, *98*(2), 561S-571S.

Pantazi, A. C., Mihai, C. M., Balasa, A. L., Chisnoiu, T., Lupu, A., Frecus, C. E., Mihai, L., Ungureanu, A., Kassim, M. A. K., Andrusca, A., Nicolae, M., Cuzic, V., Lupu, V. V., and Cambrea, S. C. (2023). Relationship between Gut Microbiota and Allergies in Children: A Literature Review. *Nutrients*, *15*(11), 1–15. https://doi.org/10.3390/nu15112529

Peredo-Lovillo, A., Romero-Luna, H. E., and Jiménez-Fernández, M. (2020). Health promoting microbial metabolites produced by gut microbiota after prebiotics metabolism. *Food Research International*, *136*(April), 109473. https://doi.org/10.1016/j.foodres.2020.109473

Piccioni, A., Covino, M., Candelli, M., Ojetti, V., Capacci, A., Gasbarrini, A., Franceschi, F., and Merra, G. (2023). How do diet patterns, single foods, prebiotics and probiotics impact gut microbiota? *Microbiology Research*, *14*(1), 390–408.

Pontes, M. V, Ribeiro, T. C. M., Ribeiro, H., Mattos, A. P. De, Almeida, I. R., Leal, V. M., Cabral, G. N., Stolz, S., Zhuang, W., and Scalabrin, D. M. F. (2016). Cow ' s milk-based beverage consumption in 1- to 4-year-olds and allergic manifestations: an RCT. *Nutrition Journal*, 1–10. https://doi.org/10.1186/s12937-016-0138-0

Ranucci, G., Buccigrossi, V., Borgia, E., Piacentini, D., Visentin, F., Cantarutti, L., Baiardi, P., Felisi, M., Spagnuolo, M. I., Zanconato, S., Baraldi, E., Giaquinto, C., and Guarino, A. (2018). Galacto-oligosaccharide/polidextrose enriched formula protects against respiratory infections in infants at high risk of atopy: A randomized clinical trial. *Nutrients*, *10*(3). https://doi.org/10.3390/nu10030286

Rinne, M. M., Gueimonde, M., Kalliomäki, M., Hoppu, U., Salminen, S. J., and Isolauri, E. (2005). Similar bifidogenic effects of prebiotic-supplemented partially hydrolyzed infant formula and breastfeeding on infant gut microbiota. *FEMS Immunology and Medical Microbiology*, *43*(1), 59–65.

Rø, A. D. B., Simpson, M. R., Rø, T. B., Storrø, O., Johnsen, R., Videm, V., and Øien, T. (2017). Reduced Th22 cell proportion and prevention of atopic dermatitis in infants following maternal probiotic supplementation. *Clinical and Experimental Allergy*, *47*(8), 1014–1021.

Roberfroid, M., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., Wolvers, D., Watzl, B., Szajewska, H., and Stahl, B. (2010). Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition*, *104*(S2), S1–S63.

Sabater-Molina, M., Larqué, E., Torrella, F., and Zamora, S. (2009). Dietary fructooligosaccharides and potential benefits on health. *Journal of Physiology and Biochemistry*, *65*, 315–328.

Schmelzle, H., Wirth, S., Skopnik, H., Radke, M., Knol, J., Böckler, H.-M., Brönstrup, A., Wells, J., and Fusch, C. (2003). Randomized Double-Blind Study of the Nutritional Efficacy and Bifidogenicity of a New Infant Formula Containing Partially Hydrolyzed Protein, a High β-Palmitic Acid Level, and Nondigestible Oligosaccharides. *Journal of Pediatric Gastroenterology and Nutrition*, *36*(3). https://journals.lww.com/jpgn/fulltext/2003/03000/randomized_double_blind_study_of_the_nutritional.8.aspx

Scholtens, P. A. M. J., Alles, M. S., Bindels, J. G., van der Linde, E. G. M., Tolboom, J. J. M., and Knol, J. (2006). Bifidogenic effects of solid weaning foods with added prebiotic oligosaccharides: a randomised controlled clinical trial. *Journal of Pediatric Gastroenterology and Nutrition*, *42*(5), 553–559.

Schouten, B., Van Esch, B. C. A. M., Kormelink, T. G., Moro, G. E., Arslanoglu, S., Boehm, G., Knippels, L. M. J., Redegeld, F. A., Willemsen, L. E. M., and Garssen, J. (2011). Non-digestible oligosaccharides reduce immunoglobulin free light-chain concentrations in infants at risk for allergy. *Pediatric Allergy and Immunology*, *22*(5), 537–542.

Scott, K. P., Gratz, S. W., Sheridan, P. O., Flint, H. J., and Duncan, S. H. (2013). The influence of diet on the gut microbiota. *Pharmacological Research*, *69*(1), 52–60.

Sestito, S., D'Auria, E., Baldassarre, M. E., Salvatore, S., Tallarico, V., Stefanelli, E., Tarsitano, F., Concolino, D., and Pensabene, L. (2020). The Role of Prebiotics and Probiotics in Prevention of Allergic Diseases in Infants. *Frontiers in Pediatrics*, *8*(December). https://doi.org/10.3389/fped.2020.583946

Shirsath, N. R., and Zawar, L. R. (2024). Role of Food Supplements in Gut Health. In *Food Supplements and Dietary Fiber in Health and Disease* (pp. 337–357). Apple Academic Press

Sierra, C., Bernal, M.-J., Blasco, J., Martínez, R., Dalmau, J., Ortuno, I., Espín, B., Vasallo, M.-I., Gil, D., and Vidal, M.-L. (2015). Prebiotic effect during the first year of life in healthy infants fed formula containing GOS as the only prebiotic: a multicentre, randomised, double-blind and placebo-controlled trial. *European Journal of Nutrition*, *54*, 89–99.

Slavin, J. (2013). Fiber and prebiotics: mechanisms and health benefits. *Nutrients*, *5*(4), 1417–1435.

Song, C., Chai, Z., Chen, S., Zhang, H., Zhang, X., and Zhou, Y. (2023). Intestinal mucus components and secretion mechanisms: what we do and do not know. *Experimental and Molecular Medicine*, *55*(4), 681–691. https://doi.org/10.1038/s12276-

023-00960-y

Stec, A., Sikora, M., Maciejewska, M., Paralusz-Stec, K., Michalska, M., Sikorska, E., and Rudnicka, L. (2023). Bacterial metabolites: a link between gut microbiota and dermatological diseases. *International Journal of Molecular Sciences*, *24*(4), 3494.

Thomas, D. W., Greer, F. R., Bhatia, J. J. S., Abrams, S. A., Daniels, S. R., Schneider, M. B., Silverstein, J., Stettler, N., Heitlinger, L. A., Baker, R. D., Gremse, D. A., Gugig, R., and Lightdale, J. R. (2010). Clinical report - Probiotics and prebiotics in pediatrics. *Pediatrics*, *126*(6), 1217–1231. https://doi.org/10.1542/peds.2010-2548

Thomsen, S. F. (2015). Epidemiology and natural history of atopic diseases. *European Clinical Respiratory Journal*, *2*(1), 24642. https://doi.org/10.3402/ecrj.v2.24642

Wopereis, H., Sim, K., Shaw, A., Warner, J. O., Knol, J., and Kroll, J. S. (2018). Intestinal microbiota in infants at high risk for allergy: Effects of prebiotics and role in eczema development. *Journal of Allergy and Clinical Immunology*, *141*(4), 1334-1342.e5. https://doi.org/https://doi.org/10.1016/j.jaci.2017.05.054

Zhou, P., Chen, C., Patil, S., and Dong, S. (2024). Unveiling the therapeutic symphony of probiotics, prebiotics, and postbiotics in gut-immune harmony. *Frontiers in Nutrition*, *11*, 1355542.

Ziegler, E., Vanderhoof, J. A., Petschow, B., Mitmesser, S. H., Stolz, S. I., Harris, C. L., and Berseth, C. L. (2007). Term infants fed formula supplemented with selected blends of prebiotics grow normally and have soft stools similar to those reported for breast-fed infants. *Journal of Pediatric Gastroenterology and Nutrition*, *44*(3), 359–364

Chapter 16

Role of Probiotics in Prevention of Avian Coccidiosis

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ABSTRACT

Coccidiosis is one of the most economically important protozoal diseases of avian which is caused by the protozoan genus *Emeria spp*. The probiotics have an important effect on the genus *Emeria*, also helping in the growth of beneficial microbiota in the gut. These have an incredible role in managing the gut microbiota and improve the immune potential of the host against pathogens in the gut. The probiotics are beneficial prokaryotes like bacteria which help in the control of coccidiosis in the avians. The key insight of this very chapter is to discuss how the probiotics acts and help to reduce coccidiosis by preventing the colonization of the *Emeria spps*. in the poultry gut. The prevention against coccidiosis is through competitive exclusion, immunomodulation, and formation of antimicrobial compounds in the gut. This chapter also sheds light on the role of coccidiostats, immunization against coccidia, bioactive compounds and natural alternative in the control of avian coccidiosis. The vaccine helps in the development of the body immunity which last longer and thus reduce the use of antibiotics for the disease control. The mentioned strategies will help in the prevention of coccidiosis and will lead to healthy chicken and food safety and security.

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INTRODUCTION

Poultry production is the source for the provision of chicken meat which is a cheap source of animal protein around the globe (Govoni et al., 2021; Nkukwana, 2018). The USDA reported that 102.9 million tons of poultry meat was produced in January 2020, which was reflected as a 3.9% increase as compared to the previous year (Mesa-Pineda et al., 2021). An increase in the population is expected to reach nine billion by 2050, which is a horrifying sign to produce sustainable and safe protein (Watson et al., 2018). A high stocking density is the predisposing factor for stress and disease prevalence, and it affects the poultry industry (Ahmad et al., 2022). Hence, a disease that impairs the productivity of the earlier indicated production system could be dangerous for the whole food chain (Aganovic et al., 2021).

The poultry sector faces significant losses due to coccidiosis, a hidden enemy caused by intracellular parasites (Blake et al., 2020). In the US, 127 million USD is invested in coccidiosis-related costs annually, with China exceeding 73 million USD, accounting for almost 30% of the total (Geng et al., 2021; Lahlou et al., 2021). Seven species of *Eimeria*, including *Emeria tenella*, *E. acervulina, E. maxima, E. necatrix*, and *E. mitis,* are associated with coccidiosis in poultry. These species attack the bird's intestines and exhibit specific cytotoxicity, preventing the body from metabolizing proteins and nutrients (Kers et al., 2018). The preventive immunization is crucial for the poultry industry's development and income (Hamid et al., 2018). In Europe, antiparasitic drugs with feed are used for broiler chickens, simplifying EU regulations for poultry health and wellbeing (Martins et al., 2022).

Since 1930, synthetic anticoccidial medications and ionophores have been used to combat dangerous parasites in chickens (Nogueira et al., 2009). However, antiparasitic resistance develops due to drug metabolites, negatively impacting human health (Nahed et al., 2022). The coccidiostats, synthetic drugs that inhibit Emeria's growth, are widely used. Coccidiosis, which is the major economic disease of poultry, is caused by the protozoan called *Emeria* (Noack et al., 2019). There are two classes of about ten coccidiostats which function as a feed additive approved by the European union for the use in poultry. Probiotics are initially provided to the birds from their first day of life to seven days to prevent the disease caused by the protozoan oocyst before they are marketed as meat for human consumption. This managemental procedure is very applicable and this helps the birds from coccidiosis. The probiotics which fortified the beneficial microorganisms including yeast and fungi have proved to boost performance and immunity of the intestine in the host and improve the gut microflora that reducing the risk of coccidiosis in the birds (Ahmad et al., 2022). Additionally, probiotics also can reduce the growth of other infectious agents in the gut and hence protect the birds' intestinal villi from damage by the toxins producing organism in the feed. In another study it has been found that the use of *Bacillus* strain orally has a significant effect on reducing the colonization of *Emeria* in the intestine of broiler birds and hence the use of bacillus strain is indispensable in the broiler feed (Gururajan et al., 2021).

The actual mechanism of action of probiotics bacterial strains acts as a competitor with *Emeria* for attaching to the intestinal epithelium receptors and thus occupy the receptors and inhibit the attachment of the *Emeria* to the epithelium its replication and oocyst shedding to the environment. Sometimes in case of acute coccidiosis the effect of probiotics and prebiotics are not proficient and so in such a case alternative needs to be identified (Nesse et al., 2019).

Probiotics and Gut Microbiota

The probiotics are compounds which play a key role in the composition of the microflora in the gastrointestinal tract. These beneficial microbes bind with the receptors in the intestinal mucosa and compete with the pathogens including *Emeria* and thus produce antimicrobial compounds which inhibit the growth of these pathogenic organisms (Abd El-Hack et el., 2020). The probiotics work as an antagonist by producing organic acids, change the gut pH and immunomodulation and have a significant positive effect on the epithelial cells, transduction pathways of microflora, intestinal surface integrity and immunity (Arif et al., 2021; Rajput et al., 2020).

The use of different molecular techniques like, metagenomic sequencings, *in-vivo* assay and culture have revealed the effect of the probiotics on the shift, function, and arrangement microflora of the GIT. Nevertheless, the effective and efficient method of getting the beneficial effect is the application through *in-vivo* method (Foligne et al., 2007). The *in-vivo* administration of the probiotic's strains like *Bacillus bifidum*, *B. animalis*, *Bifidobacterium longum* and *Bifidobacterium infants*. The *Lactobacillus* and *Bifidobacterium* have improved the bacterial population of the ileum by enhancing the intestinal colonization fermentation and reducing the coliforms bacteria (El-Moneim et al., 2020).

Mode of Action (Probiotics)

The unique mode of action of probiotics in gastrointestinal tract is competition with other pathogens by covering the specific receptors for their attachment in the intestinal epithelium that help eliminate the other pathogens to enter the epithelium and damage the gut microflora by bacterial belligerence or competitive exclusion. Interestingly this mode of action mostly possesses all the probiotics, prebiotics and symbiotics (Abd El-hack et al., 2020). Apart from this there is another concept called "Nurmi" in which resistance is developed in the gut microbiota by injecting the infectious agent into the chicken GIT (Bajagai et al., 2016). Similarly, probiotics attached to the epithelial surface both inner and outer and help in development and improve the digestion which is mostly investigated in the caeca and intestine of the avians (Ahmad et al., 2022; Agyare et al., 2018; Zaefarian et al., 2016).

The *Bacillus amyloliquefaciens* (BAP) accelerates digestion, nutrients absorption and availability in GIT, thus BAP mixed feed for 35 days (20g/kg) drastically accelerated the growth of broilers. The oral administration of spores of the genus *Bacillus* is one technique of competitive exclusion that may strengthen and promote host defense against coccidiosis.

Approaches to Control Avian Coccidiosis

To prevent the coccidiosis in the farm, there are different strategies implied like coccidial vaccination, use of feed additives, prophylactic use of anticoccidial drugs and farm management especially the litter and beddings (Broom, 2021). To ensure healthy poultry it is important to follow all the handling and managemental to minimize the stress and to produce a high-quality healthy poultry product (Dhaka et al., 2023).

The proper management of the farms' birds includes provision of stress-free environment, superior quality feed, water, feed supplements, optimum lighting, proper ventilation, and temperature. To control and prevent coccidiosis in the poultry farm it is utmost important to practice farm biosecurity. Maintaining litter conditions, reducing oocyst sporulation, and using anticoccidials (prophylaxis) are also essential for producing high-quality chickens. Regular cleaning, regular disinfection, and clean water usage are also essential for maintaining a healthy poultry farm (Tilli et al., 2022; Abebe and Gugsa, 2018).

Use of Coccidiostats

Since the 1950s, poultry and turkeys have been fed anticoccidial feed additives to prevent growth. Agri Stats Inc reports that 99% birds were administered anticoccidial drugs in the late 1900s (Chapman, 2009). However, 60% of broiler meat in the US is produced without these agents (Mesa-Pineda, et al., 2021). Anticoccidial agents are categorized as coccidiostats or coccidiocides based on their mode of action. Coccidiostats limit microbe growth and reproduction, while coccidiocides destroy pathogens and cause irreversible damage (Nahed et al., 2022).

Coccidiostats are two types of antibiotics, primarily synthetic compounds and ionophores. Streptomycetacae family bacteria produce natural substances like polyether ionophores. Synthetic coccidiostats, also known as chemicals, change ion concentration ratios on cell membranes through dimerization and binding (Dembitsky, 2022; Clarke et al., 2014;

Muthamilselvan et al., 2016). They modulate ion concentrations, leading to less cytotoxicity and energy production (Miller and Zachary, 2017). The EU has authorized eleven coccidiostats, primarily synthetic compounds and ionophores, to prevent disease spread, reduce parasite multiplicity, and strengthen the immune system. Ionophores target sporozoites before host cell penetration, allowing some to survive and develop host immunity (Nesse et al., 2019; Noack et al., 2019).

Vaccines

The coccidiosis control strategies rely on vaccination, which stimulates the immune system to defend against *Eimeria* hazards (Lee et al., 2022; Shivaramaiah et al., 2014). The vaccinations are a crucial substitute for eradicating coccidiosis, but they must be effective and provide adequate protection to poultry. Vaccines contain oocysts from *Eimeria* strains, with *E. maxima* oocysts causing the highest immune response (Attree et al., 2021). The adaptive immune response can be stimulated in 3-4 weeks, depending on the host's genetic makeup, infection duration, and parasite concentration (Martins et al., 2022). However, the current vaccination program is challenging due to the uncertainty of exposure to the same amount of coccidian. The *in-ovo* immunization, administered to 18-day embryonated chicken eggs, is a recent advancement that ensures accurate and consistent administration of vaccines to the embryo's amniotic sac (Williams, 2005).

The *in-ovo* inoculation is a method that delivers chemicals directly to chicken embryos during incubation stages, potentially controlling their gastrointestinal growth. Introduced in 2003, it involves injecting nutrients and chemicals into embryonic amnion to stimulate growth (Arain et al., 2022). In a research investigation Lee et al. (2022) stated that a micronutrient, selenium can modulate the immune response of broilers exposed to the *E. maxima* and *C. perfreingens*. Because the high immune response against the exposed pathogen were recorded in the selenium treated group as compared to the control and hence very minute intestinal damage and small number of oocysts were recorded. Similarly in another study conducted by Stadnicka et al. (2020) reported that the use of raffinose from lupine seed has a significant effect on the growth of pathogenic bacteria *C. perfreingens* and *Emeria* oocyst shedding.

The use of probiotics *in-ovo* 17 days post incubation have significant effects on the colonization of all *Emeria* species and thus limit their pathogenesis (Pender et al., 2016). Another study by Sokale et al. (2017) reported that *in-ovo* vaccination before hatching against coccidiosis in broiler has a significant effect on immunity development and prevention against subsequent exposure. The use of live vaccines (Inovocox, Pfizer) during incubation produces protective immunity in the birds (Zaheer et al., 2022).

The use of recombinant DNA vaccine (EtMIC2) has also a significant effect on the boosting of immune response against the coccidiosis in the gastrointestinal tract of the poultry birds (Huang et al., 2020). Similarly, Yuan et al. (2022) found that the *in-ovo* use of recombinant protein based vaccines is highly effective immunity booster. The feed ionophores combination during inoculation in commercial poultry improve the bird’s performance (Hamid et al., 2018). The vaccines can be offered topically, directly, or in the hatchery (Blake et al., 2021). The EU initiated vaccination programs for laying pullets, commercial broilers, and replacement breeders in 1992 and 2000 (Abebe and Gugsa, 2018). Common vaccination forms include attenuated, non-attenuated, and recombinant (Arczewska-Włosek et al., 2022).

Natural Alternatives

Several alternative coccidiosis control techniques are accessible that capitalize on less veterinary drugs in the feed. Natural remedies such as prebiotics and probiotics, plant and fungal extracts and essential oils are examples of alternative pharmaceutical methodologies. Normally, natural compounds modify GIT flora and the immune system instead of tackling parasites (Abd El-Hack et al., 2022).

Garlic (*Allium sativum*), a medicinal herb, contains allicin, a significant organosulfur component, which contributes over 70% of all thiosulphates and gives it its scent (Kovarovič et al., 2019). It also contains diallyl sulfide and diallyl trisulphide, which offer garlic anti-inflammatory and antioxidant properties. Garlic's anticoccidial property is linked to its immune-suppressive activity (Kim et al., 2013). Aqueous garlic extract contains phenols, flavonoids, and other sulfur compounds, which alter the cytoplasmic membrane's permeability, affecting molecular physiological activities, reducing membrane potential, cellular loss, and cellular death (Bhavaniramya et al., 2019; Jang et al., 2018; Christaki, et al., 2004).

The anti-inflammatory, antiviral, immunomodulatory and antioxidant are the medicinal properties possess by the garlic powder and its different extracts including flavonoids, phenols, diallyl disulfhide, and essential oils (Ali et al., 2019; Alnassan et al., 2015).

The herbal and medicinal plant *Artemisia annua* belongs to the family *Asteracae* and is a perineal plant that has antimalarial and anticoccidial properties (Coroian et al., 2022; Hong 2014). On contrary to Artemisinin the plant ingredient combination does not reduce malaria (Li et al., 2018; Cai et al., 2017).

The supplementation of the *A. annua* improves the feed conversion ratio in the layer by reducing the body weight compared to the control group (Lang et al., 2019).

Another member of the family *Asteracae* is *Biden Pilosa* (BP) is a medicinal plant used for more than 41 types of infectious diseases including coccidiosis and it also promotes the gut microflora and inhibit the growth of pathogenic microbes in the GI tract of the poultry (Mtenga and Ripanda, 2022; Khater et al., 2020; Uysal et al., 2018).

The use of BP at dose rate of 0.025% significantly improves the growth performance of the birds by inhibiting the colonization of the *Emeria* and shedding of oocysts in the faces. The BP in combination with probiotics in the poultry feed significantly inhibit the colonization of the *Emeria* infection and thus function as potent coccidiostat (Memon et al., 2021).

Bioactive Compound

Prebiotics

The commonly used prebiotics like fructo-oligosaccharides, oxylo oligosaccharides, inulines and mannan oligosaccharides in the poultry is to control the coccidiosis, these prebiotics help promotes the multiplication and activation of probiotics bacteria which inhibit the growth of the *Emeria* infection. The actual mechanism by which these prebiotics are working is that they stimulate the gut associated inflammatory response and activation of tissue macrophages and thus limiting the infectivity and virulency of the *Emeria* in the intestine and help in the control of the coccidiosis in the poultry birds (Santos et al., 2022; Adhikari et al., 2020; Gadde et al., 2017; Assis et al., 2010).

Probiotics

The probiotics include beneficial bacteria, fungi and yeast have a significant effect on the *Emeria* species which are responsible for coccidiosis in the poultry. These beneficial microbes promote the growth of gut microflora, stimulate immunity, and enhance the bird's performance by reducing the feed conversion ratio of the flock (Ahmad et al., 2022). The report of the Yin et al. (2014) indicated that probiotics like *Pediococcus* showed significant protection against *E. tenella* in challenge study in birds. Similarly, if the probiotics are used in a combined form significantly modulate the immune system and protect the birds from the Emeria infection (Wang et al., 2019).

The unique mode of action of probiotics in gastrointestinal tract is competition with other pathogens by covering the specific receptors for their attachment in the intestinal epithelium that help eliminate the other pathogens to enter the epithelium and damage the gut microflora by bacterial belligerence or competitive exclusion. Interestingly, this mode of action mostly possesses all the probiotics, prebiotics and symbiotics (Abd El-hack et al., 2020).

REFERENCES

Abd El-Hack, M. E., El-Saadony, M. T., Salem, H. M., El-Tahan, A. M., Soliman, M. M., Youssef, G. B., and Swelum, A. A. (2022). Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird health and production. *Poultry Science*, *101*(4), 101696.

Abd El-Hack, M. E., El-Saadony, M. T., Shafi, M. E., Qattan, S. Y., Batiha, G. E., Khafaga, A. F., and Alagawany, M. (2020). Probiotics in poultry feed: A comprehensive review. *Journal of Animal Physiology and Animal Nutrition*, *104*(6), 1835-1850.

Abebe, E., and Gugsa, G. (2018). A review on poultry coccidiosis. *Abyssinia Journal of Science and Technology*, *3*(1), 1-12.

Adhikari, P., Kiess, A., Adhikari, R., and Jha, R. (2020). An approach to alternative strategies to control avian coccidiosis and necrotic enteritis. *Journal Applied Poultry Research* 29:515-34. https://doi.org/ 10.1016/j.japr.2019.11.005

Aganovic, K., Hertel, C., Vogel, R. F., Johne, R., Schlüter, O., Schwarzenbolz, U., and Heinz, V. (2021). Aspects of high hydrostatic pressure food processing: Perspectives on technology and food safety. *Comprehensive Reviews in Food Science and Food Safety*, *20*(4), 3225-3266. https://doi.org/10.1111/1541-4337.12763.

Agyare, C., Boamah, V. E., Zumbi, C. N., and Osei, F. B. (2018). Antibiotic use in poultry production and its effects on bacterial resistance. *Antimicrobial resistance—A global threat*, 33-51.

Ahmad, R., Yu, Y. H., Hsiao, F. S. H., Su, C. H., Liu, H. C., Tobin, I., and Cheng, Y. H. (2022). Influence of heat stress on poultry growth performance, intestinal inflammation, and immune function and potential mitigation by probiotics. *Animals*, *12*(17), 2297.

Ali, M., Chand, N., Khan, R. U., Naz, S., and Gul, S. (2019). Anticoccidial effect of garlic (Allium sativum) and ginger (Zingiber officinale) against experimentally induced coccidiosis in broiler chickens. *Journal of Applied Animal Research*, 47(1), 79-84. https://doi.org/10.1080/09 712119.2019.1573731.

Alnassan, A. A., Thabet, A., Daugschies, A., and Bangoura, B. (2015). In vitro efficacy of allicin on chicken Eimeria tenella sporozoites. *Parasitology Research*, *114*, 3913-3915.

Arain, M. A., Nabi, F., Marghazani, I. B., Hassan, F. U., Soomro, H., Kalhoro, H., and Buzdar, J. A. (2022). In ovo delivery of nutraceuticals improves health status and production performance of poultry birds: a review. *World's Poultry Science Journal*, *78*(3), 765-788.

Arczewska-Włosek, A., Świątkiewicz, S., Ognik, K., andJózefiak, D. (2022). Effects of a dietary multi-strain probiotic and vaccination with a live anticoccidial vaccine on growth performance and haematological, biochemical, and redox status indicators of broiler chickens. *Animals*, *12*(24), 3489.

Arif, M., Akteruzzaman, M., Islam, S. S., Das, B. C., Siddique, M. P., and Kabir, S. L. (2021). Dietary supplementation of Bacillus-based probiotics on the growth performance, gut morphology, intestinal microbiota, and immune response in low biosecurity broiler chickens. *Veterinary and Animal Science*, *14*, 100216.

Assis, R. C. L., Luns, F. D., Beletti, M. E., Assis, R. L., Nasser, N. M., Faria, E. S. M., and Cury, M. C. (2010). Histomorphometry and macroscopic intestinal lesions in broilers infected with Eimeria acervulina. *Veterinary Parasitology*, 168(3-4), 185-189. https://doi. org/10.1016/j.vetpar.2009.11.017

Attree, E., Sanchez-Arsuaga, G., Jones, M., Xia, D., Marugan-Hernandez, V., Blake, D., and Tomley, F. (2021). Controlling the causative agents of coccidiosis in domestic chickens; an eye on the past and considerations for the future. *CABI Agriculture and Bioscience*, *2*, 1-16.

Bajagai, Y. S., Klieve, A. V., Dart, P. J., and Bryden, W. L. (2016). Probiotics in animal nutrition: production, impact, and regulation. *FAO Animal Production and Health Paper (FAO) eng no. 179*.

Bhavaniramya, S., Vishnupriya, S., Al-Aboody, M. S., Vijayakumar, R., and Baskaran, D. (2019). Role of essential oils in food safety: Antimicrobial and antioxidant applications. *Grain and Oil Science and Technology*, *2*(2), 49-55

Blake, D. P., Knox, J., Dehaeck, B., Huntington, B., Rathinam, T., Ravipati, V., and Tomley, F. M. (2020). Re-calculating the cost of coccidiosis in chickens. *Veterinary Research*, *51*, 1-14.

Blake, D. P., Marugan-Hernandez, V., and Tomley, F. M. (2021). Spotlight on avian pathology: Eimeria and the disease coccidiosis. *Avian Pathology*, *50*(3), 209-213.

Broom, L. J. (2021). Evidence-based consideration of dietary 'alternatives' to anticoccidial drugs to help control poultry coccidial infections. *World's Poultry Science Journal*, *77*(1), 43-54.

Cai, T. Y., Zhang, Y. R., Ji, J. B., and Xing, J. (2017). Investigation of the component in Artemisia annua L. leading to enhanced antiplasmodial potency of artemisinin via regulation of its metabolism. *Journal of Ethnopharmacology*, *207*, 86-91.

Chapman, H. D. (2009). A landmark contribution to poultry science—prophylactic control of coccidiosis in poultry. *Poultry Science*, *88*(4), 813-815.

Christaki, E., Florou-Paneri, P., Giannenas, I., Papazahariadou, M., Botsoglou, N. A., andSpais, A. B. (2004). Effect of a mixture of herbal extracts on broiler chickens infected with Eimeria tenella. *Animal Research*, *53*(2), 137-144.

Clarke, L., Fodey, T. L., Crooks, S. R., Moloney, M., O'Mahony, J., Delahaut, P., and Danaher, M. (2014). A review of coccidiostats and the analysis of their residues in meat and other food. *Meat Science*, *97*(3), 358-374.

Coroian, M., Pop, L. M., Popa, V., Friss, Z., Oprea, O., Kalmár, Z., and Györke, A. (2022). Efficacy of Artemisia annua against Coccidiosis in Broiler Chickens: A Field Trial. *Microorganisms*, *10*(11), 2277.

Dembitsky, V. M. (2022). Natural polyether ionophores and their pharmacological profile. *Marine Drugs*, *20*(5), 292.

Dhaka, P., Chantziaras, I., Vijay, D., Bedi, J. S., Makovska, I., Biebaut, E., and Dewulf, J. (2023). Can improved farm biosecurity reduce the need for antimicrobials in food animals? A scoping reviews. *Antibiotics*, *12*(5), 893.

El-Moneim, A. E. M. E. A., El-Wardany, I., Abu-Taleb, A. M., Wakwak, M. M., Ebeid, T. A., and Saleh, A. A. (2020). Assessment of in ovo administration of Bifidobacterium bifidum and Bifidobacterium longum on performance, ileal histomorphometry, blood hematological, and biochemical parameters of broilers. *Probiotics and Antimicrobial Proteins*, 12, 439-450.

Foligne, B., Nutten, S., Grangette, C., Dennin, V., Goudercourt, D., Poiret, S., and Pot, B. (2007). Correlation between in vitro and in vivo immunomodulatory properties of lactic acid bacteria. *World Journal of Gastroenterology: WJG*, *13*(2), 236.

Gadde, U., Kim, W. H., Oh, S. T., and Lillehoj, H. S. (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. *Animal Health Research Reviews*, 18(1), 26-45. https://doi.org/10.1017/S1466252316000207

Geng, T., Ye, C., Lei, Z., Shen, B., Fang, R., Hu, M., and Zhou, Y. (2021). Prevalence of Eimeria parasites in the Hubei and Henan provinces of China. *Parasitology Research*, 120, 655-663. https://doi.org/10.1007/s00436-020-07010-w.

Govoni, C., Chiarelli, D. D., Luciano, A., Ottoboni, M., Perpelek, S. N., Pinotti, L., and Rulli, M. C. (2021). Global assessment of natural resources for chicken production. *Advances in Water Resources*, *154*, 103987.

Gururajan, A., Rajkumari, N., Devi, U., and Borah, P. (2021). Cryptosporidium and waterborne outbreaks–A mini review. *Tropical Parasitology*, 11(1), 11-15. https://doi.org/10.4103/tp.TP_68_20.

Hamid, P. H., Kristianingrum, Y. P., Wardhana, A. H., Prastowo, S., and Silva, L. M. R. D. (2018). Chicken coccidiosis in Central Java, Indonesia: A recent update. *Veterinary Medicine International*, *2018*.

Hong, S. C. (2014). Artemisia Annua, Artemisinin, ACTs, and Malaria Control in Africa: Tradition, Science, and Public Policy. By Dana G. Dalrymple. Washington DC: Politics and Prose. 2012. Pp. 254. $18.00, paper. *The Journal of Economic History*, *74*(1), 304-306.

Huang, H., Jiang, Y., Zhou, F., Shi, C., Yang, W., Wang, J., and Yang, G. (2020). A potential vaccine candidate towards chicken coccidiosis mediated by recombinant Lactobacillus plantarum with surface displayed EtMIC2 protein. *Experimental Parasitology*, *215*, 107901.

Jang, H. J., Lee, H. J., Yoon, D. K., Ji, D. S., Kim, J. H., and Lee, C. H. (2018). Antioxidant and antimicrobial activities of fresh garlic and aged garlic by-products extracted with different solvents. *Food Science and Biotechnology*, *27*, 219-225.

Kers, J. G., Velkers, F. C., Fischer, E. A., Hermes, G. D., Stegeman, J. A., and Smidt, H. (2018). Host and environmental factors affecting the intestinal microbiota in chickens. *Frontiers in Microbiology*, *9*, 322066.

Khater, H. F., Ziam, H., Abbas, A., Abbas, R. Z., Raza, M. A., Hussain, K., and Selim, A. (2020). Avian coccidiosis: Recent advances in alternative control strategies and vaccine development. *Agrobiology Record*, 1, 11-25. https://doi.org/10.47278/ journal.abr/2020.003 .

Kim, D. K., Lillehoj, H. S., Lee, S. H., Lillehoj, E. P., and Bravo, D. (2013). Improved resistance to Eimeria acervulina infection in chickens due to dietary supplementation with garlic metabolites. *British Journal of Nutrition*, *109*(1), 76-88.

Kovarovič, J., Bystricka, J., Vollmannova, A., Toth, T., and Brindza, J. (2019). Biologickyhodnotnélátky v cesnaku (Allium sativum L.)–Prehľad. *Journal of Central European Agriculture*, *20*(1), 292-304.

Lahlou, R. A., Bounechada, M., Mohammedi, A., Silva, L. R., and Alves, G. (2021). Dietary use of Rosmarinus officinalis and Thymus vulgaris as anticoccidial alternatives in poultry. *Animal Feed Science and Technology*, 273, 114826. https://doi.org/10.1016/j.anifeedsci.2021. 114826.

Lang, S. J., Schmiech, M., Hafner, S., Paetz, C., Steinborn, C., Huber, R., and Simmet, T. (2019). Antitumor activity of Artemisia annua herbal preparation and identification of active ingredients. *Phytomedicine*, *62*, 152962.

Lee, Y., Lu, M., and Lillehoj, H. S. (2022). Coccidiosis: recent progress in host immunity and alternatives to antibiotic strategies. *Vaccines*, *10*(2), 215.

Li, J., Zhang, C., Gong, M., and Wang, M. (2018). Combination of artemisinin-based natural compounds from Artemisia annua L. for the treatment of malaria: Pharmacodynamic and pharmacokinetic studies. *Phytotherapy Research*, *32*(7), 1415-1420.

Martins, R. R., Silva, L. J., Pereira, A. M., Esteves, A., Duarte, S. C., and Pena, A. (2022). Coccidiostats and poultry: A comprehensive review and current legislation. *Foods*, *11*(18), 2738.

Memon, F. U., Yang, Y., Lv, F., Soliman, A. M., Chen, Y., Sun, J., and Si, H. (2021). Effects of probiotic and Bidens pilosa on the performance and gut health of chicken during induced Eimeria tenella infection. *Journal of Applied Microbiology*, 131(1), 425-434. https://doi.org/10.1111/jam.14928

Mesa-Pineda, C., Navarro-Ruíz, J. L., López-Osorio, S., Chaparro-Gutiérrez, J. J., and Gómez-Osorio, L. M. (2021). Chicken coccidiosis: from the parasite lifecycle to control of the disease. *Frontiers in Veterinary Science*, *8*, 787653.

Miller, M. A., and Zachary, J. F. (2017). Mechanisms and morphology of cellular injury, adaptation, and death. *Pathologic Basis of Veterinary Disease* 2.

Mtenga, D. V., and Ripanda, A. S. (2022). A review on the potential of underutilized Blackjack (Biden Pilosa) naturally occurring in sub-Saharan Africa. *Heliyon*, 8(6). https://doi.org/10. 1016/j.heliyon. 2022 e09586.

Muthamilselvan, T., Kuo, T. F., Wu, Y. C., and Yang, W. C. (2016). Herbal remedies for coccidiosis control: A review of plants, compounds, and anticoccidial actions. *Evidence-based Complementary and Alternative Medicine: eCAM*, *2016*.

Nahed, A., Abd El-Hack, M. E., Albaqami, N. M., Khafaga, A. F., Taha, A. E., Swelum, A. A., and Elbestawy, A. R. (2022). Phytochemical control of poultry coccidiosis: a review. *Poultry Science*, 101(1), 101542. https://doi.org/10.1016/j.psj.2021.101542

Nesse, L. L., Bakke, A. M., Eggen, T., Hoel, K., Kaldhusdal, M., Ringø, E., and Krogdahl, Å. (2019). The risk of development of antimicrobial resistance with the use of coccidiostats in poultry diets.

Nkukwana, T. T. (2018). Global poultry production: Current impact and outlook on the South African poultry industry. *South African Journal of Animal Science*, *48*(5), 869-884.

Noack, S., Chapman, H. D., and Selzer, P. M. (2019). Anticoccidial drugs of the livestock industry. *Parasitology Research*, *118*, 2009-2026.

Nogueira, V. A., França, T. N., and Peixoto, P. V. (2009). Ionophore poisoning in animals. *Pesquisa Veterinária Brasileira*, 29, 191-197. https://doi.org/10. 1590/S0100-736X2009000300001

Pender, C. M., Kim, S., Potter, T. D., Ritzi, M. M., Young, M., and Dalloul, R. A. (2016). Effects of in ovo supplementation of probiotics on performance and immunocompetence of broiler chicks to an Eimeria challenge. *Beneficial Microbes,* 7(5), 699-705.

Rajput, D. S., Zeng, D., Khalique, A., Rajput, S. S., Wang, H., Zhao, Y., and Ni, X. (2020). Pretreatment with probiotics ameliorates gut health and necrotic enteritis in broiler chickens, a substitute to antibiotics. *Amb Express*, *10*, 1-11.

Santos, R. R., Velkers, F. C., Vernooij, J. C. M., Star, L., Heerkens, J. L. T., van Harn, J., and de Jong, I. C. (2022). Nutritional interventions to support broiler chickens during Eimeria infection. *Poultry Science*, 101(6), 101853. https://doi.org/10.1016/j.psj.2022. 101853.

Shivaramaiah, C., Barta, J. R., Hernandez-Velasco, X., Téllez, G., and Hargis, B. M. (2014). Coccidiosis: recent advancements in the immunobiology of Eimeria species, preventive measures, and the importance of vaccination as a control tool against these Apicomplexan parasites. *Veterinary Medicine: Research and Reports*, 23-34.

Sokale, A. O., Zhai, W., Pote, L. M., Williams, C. J., and Peebles, E. D. (2017). Effects of coccidiosis vaccination administered by in ovo injection on Ross 708 broiler performance through 14 days of post-hatch age. *Poultry Science*, *96*(8), 2546-2551.

Stadnicka, K., Bogucka, J., Stanek, M., Graczyk, R., Krajewski, K., Maiorano, G., and Bednarczyk, M. (2020). Injection of raffinose family oligosaccharides at 12 days of egg incubation modulates the gut development and resistance to opportunistic pathogens in broiler chickens. *Animals*, 10(4), 592.

Tilli, G., Laconi, A., Galuppo, F., Mughini-Gras, L., and Piccirillo, A. (2022). Assessing biosecurity compliance in poultry farms: a survey in a densely populated poultry area in northeast Italy. *Animals*, *12*(11), 1409.

Uysal, S., Ugurlu, A., Zengin, G., Baloglu, M. C., Altunoglu, Y. C., Mollica, A., and Mahomoodally, M. F. (2018). Novel in vitro and in silico insights of the multi-biological activities and chemical composition of Bidens tripartita L. *Food and Chemical Toxicology*, 111, 525-536. https://doi.org/10.1016/j.fct.2017.11.058

Wang, X., Farnell, Y. Z., Kiess, A. S., Peebles, E. D., Wamsley, K. G., and Zhai, W. (2019). Effects of Bacillus subtilis and coccidial vaccination on cecal microbial diversity and composition of Eimeria-challenged male broilers. *Poultry Science*, *98*(9), 3839-3849.

Williams, R. B. (2005). Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. *Avian Pathology*, *34*(3), 159-180.

Watson, R. R., Singh, R. B., and Takahashi, T. (2018). The role of functional food security in global health. Academic Press.

Yin, G., Lin, Q., Wei, W., Qin, M., Liu, X., Suo, X., and Huang, Z. (2014). Protective immunity against Eimeria tenella infection in chickens induced by immunization with a recombinant C-terminal derivative of EtIMP1. *Veterinary Immunology and Immunopathology*, 162(3-4), 117-121. https://doi. org/10.1016/j.vetimm.2014.10.009.

Yuan, B., Sun, Z., Lu, M., Lillehoj, H., Lee, Y., Liu, L., and Li, C. (2022). Immunization with pooled antigens for Clostridium perfringens conferred partial protection against experimental necrotic enteritis in broiler chickens. *Vaccines*, *10*(6), 979.

Zaefarian, F., Abdollahi, M. R., and Ravindran, V. (2016). Particle size and feed form in broiler diets: impact on gastrointestinal tract development and gut health. *World's Poultry Science Journal*, *72*(2), 277-290.

Zaheer, T., Abbas, R. Z., Imran, M., Abbas, A., Butt, A., Aslam, S., and Ahmad, J. (2022). Vaccines against chicken coccidiosis with reference to previous decade: progress, challenges, and opportunities. *Parasitology Research*, *121*(10), 2749-2763

Chapter 17

Use of Prebiotic, Probiotic and Synbiotic Growth Promoters in the Modern Poultry Farming: An Updated Review

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ABSTRACT

One of the biggest and rapidly emerging global health challenges is antimicrobial resistance (AMR) which is included among the top ten priorities of the WHO. Several factors, such as the overuse, misuse, or underutilization of antibiotics in clinical settings, are associated with antibiotic resistance. Furthermore, overwhelming use of antibiotics is a major factor in the rise in antibiotic resistance in the environment and in foodborne pathogens. Probiotics, prebiotics, and synbiotics provide the most sustainable substitutes for antibiotics in these restrictive and difficult circumstances. Prebiotics combined with probiotics have a beneficial effect in terms of growth performance, carcass yield and intestinal morphology of the modern poultry farming, thereby avoiding the rising AMR.

KEYWORDS
Broilers, Non-antibiotic, Growth promoters, Antimicrobial resistance

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INTRODUCTION

The widespread use of antibiotic growth promoters in poultry reduces the intestinal pathogens but leads to the antibiotics residue in poultry products (Mathur and Singh, 2005), thereby increasing the incidence of AMR. Currently, researchers are in continuous search for an effective antibiotic substitute such as prebiotics, probiotics, acidifiers, and phenolic compounds (Baurhoo et al., 2009).

The medical use of antibiotics has been constantly increasing and their effectiveness against diseases is decreasing which results in widespread AMR. One of the major factors of AMR is the widespread use of antibiotics in cattle and poultry production. Therefore, to improve poultry performance and prevent and treat infections, it becomes necessary to discover alternatives to the usage of antibiotics such as probiotics, prebiotics, acidifiers, and phenolic compounds for poultry diet as growth promoter (Baurhoo et al., 2009). Use of antibiotics and probiotics resulted in better FCR in birds fed probiotics supplemented diet (Gao et al., 2017). Probiotics have better effect than salinomycin on production performance in broiler and as use of probiotic reduced the *E. Coli* (Ritzi et al., 2014). Probiotics has an impact on growth performance such that birds fed a diet containing protexin, primalac and calciparine had greater weight gain and improved FCR (Shabani et al., 2012). Probiotics had a beneficial effect on digestibility, performance and microflora composition (Mountzouris et al., 2010).

Probiotics are defined as "dietary supplements containing live microorganisms that positively impact the host organism through their advantageous effects linked to the enhancement of the microbial balance within the intestine." The performance of broilers is enhanced when probiotics are added to their diet. The livability of birds fed supply with *Lactobacillus spp*. or *Lactococcus lactis* was enhanced (Brzoska et al., 2012). Prebiotics are indigestible dietary supplements that particularly stimulate the growth or activity of one or more types of bacteria in the gastrointestinal tract and improving the health of the host (Hajati and Rezaei, 2010). Prebiotics are also thought to aid in the growth of chickens. Broilers fed a prebiotic-rich diet consumed more feed and had greater feed conversion rates (FCRs) (Kamran et al., 2013).

Prebiotics supplementation improved the breast and thigh yield (Piray et al., 2007) and reduced the cost of production in broiler diet (Peric et al., 2009).

Two naturally occurring functional polysaccharides with well acknowledged health-improving qualities are abundant in yeast cell walls: Oligosaccharides mannan and β-D-glucan. *Mannan oligosaccharides,* present on the outer layer of autolyzed yeast cell walls, bind harmful bacteria to stop them from colonizing the stomach, preventing infections and the release of toxins (Fowler et al., 2015). Mannan oligosaccharides also enhance gut health by improving the functional structure of the intestines (Ganner and Schatzmayr, 2012). Recent researches had focused on the importance of MOS to improve the microbial profiles, intestinal microarchitecture, production performance and humoral immunity in the broiler (Sohail et al., 2012).

The outermost layer of yeast (*Saccharomyces cerevisiae*) cell walls is the source of commercial mannan oligosaccharides, which are feed additives. Utilizing 0.05% MOS reduced *E. coli* and increased lactobacilli levels in the intestines of birds (Kim et al., 2011). Additionally, enzymatic hydrolysis of agricultural wastes like copra meal produces *mannan oligosaccharides*. (Ariandi and Meryandini, 2015).

Prevalent use of Antibiotics in Broiler Diet

Antibiotics are compounds with antibacterial properties that are being used in veterinary and human medicine to treat and prevent infections. They also work as growth boosters in animal feed. The physiological and metabolic capabilities of food-producing animals are compromised by antibiotics, which either eradicate or suppress the growth of bacteria and related microbes. Antibiotics come in two varieties: bactericidal and bacteriostatic. Antibiotics are bactericidal act by eradicating the microorganisms they are intended to combat. Antibiotics that are bacteriostatic stop the organism's cells from proliferating rather than killing it, maintaining a steady population level. These authors also mentioned that a lot of antibiotics function by attaching themselves to the enzymes' active sites, which makes the enzymes inactive. Thus, an antibiotic can halt essential cellular functions or prevent the production of new proteins during cell growth by blocking an enzyme (Phillip et al., 2004; Mathur and Singh, 2005).

The supplementation of 20g/ton of virginiamycin had improved weight gain than control diet. Likewise, bird fed with diet having 50 g/ton of narrow spectrum antibiotic BMD had higher weight gain. The supplementation of antibiotics had beneficial effect on growth performance. The addition of antibiotics in broiler diet increased weight gain. Intestinal length and weight were greater in birds fed with virginiamycin than bacitracin methylene disalicylate (Miles et al., 2006; Jian-mei et al., 2010).

Antibiotics have been extensively used in the livestock and poultry sectors during a period of fast management change from low-performance, free-range farming to a more controlled and intensive husbandry sector since their discovery more than 50 years ago. Certain antibiotics have been shown to suppress and restrict the growth of bacteria (*Clostridium perfringens*) that are known to be detrimental to chickens (Ferket et al., 2002).

Alternatives for Antibiotic use in Poultry

Reducing the use of antibiotics in human healthcare has been seriously considered due to the fear that antibiotics used in cattle and poultry production decrease their effectiveness. In nations that have discontinued utilizing antibiotic growth promoters, the prevalence of necrotic enteritis linked to Clostridium perfringens in poultry has increased (Immerseel et al., 2004). *Clostridium perfringens* is a significant food-borne pathogen that is thought to be responsible for 248,000 instances of food-borne disease in the US each year (Mead et al., 1999). Therefore, to improve poultry performance and prevent and treat infections, alternative methods to the usage of antibiotics must be found. Probiotic bacteria and organic acids have the potential to serve as viable substitutes for antibiotics in the growth stimulation and feed conversion efficiency enhancement of agricultural animals (Spring, 2003).

Use of Probiotics in Broiler Diet

Since the public's concern over antibacterial growth-promoting substances has grown and some farmers are choosing to use probiotics instead of antibiotics, the use of probiotics in the chicken business is of great interest (Jian-mei et al., 2010). Probiotics have been shown to improve newborn survival, reduce or prevent diarrhea, accelerate growth, improve feed efficiency, and strengthen the immune system as shown in Table 1 (Tollba et al., 2004).

Probiotics have a variety of advantageous effects, such as immuno-stimulation, the inhibition of enteric pathogens, and the preservation of a balanced gut microbiota (Tannock, 2004). Breeders supplementing their diet with probiotics saw increased egg production, decreased cracked eggs, and improved weight gain and feed conversion in their broiler chickens (Fuller, 1989).

Using a lactobacillus-based probiotic supplement for 42 days significantly improved the broiler's weight gain and food conversion ratio (Jin et al., 1988). When Bacillus subtilis was added, laying hens' eggshell thickness and feed conversion increased, which reduced the number of cracked eggs (Pedroso et al., 1999). It has been discovered that probiotics added to chicken feed increase egg weight, food conversion ratio, and egg output. Layers that were fed a meal containing 153g CP/kg of *Lactobacillus* produced larger eggs than those that were fed a diet that was comparable but did not contain *Lactobacillus* (Nahashon et al., 1996). The improved performance of chickens given probiotic supplements may be linked to changes in the microstructure of the gut, namely in the areas of villus height, goblet cell count, and crypt depth. Probiotics are therefore competitively priced, and their usage of antibiotic growth promoters makes them equally alluring as the growth promoters themselves. Probiotics may be beneficial for health in a number of ways, such as immuno-stimulation, anti-inflammatory responses, pathogen exclusion and death in the digestive system, and decreased bacterial contamination of processed broiler carcasses (Edens, 2003).

Probiotics lessen the incidence of enteric infections in chickens and the consequent contamination of poultry products (Patterson and Burkholder 2003). Antibiotics and probiotics increase the cecal microbiomes of broilers that increased Enterobacteriaceae and reduce *Brucellaceae, Erysipelotrichaceae, Coriobacteriaceae* and *Clostridiales* than those fed antibiotic (Neveling et al., 2017). Probiotics result in increased immunity and economic benefits (Gao et al., 2017).

Mode of Action of Probiotics

Being nonpathogenic and having the ability to stimulate the gut microbiota in their host are desirable qualities in a probiotic (Smith, 2014). This is significant because it establishes a symbiotic or commensal interaction. Commensal interactions describe the coexistence of nonpathogenic bacteria and their host, while clear advantages are not always evident. When two distinct species coexist peacefully and at least one of them gains benefits without harming the other, this is known as a symbiotic connection (Hooper and Gordon, 2001). For instance, oral *Lactobacillus plantarum* inoculation resulted in large quantities of immunoglobulin G specific to tetanus toxin fragment C, which triggered immune responses against the 6 produced antigen due to a symbiotic association (Shaw et al., 2000). Conversely, in patients with impaired immune systems, probiotics can occasionally result in infections and GIT disorders.

In the projected 45-day production phase, the GIT is critical to the health and growth of the birds. Gut associate microbial populations are very crucial for properly metabolic physiology of host. For example, probiotics or live Bacillus subtilis strains are being given to chickens to improve GIT physiology including duodenal IgA secretions and feed conversion ratio (FCR) (Sohail et al., 2012; Amerah et al., 2013).

Probiotics are thought to stimulate gut microbiota via a number of mechanisms, such as binding microbial specific receptors within the intestinal mucosa, competing microorganisms for nutrients, and producing antimicrobial agents to suppressing the growth of other microbial population (Hemarajata and Versalovic, 2013; Abd El-Moneim and Sabic, 2019; Abd El-Moneim et al., 2020). There are other possible pathways that probiotics can stimulate against pathogenic microbial populations such as lowering of pH, generation of organic acid and immune regulation of host (Sherman et al., 2009; Abd El-Moneim et al., 2020). They are also believed to be involved in maintaining the integrity of intestinal barrier and immunological tolerance that can negatively affect translocation of pathogenic microbes across intestinal defense (Lee and Bak, 2011). The mechanisms of probiotic action are illustrated in the Fig. 1.

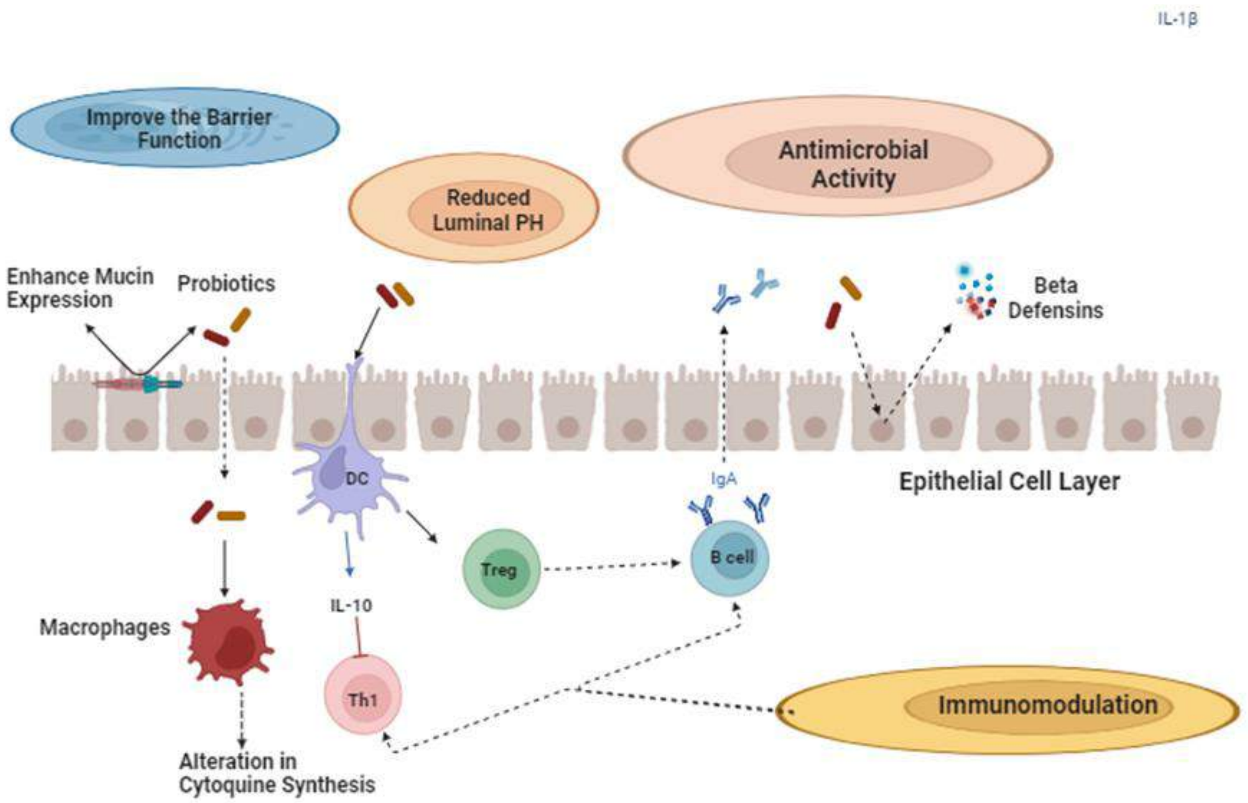

Fig. 1: Mechanism of action of probiotics in broiler diet for better performance and production showing immunomodulation, improved barrier function, antimicrobial activity and reduced luminal pH

Use of Prebiotics in Broiler Diet

Prebiotics are non-digestible food ingredients that specifically act as substrates for microbes (Swanson et al., 2020). The potential to optimize the effects of probiotics in prebiotic formulations is presented by the formation of new molecules upon the combination of prebiotics and probiotics in a single matrix (Cunningham et al., 2021). Lactulose, oligosaccharides (XOS), oligogalactose (GOS), inulin and oligofructose (FOS) are some of the most often occurring prebiotics in diets (Casarotti et al., 2018; Peng et al., 2020). Prebiotics added to meat products improve their nutritional content and affect the way different foods look on the technical side (Pogorzelska-Nowicka et al., 2018).

It has been demonstrated that oligosaccharides reduce the risk of illness, most likely by improving the digestion of some feed components and inhibiting the proliferation of pathogenic species. FOS and MOS are two common oligosaccharides found in animal diets. It has been demonstrated that the majority of prebiotics work to benefit the host by feeding the helpful bacteria while suppressing the pathogenic ones. Prebiotics appear to improve the intestinal bacterial balance in broilers by promoting the growth of nonpathogenic microorganisms and decreasing the colonization of bacteria like *Salmonella* and *E. coli* (Yusrizal and Chen, 2003; Chung and Day, 2004).

Prebiotic had similar effect in replacement to antibiotics in broiler in term of feed consumption and body weight gain (Alonge et al., 2017). Yeast cell wall had greater weight gain and better FCR (Fowler et al., 2015).

Antibiotics can be replaced with MOS without having an adverse effect on the growth performance of broilers (Kamran et al., 2013). Under a *Clostridium perfringens* challenge, dietary mannan oligosaccharide (MOS) at 0.05% can be added to broiler diets to protect them from antimicrobial growth boosters without compromising the broiler's ability to grow (Abudabos and Yehia 2013). Mannan oligosaccharide had similar effect on weight gain and FCR to antibiotics group (Eseceli et al., 2010).

Mannan oligosaccharide had no influence on carcass yield (Leblebicier and Aydoğan 2018). Prebiotic had improved the population of cecal microbiota, enhanced lactic acid producing bacteria and decreased *E. Coli* bacteria (Tayeri et al., 2018). *Bifidobacteria* concentrations are higher in birds fed with *mannan oligosaccharide* (Baurhoo et al., 2009). Further, mannan oligosaccharide improved microbial ecology and morphological development. It does not affect overall performance of birds (Leblebicier and Aydoğan 2018) which shows no influence on feeding behavior, weight gain, and FCR (Yalçinkaya et al., 2008).

Mode of Action of Prebiotics

Prebiotics can affect poultry directly by affecting host systems like immunological responses, or indirectly by changing the gut microbiota's fermentation patterns and composition. When prebiotics are fermented by gastrointestinal bacteria, short chain fatty acids are thought to be the main inhibitory mechanism against pathogens; however, other processes, such as interference with adhesion, may also take place. Prebiotics are linked to a wide range of processes and roles in the avian GIT microbiota, such as immune system interaction, GIT morphological modification, and competitive exclusion of pathogens (Ricke, 2018).

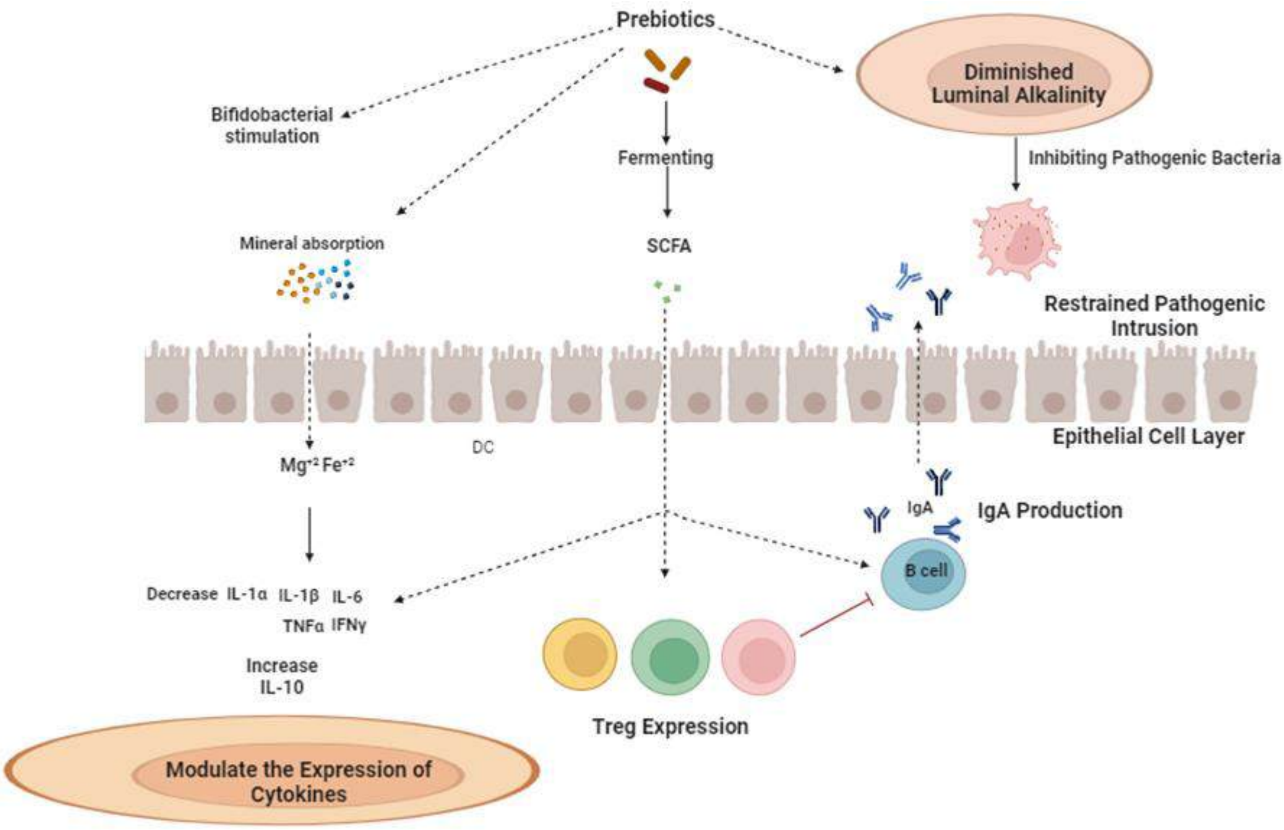

Fig. 2: Mode of action of prebiotics in improved poultry production by diminishing luminal alkalinity, IgA production, elevated mineral absorption, bifidobacterial stimulation and modulating the expression of cytokines.

Fructooligosaccharides (FOS) Role in Poultry Production

FOS, which are naturally occurring substances derived from a range of plants, have been added to chicken and swine feed as prebiotic additions. Their potential to enhance animal performance and health has been well investigated (Kumar and Pandey, 2020). They could be substituted for antibiotics at subtherapeutic concentrations. Furthermore, the broiler chicks fed diets supplemented with fructo oligosaccharides show a decreased incidence of intestinal colonization with Salmonella enteritidis compared to those on a control diet. It has been reported that female birds if treated with FOS can improve FCR, weight gain including carcass weight, and overall carcass percentage with longer small intestine (Shang et al., 2015; Yusrizal and Chen 2003).

Mannan Oligosaccharides (MOS)

Use of MOS improve body weight, feed conversion ratio and livability in broiler chickens to help to optimize gut health and bird performance as explained in Table 1 (Spring, 2003; Hooge, 2004). It gives enteric pathogens specialized binding sites (D-Mannose), which lessens the likelihood of the pathogens adhering to the intestinal system. According to Waldroup et al. (2003), there is no discernible impact on feed conversion or body weight. MOS was added to the diet eaten for 0-42 days and 42-56 days, respectively, at an 1g/kg and 0.75g/kg concentration. When MOS was added to broiler feeds, there was an improvement in feed conversion and body weight increase (Parks et al*.,* 2001). When compared to birds fed with control diet, the weight gain, feed conversion, and mortality of birds supplemented with MOS were dramatically enhanced (Hooge, 2004). The performance of the birds treated with MOS and those given antibiotic growth boosters was comparable, but the birds fed MOS had a reduced fatality rate. For chicken production to be lucrative and of excellent quality, gut health must be maintained (Parks et al., 2001). MOS has demonstrated potential in immune system modulation and intestinal pathogen suppression (Spring, 2003). When it came to preventing *Salmonella enteritidis* colonization in chicks, chickens fed with MOS or PKM had hen caecal contents (HCC) that were more efficient than hens fed control feed. In challenged three-day-old chicks, MOS decreased the concentration of *S. typhimurium* 29E and *S. dublin* (Fernandez et al*.,* 2002).

The Nutritional Benefits of Probiotics

The major way that prebiotic supplementation affects the host's nutrition is by its fermentation into short-chain fatty acids (SCFAs), primarily lactate and acetic, propionic, and butyric acids, in the hindgut. By passive diffusion, SCFAs are absorbed through the cecal epithelium and provide mature birds with up to 11% of their metabolizable energy. Since SCFAs lower intestinal pH and encourage protein and mineral solubilization, they may also increase the availability of these nutrients (Feng et al., 2005). Microbial prebiotic-mediated alterations affect the production of vitamins and nitrogen compounds, the breakdown of indigestible feed components, and the facilitation of the elimination of undesirable food components. Cecal microbes contain up to 5% of the genes involved in cofactor and vitamin production and 10% involved in protein and amino acid metabolism (Danzeisen et al., 2011). These genes could be used by the microbes themselves or by the host (Pan and Yu, 2014). Additionally, the grill metagenomics analysis revealed the presence of genes encoding lactase, cellulase, hemicellulase, and arabinoxylanse activity, supporting the microbial digestion of these indigestible food components for the generation of SCFAs as well as amylase and protease activity (Sergeant et al., 2014).

Effect of Prebiotic on Breeder, Broiler, and Layer Performance

MOS addition in the basal diet can improve the immune response in both broilers and layers that can also be analyzed by immune organ indexing (Raju and Devegowda, 2002). The ether extract content in breast muscle was lower with the herbs than with AGP, whereas the EE level in leg muscles was unaffected (Pisarski and Szkucik, 2007).

Prebiotics and BMD can be added to diets to boost growth and improve performance of birds, but better performance has been reported in control group if treated with herbes. The treatment group exhibited considerably higher live weight gain, survival rate, dressing percentage, and profitability in comparison to the control group (Shivakumar and Javed 2005). Consequently, the performance of birds leading to profitability can be enhanced by providing additional herbes as growth promoter.

Synbiotics

Nutritional supplements that combine probiotics and prebiotics in a synergistic manner are referred as synbiotics. The idea that a probiotic cannot thrive in the digestive system without its prebiotic substrate is the main justification for utilizing a synbiotic. The probiotic will be more sensitive to low pH, oxygen, and temperature if it does not receive the essential nutrition supply. Prebiotics maintain the colonies of these "good" bacteria by providing better circumstances for probiotics to grow. By taking both probiotics and prebiotics, one can see improvements in one's health state and an increase in the good bacteria in the digestive system. Synbiotics have these beneficial effects in two ways: (1) by enhancing probiotic viability and (2) by optimizing specific health benefits (Sekhon and Jairath, 2010). In commercial broilers, the combination of prebiotics and probiotics improved growth performance and carcass yield with simultaneous growth in intestinal morphology, as compared with antibiotic growth promoters (Ali et al., 2023).

Mechanism of Action

Prebiotics and probiotics combined (known as synbiotics) have a synergistic effect that enhances poultry health. They have the following benefits when added to broiler chicken feed; thicken the intestinal wall, boost resistance, boost the absorption of glucose, increase the levels of short chain fatty acids and lactic acid, reduce the concentration of branched chain fatty acids, encourage the growth of lymphoid tissue associated with the gut and enhance colonization of GALT by T and B cells (Jiang et al., 2022; Nguyen et al., 2022). Combination of prebiotic (MOS) and probiotic (*Bacillus subtilis*) fed to commercial broilers increased the dressing percentage, breast yield percentage and decreased FCR, thereby increasing the surface area for nutrient absorption by increasing villus height and villus area (Ali et al., 2023).

There are two varieties of synbiotics: synergistic and complementary synbiotics. Probiotics and prebiotics, of which more than one can be utilized, form complementary synbiotics. These microorganisms function separately to provide various health advantages. The live bacteria and a selectively fertilized substrate are the two parts of a synergistic synbiotic (Zubair, 2022)**.**

Table 1: Summarized effects of probiotics, prebiotics and synbiotics on birds

	Commonly used	Growth performance	Immune system	Gastrointestinal system	References
Probiotics	*Lactobacillus bulgaricus, Enterococcus faecium, Lactobacillus spp. Bacillus spp. Rhodopseudomonas palustris, Aspergillus oryzae, Candida pinpolopesi.*	Increased growth performance, improved FCR, Plasma cholesterol and triglyceride concentrations reduced Better meat composition, increased feed intake and better weight gain	Elevated Serum KLH-specific IgA, antibody response to ND virus, increased spleen and bursa relative weights.	Digestibility of fat and nitrogen, elevated ceaca microbiota, increased ileal and duodenal villus heights, Improved intestinal microbiota and gizzard weights, Decreased ammonia emission.	(Ricke et al., 2020, Rehman et al.,2020; Sheheta et al., 2022; Rashid et al., 2023)
Prebiotics	*Bacillus licheniformis and Bacillus subtilis,* Fructooligosaccharide and *Bacillus subtilis, Enterococcus faecium, Lactococcus lactis*	Increased growth performance, improved FCR, better weight gain	Improved immunity against salmonella, Development of central and peripheral lymphatic organs, improved gene expression regulation in spleen and ceacal tonsils	Increased ammonia concentration, Enhanced Caeca microbiota, Phenol and cresol decreased in caeca, Elevated acetic acid and butyric acid in caeca	(Ajuwon, K.M., 2016; krysiak et al., 2021; Wu et al., 2022)
Synbiotics	Oligosaccharides, Galactooligosaccharides, Insulin, Sucrose thermal oligosaccharides	Increased weight gain, FCR, feed intake, dressing percentage, carcass, wing, breast, back, thigh, and drumstick percentages, Improved heart, liver and gizzard weights	Development of peripheral and central lymphatic organs, Improvement in gene expression regulation in spleen, GALT development	Increased villus length to crypt depth ratio in the jejunal mucosa, improved villus heights	(Jha et al., 2020; Yaqoob et al., 2021; Khomayezi and Adewole, 2022; Rashid et al., 2023)

Conclusion

From the above discussion, it is concluded that the addition of prebiotics, probiotics, synbiotics or their combination (replacing antibiotics) reduces the risk of AMR. Furthermore, it has a beneficial effect on the poultry in terms of growth performance, carcass yield and intestinal morphology, leading to higher profitability. However, much research is still needed to fully understand the mechanisms involved to maximize the health benefits of probiotics, prebiotics and synbiotics on poultry health and production.

REFERENCES

Abudabos, A. M., and Yehia, H. M. (2013). Effect of dietary mannan oligosaccharide from Saccharomyces cerevisiae on live performance of broilers under Clostridium perfringens challenge. *Italian Journal of Animal Science*, *12*(2), e38.

Abd El-Moneim, A. E., and Sabic, E. M. (2019). Beneficial effect of feeding olive pulp and Aspergillus awamori on productive performance, egg quality, serum/yolk cholesterol and oxidative status in laying Japanese quails. *Journal of Animal and Feed Sciences*, *28*(1), 52-61.

Ajuwon, K. M. (2016). Toward a better understanding of mechanisms of probiotics and prebiotics action in poultry species. *Journal of Applied Poultry Research*, *25*(2), 277-283.

Ali, A., Qureshi, A. S., Rehan, S., Deeba, F., and Usman, M. (2023). Prebiotic, probiotic, and antibiotic growth promoters use in commercial broilers: A comparative study: Gut histology of broilers and growth promoters. *Journal of the Hellenic Veterinary Medical Society*, *74*(3), 5929-5936.

Ali, M. A., Soner, M. A. S., and Rahman, M. A. (1994). Effect of feeding Livol on the performance of broilers.

Alloui, M. N., Szczurek, W., and Świątkiewicz, S. (2013). The usefulness of prebiotics and probiotics in modern poultry nutrition: A review/Przydatność prebiotyków i probiotyków w nowoczesnym żywieniu drobiu–przegląd. *Annals of Animal Science*, *13*(1), 17-32..

Alonge, E. O., Eruvbetine, D., Idowu, O. M. O., Obadina, A. O., Olukomaiya, O. O., Omotainse, O. S., and Abiola, Y. O. (2017). Effects of antibiotic, probiotic and prebiotic supplementation in broiler diets on performance characteristics and apparent nutrient digestibility. *Journal of Applied Sciences and Environmental Management*, *21*(7), 1297-1300.

Amerah, A. M., Quiles, A., Medel, P., Sánchez, J., Lehtinen, M. J., and Gracia, M. I. (2013). Effect of pelleting temperature and probiotic supplementation on growth performance and immune function of broilers fed maize/soy-based diets. *Animal Feed Science and Technology*, *180*(1-4), 55-63.

Meryandini, A. N. J. A. (2015). Enzymatic hydrolysis of copra meal by mannanase from Streptomyces sp. BF3. 1 for the production of mannooligosaccharides. *HAYATI Journal of Biosciences*, *22*(2), 79-86.

Ahsan-ul-Haq-Gilari, A. H. (1991). Comparative efficiency of various feed additives in broiler chicks. *Sarhad Journal of Agriculture*, *7*, 33-38.

Baurhoo, B., Ferket, P. R., and Zhao, X. (2009). Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. *Poultry Science*, *88*(11), 2262-2272.

Brzoska, F., Śliwiński, B., and Stecka, K. (2012). Effect of Lactococcus lactis vs. Lactobacillus Spp. bacteria on chicken body weight, mortality, feed conversion and carcass quality. *Annals of Animal Science*, *12*(4), 549-559.

Cardoso, M. A. B., Flemming, J. S., and Flemming, F. F. (2002). Utilization of halquinol as a growth promoter and coadjuvant for coccidiosis control in broilers.

Casarotti, S. N., Borgonovi, T. F., Batista, C. L., and Penna, A. L. B. (2018). Guava, orange and passion fruit by-products: Characterization and its impacts on kinetics of acidification and properties of probiotic fermented products. *Lwt*, *98*, 69-76.

Cavazzoni, V., Adami, A., and Castrovilli, C. (1998). Performance of broiler chickens supplemented with Bacillus coagulans as probiotic. *British Poultry Science*, *39*(4), 526-529..

Choi, K. H., Namkung, H., and Paik, I. K. (1994). Effects of dietary fructooligosaccharides on the suppression of intestinal colonization of Salmonella typhimurium in broiler chickens..

Chung, C. H., and Day, D. F. (2004). Efficacy of Leuconostoc mesenteroides (ATCC 13146) isomaltooligosaccharides as a poultry prebiotic. *Poultry Science*, *83*(8), 1302-1306.

Cunningham, C., Ederer, F., and Ma, S. (2021). Killer acquisitions. *Journal of Political Economy*, *129*(3), 649-702.

Dalloul, R. A., Lillehoj, H. S., Shellem, T. A., and Doerr, J. A. (2003). Enhanced mucosal immunity against Eimeria acervulina in broilers fed a Lactobacillus-based probiotic. *Poultry Science*, *82*(1), 62-66.

Danzeisen, J. L., Kim, H. B., Isaacson, R. E., Tu, Z. J., and Johnson, T. J. (2011). Modulations of the chicken cecal microbiome and metagenome in response to anticoccidial and growth promoter treatment. *PloS one*, *6*(11), e27949.

El-Moneim, A. E. M. E. A., El-Wardany, I., Abu-Taleb, A. M., Wakwak, M. M., Ebeid, T. A., and Saleh, A. A. (2020). Assessment of in ovo administration of Bifidobacterium bifidum and Bifidobacterium longum on performance, ileal histomorphometry, blood hematological, and biochemical parameters of broilers. *Probiotics and Antimicrobial Proteins*, *12*, 439-450.

E Edens, F. W. (2003). An alternative for antibiotic se in poultry: probiotics. *Brazilian Journal of Poultry Science*, *5*, 75-97.

Eseceli, H., Değirmencioğlu, N., Demir, E., and Bilgic, M. (2010). The effects of bio-mos (r) mannan oligosaccharide and antibiotic growth promoter performance of broilers..

Feng, J., Lee, S. J., and Resta-Lenert, S. (2005). Effect of probiotic and commensal bacteria on vitamin D-receptor and calcium transport protein expression in intestinal epithelial cells in vitro. *Gastroenterology*, *128*(4), A101-A101.

Ferket, P. R., Parks, C. W., and Grimes, J. L. (2002, May). Benefits of dietary antibiotic and mannanoligosaccharide supplementation for poultry. In *Multi-State Poultry Meeting* (Vol. 14, pp. 1-22). Raleigh, NC: Department of Poultry Science North Carolina State University.

Fernandez, F., Hinton, M., and Gils, B. V. (2002). Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to Salmonella Enteritidis colonization. *Avian Pathology*, *31*(1), 49-58.

Fowler, J., Kakani, R., Haq, A., Byrd, J. A., and Bailey, C. A. (2015). Growth promoting effects of prebiotic yeast cell wall products in starter broilers under an immune stress and Clostridium perfringens challenge. *Journal of Applied Poultry Research*, *24*(1), 66-72.

Fuller, R. (1989). Probiotics in man and animals. *The Journal of Applied Bacteriology*, *66*(5), 365-378.

Ganner, A., and Schatzmayr, G. (2012). Capability of yeast derivatives to adhere enteropathogenic bacteria and to modulate cells of the innate immune system. *Applied Microbiology and Biotechnology*, *95*(2), 289-297.

Gao, P., Ma, C., Sun, Z., Wang, L., Huang, S., Su, X., and Zhang, H. (2017). Feed-additive probiotics accelerate yet antibiotics delay intestinal microbiota maturation in broiler chicken. *Microbiome*, *5*, 1-14.

Hajati, H., and Rezaei, M. (2010). The application of prebiotics in poultry production. *International Journal Poultry*

Science, *9*(3), 298-304.

Hemarajata, P., and Versalovic, J. (2013). Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. *Therapeutic Advances in Gastroenterology*, *6*(1), 39-51.

Hooge, D. M. (2004). Meta-analysis of broiler chicken pen trials evaluating dietary mannan oligosaccharide, 1993-2003. *International Journal Poultry Science*, *3*(3), 163-174.

Hooper, L. V., and Gordon, J. I. (2001). Commensal host-bacterial relationships in the gut. *Science*, *292*(5519), 1115-1118.

Immerseel, F. V., Buck, J. D., Pasmans, F., Huyghebaert, G., Haesebrouck, F., and Ducatelle, R. (2004). Clostridium perfringens in poultry: an emerging threat for animal and public health. *Avian Pathology*, *33*(6), 537-549.

Jiang, H., Cai, M., Shen, B., Wang, Q., Zhang, T., and Zhou, X. (2022). Synbiotics and gut microbiota: new perspectives in the treatment of type 2 diabetes mellitus. *Foods*, *11*(16), 2438.

Jian-mei, W., L. Lin, L. Su-fen, Z. Ming, Z. Ke-ying and L. Xu-gang. (2010). Effects of different antibiotics on growth and slaughter performance and meat quality in broiler chickens. C*hinese Journal of Animal Science, 1*,48-52.

Jin, L. Z., Ho, Y. W., Abdullah, N., and Jalaludin, S. (1998). Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing Lactobacillus cultures. *Poultry Science*, *77*(9), 1259-1265.

Kamran, Z., Mirzaa, M. A., Ahmad, S., Samad, H. A., Sohail, M. U., and Saadullahb, M. (2013). Performance of broiler chickens fed mannan oligosaccharides as alternatives to antibiotics from one to twenty-two days of age.

Kim, G. B., Seo, Y. M., Kim, C. H., and Paik, I. K. (2011). Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. *Poultry Science*, *90*(1), 75-82.

Kumar, S., and Pandey, G. (2020). Biofortification of pulses and legumes to enhance nutrition. *Heliyon*, *6*(3).

Leblebicier, Ö. D. Y., and Aydoğan, İ. (2018). The effects of mannan oligosaccharide and chitosan oligosaccharide on performance and blood parameters of broilers. *Journal of Poultry Research*, *15*(1), 18-22.

Lee, B. J., and Bak, Y. T. (2011). Irritable bowel syndrome, gut microbiota and probiotics. *Journal of Neurogastroenterology and Motility*, *17*(3), 252.

Mathur, S., and Singh, R. (2005). Antibiotic resistance in food lactic acid bacteria—a review. *International Journal of Food Microbiology*, *105*(3), 281-295.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., and Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases*, *5*(5), 607.

Miles, R. D., Butcher, G. D., Henry, P. R., and Littell, R. C. (2006). Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. *Poultry Science*, *85*(3), 476-485.

Mountzouris, K. C., Tsitrsikos, P., Palamidi, I., Arvaniti, A., Mohnl, M., Schatzmayr, G., and Fegeros, K. (2010). Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. *Poultry Science*, *89*(1), 58-67.

Nahashon, S. N., Nakaue, H. S., and Mirosh, L. W. (1996). Nutrient retention and production parameters of Single Comb White Leghorn layers fed diets with varying crude protein levels and supplemented with direct-fed microbials. *Animal Feed Science and Technology*, *61*(1-4), 17-26.

Neveling, D. P., Van Emmenes, L., Ahire, J. J., Pieterse, E., Smith, C., and Dicks, L. M. T. (2017). Safety assessment of antibiotic and probiotic feed additives for Gallus gallus domesticus. *Scientific Reports*, *7*(1), 12767.

Nguyen, T. T., Nguyen, P. T., Pham, M. N., Razafindralambo, H., Hoang, Q. K., and Nguyen, H. T. (2022). Synbiotics: a new route of self-production and applications to human and animal health. *Probiotics and Antimicrobial Proteins*, *14*(5), 980-993.

Pan, D., and Yu, Z. (2014). Intestinal microbiome of poultry and its interaction with host and diet. *Gut Microbes*, *5*(1), 108-119.

Parks, C. W., Grimes, J. L., Ferket, P. R., and Fairchild, A. S. (2001). The effect of mannanoligosaccharides, bambermycins, and virginiamycin on performance of large white male market turkeys. *Poultry Science*, *80*(6), 718-723.

Patterson, J. A., and Burkholder, K. M. (2003). Application of prebiotics and probiotics in poultry production. *Poultry Science*, *82*(4), 627-631.

Pedroso, A. A., Moraes, V. M. B., and Ariki, J. (1999). Effects of protein and probiotic (Bacillus subtilis) levels in the diets of pullets and laying hens.

Perić, L., Žikić, D., and Lukić, M. (2009). Application of alternative growth promoters in broiler production. *Biotechnology in Animal Husbandry*, *25*(5-6-1), 387-397.

Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C., Jones, R., and Waddell, J. (2004). Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *Journal of Antimicrobial Chemotherapy*, *53*(1), 28-52.

Piray, A. H., Kermanshahi, H., Tahmasbi, A. M., and Bahrampour, J. (2007). Effects of cecal cultures and aspergillus meal prebiotic (Fermacto) on growth performance and organ weights of broiler chickens. *International Journal Poultry Science*, *6*(5), 340-344.

Pisarski, R. K., Szkucik, K., and Zięba, M. (2007). The chemical composition of muscles and sensory traits of meat in broiler chickens fed herbs substituted for antibiotic growth promoter.

Pogorzelska-Nowicka, E., Atanasov, A. G., Horbańczuk, J., and Wierzbicka, A. (2018). Bioactive compounds in functional meat products. *Molecules*, *23*(2), 307.

Rashid, S., Tahir, S., Akhtar, T., Altaf, S., Ashraf, R., and Qamar, W. (2023). Bacillus-based Probiotics: An Antibiotic Alternative for the Treatment of Salmonellosis in Poultry. *Pakistan Veterinary Journal*, *43*(1).

Rashid, S., Alsayeqh, A. F., Akhtar, T., Abbas, R. Z., and Ashraf, R. (2023). Probiotics: alternative to antibiotics in poultry production.

Raju, M. V. L. N., and Devegowda, G. (2002). Esterified-glucomannan in broiler chicken diets-contaminated with aflatoxin, ochratoxin and T-2 toxin: Evaluation of its binding ability (in vitro) and efficacy as immunomodulator. *Asian-Australasian Journal of Animal Sciences*, *15*(7), 1051-1056.

Ricke, S. C. (2018). Focus: nutrition and food science: impact of prebiotics on poultry production and food safety. *The Yale Journal of Biology and Medicine*, *91*(2), 151.

Ritzi, M. M., Abdelrahman, W., Mohnl, M., and Dalloul, R. A. (2014). Effects of probiotics and application methods on performance and response of broiler chickens to an Eimeria challenge. *Poultry Science*, *93*(11), 2772-2778.

Sekhon, B. S., and Jairath, S. (2010). Prebiotics, probiotics and synbiotics: an overview. *Journal of Pharmaceutical Education and Research*, *1*(2).

Sergeant, M. J., Constantinidou, C., Cogan, T. A., Bedford, M. R., Penn, C. W., and Pallen, M. J. (2014). Extensive microbial and functional diversity within the chicken cecal microbiome. *PloS one*, *9*(3), e91941.

Shabani, R., Nosrati, M., Javandel, F., Gothbi, A. A. A., and Kioumarsi, H. (2012). The effect of probiotics on growth performance of broilers. *Annals of Biological Research*, *3*(12), 5450-5452.

Shang, Y., Regassa, A., Kim, J. H., and Kim, W. K. (2015). The effect of dietary fructooligosaccharide supplementation on growth performance, intestinal morphology, and immune responses in broiler chickens challenged with Salmonella Enteritidis lipopolysaccharides. *Poultry Science*, *94*(12), 2887-2897.

Shaw, D. M., Gaerthe, B., Leer, R. J., Van Der Stap, J. G. M. M., Smittenaar, C., Heijne Den Bak-Glashouwer, M. J., and Havenith, C. E. G. (2000). Engineering the microflora to vaccinate the mucosa: serum immunoglobulin G responses and activated draining cervical lymph nodes following mucosal application of tetanus toxin fragment C-expressing lactobacilli. *Immunology*, *100*(4), 510-518.

Sherman, D. K., Cohen, G. L., Nelson, L. D., Nussbaum, A. D., Bunyan, D. P., and Garcia, J. (2009). Affirmed yet unaware: exploring the role of awareness in the process of self-affirmation. *Journal of Personality and Social Psychology*, *97*(5), 745.

Shin, M. S., Han, S. K., Ji, A. R., Kim, K. S., and Lee, W. K. (2008). Isolation and characterization of bacteriocin-producing bacteria from the gastrointestinal tract of broiler chickens for probiotic use. *Journal of Applied Microbiology*, *105*(6), 2203-2212.

Shivakumar, M. C., Mulla, J., Pugashetti, B. K., and Nidgundi, S. (2005). Influence of supplementation of herbal growth promoter on growth and performance of broilers. *Karnataka Journal of Agricultural Sciences*, *18*(2), 481.

Smith, J. M. (2014). A review of avian probiotics. *Journal of Avian Medicine and Surgery*, *28*(2), 87-94.

Sohail, M. U., Hume, M. E., Byrd, J. A., Nisbet, D. J., Ijaz, A., Sohail, A., and Rehman, H. (2012). Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. *Poultry Science*, *91*(9), 2235-2240.

Spring, P. (2003). Improving gut health through nutrition. Proceedings of the 22nd Scientific day, *WPSA Southern African Branch,* 35-47.

Swanson, K. S., Gibson, G. R., Hutkins, R., Reimer, R. A., Reid, G., Verbeke, K., and Sanders, M. E. (2020). The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. *Nature Reviews Gastroenterology and Hepatology*, *17*(11), 687-701.

Tannock, G. W. (2004). A special fondness for lactobacilli. *Applied and Environmental Microbiology*, *70*(6), 3189-3194.

Tayeri, V., Seidavi, A., Asadpour, L., and Phillips, C. J. (2018). A comparison of the effects of antibiotics, probiotics, synbiotics and prebiotics on the performance and carcass characteristics of broilers. *Veterinary Research Communications*, *42*, 195-207.

Tollba, A. A. H., Sabry, M. M., and Abuzead, S. M. M. (2004). Effect of microbial probiotics on the performance of broiler chicks under normal or heat stress conditions. 1-Lactobacillus or Pediococcus.

Waldroup, P. W., Oviedo-Rondon, E. O., and Fritts, C. A. (2003). Comparison of Bio-Mos and antibiotic feeding programs in broiler diets containing copper sulfate. *International Journal of Poultry Science*, *2*(1), 28-31.

Yalçinkaya, İ., Guengoer, T., Başalan, M., and Erdem, E. (2008). Mannan oligosaccharides (MOS) from Saccharomyces cerevisiae in broilers: Effects on performance and blood biochemistry. *Turkish Journal of Veterinary and Animal Sciences*, *32*(1), 43-48.

Yusrizal, Y., and Chen, T. C. (2003). Effect of adding chicory fructans in feed on broiler growth performance, serum cholesterol and intestinal length.

Zubair, A. (2022) A Commentary on Mechanism of Action and Types of Synbiotics. *Journal of Probiotics and Health, 10*, 1–1

Chapter 18

Use of *Saccharomyces cerevisiae* as Probiotic in Ruminants Feed

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ABSTRACT

Probiotics are live bacteria that give the host health benefits when given in sufficient doses. Yeast products have a variety of impacts, such as improving the operation of the intestinal barrier, changing the levels of bacteria in the gastrointestinal (GI) tract, and giving host bacteria useful substrates. By promoting the growth of the bacteria engaged in the process, yeast can affect the rumen's bio-hydrogenation pathway. Any alteration in WBC concentrations could indicate a better or more severe state of the illness. Cytokine production is necessary for immune system activation, which includes WBC recruitment and the induction of illness behaviour. Cachectin, B-cell stimulatory factor 2 (BSF-2), and type II interferon are examples of cytokines associated with inflammation that induce fever, illness behaviour, and the synthesis of APP. In contrast to untreated cows, nursing milking cows treated using *Saccharomyces cerevisiae* cultures or living yeast had higher serum glucose and lower nitrogen in their blood.

KEYWORDS		
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INTRODUCTION

Probiotics are live bacteria that give the host health benefits when given in sufficient doses (Czerucka et al., 2007; Kumprechtová et al., 2019). Aside from the well-researched and well-known 2 Hydroxypropanoic acid bacteria, yeasts may also function as live microorganisms for improving growth (Altmann, 2017; Fomenky et al., 2018). The interest in this topic has grown over the past few years; over 31,000 articles using the term probiotic are indexed by PubMed, and over 15,000 of those have been printed in the past five years (Fig. 1). However, probiotic *S. cerevisiae* study makes up a small portion of this, with lesser than 850 articles published by PubMed in the past five years. The printing goal is to analyze the most recent data regarding probiotic and possibly probiotic yeasts and their use in different types of health supplemented food.

Etiology

S. cerevisiae from many families have been extracted from food sources and natural environments. By producing a variety of fermented foods, these potentially probiotic yeasts can improve their nutritional and sensory qualities. On the other hand, Saccharomyces is the only yeast genus that is productive in double-masked studies (Czerucka et al., 2007). Probiotics are capable of developing at 37°C, bear the harsh conditions of the human gastrointestinal tract (such as acidic pancreatico-biliary secretion, bile acids, and an acidic pH), and improve the health of an individual's health by modulating the microbes that reside there and carrying out biological activities (Table 1). Some probiotics can even stick to the secretions of gut epithelial cells (Sanchez et al., 2014). It is one of the most extensively used commercial treatments for diarrhoea in the world, having been used since the 1950s (McFarland, 1996; Altmann, 2017).

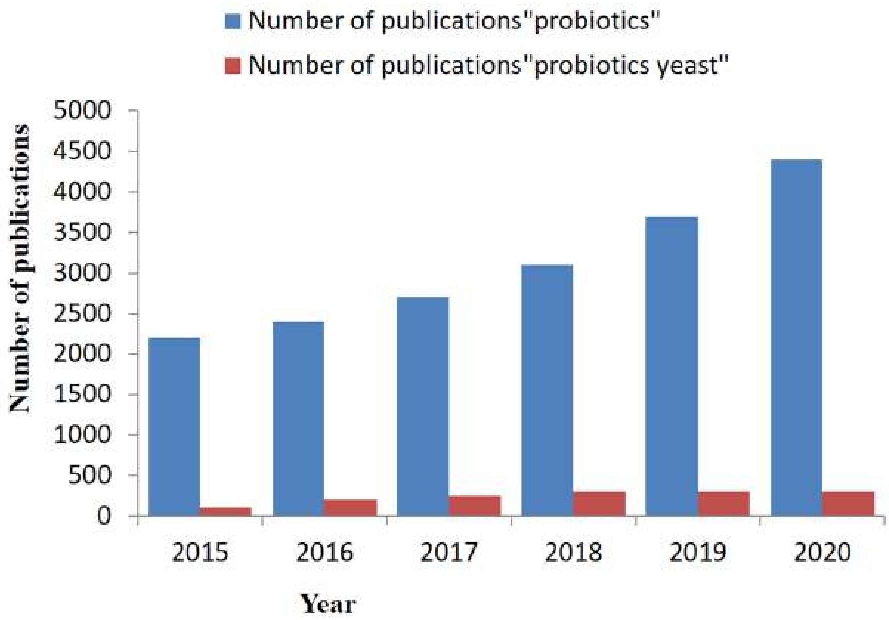

Fig. 1: Analysis of publications by PubMed in the past five years.

Table 1: Distinctive features of *Saccharomyces cerevisiae var. boulardii* and *Saccharomyces cerevisiae* (Altmann, 2017).

No.	Features	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae var. boulardii*
1.	Capability to grow at 37 ◦C	negative	positive
2.	Milk sugar used as a source of carbon	positive	Negative
3.	Ascospores generating capacity	positive	Negative
4.	Capability to withstand pH 2.5	Negative	positive
5.	Extra versions of chromosome 9	Negative	positive
6.	Increased capacity for pseudohyphal transitioning	Negative	positive
7.	No. of chromosome sets	bipartite or monoploid	double

Furthermore, compared to *S. cerevisiae*, *S. cerevisiae var. boulardii* has more copies of the genes involved in response to stressed conditions and translation These genes might be involved in pseudo- hyphal transitioning, rapid development rates, and enhanced resilience to raise pH (Czerucka et al., 2007). Heat shock proteins, elongation factors, ribosomal proteins, enzymes activating inactivated proteins, carriers, and fluoride efflux are usually encoded by duplicated and triplicated genes, and these proteins may be useful in adapting to stressful environments. Additionally, it has been found that *S. cerevisiae var. boulardii* possesses many genes related to pseudo-hyphal development, each with varying copies (Altmann, 2017).

Impact of *S. cerevisiae*

According to research, *S. cerevisiae* has no discernible impact on ammonia content, methane generation, or overall VFA concentrations (Durand-Chaucheyras et al., 1997). The type of diet being evaluated appears to have a significant impact on how yeast supplementation affects pH stabilization. Yeast augmentation has often been associated with greater rumen pH, while the regulated pH tended to be lower than 6. According to Fiems et al. (Fiems et al., 1999), sheep cater for a concentrate diet consisting of maize silage and cereals with a high dextrose and glycogen content showed a greater influence of yeast on the ruminal pH compared to sheep fed grass hay and sugar beet gloop. Yeast products have a variety of impacts, such as improving the operation of the intestinal barrier, changing the levels of bacteria in the gastrointestinal (GI) tract, and giving host bacteria useful substrates. Controlling agitation in the GI tract can have a significant positive effect on the host by improving nutrition uptake and minimizing the migration of pathogenic microorganisms and the production of toxins systemically. This frequently happens as a result of changes in the GI tract's crypt and villi properties, tight connections between epithelial cells, and mucin formation. For instance, it was observed that pigs given yeast supplements had thinner intestinal mucus than control pigs, which may enhance nutrient absorption (Bontempo et al., 2006; Di Giancamillo et al., 2007). Furthermore, newborn pigs treated with yeast showed increases in crypt depth and villus height, two indicators of good intestinal health (Di Giancamillo et al., 2007). Dairy calves' GI tract development was enhanced by YCW supplementation (Ma et al., 2020). High-grain diets are frequently fed to cattle to boost effectiveness, but doing so comes at the cost of a rise in acidification prevalence, which is linked to reductions in rumen pH and dry matter intake (DMI), as well as increases in inflammation and disarray of the gastrointestinal barrier's function (Bradford et al., 2015). Before and after the calving in cows, yeast was discovered to alter the transcription of genes associated with the immune system in the rumen. This finding suggests that yeast is controlling inflammation and gut barrier integrity during this transitional phase (Bach et al., 2018). Moreover, it has been discovered that yeast not only boosts the number of cellulolytic microorganisms in the rumen that crash incomprehensible fibre but also scavenges oxygen, which may contribute to pH stabilization and enhanced absorption of fibre-based feedstuffs (Chaucheyras-Durand et al., 2008; Pinloche et al., 2013; Habeeb, 2017). Reduced lactic acid levels in the rumen are typically linked to pH stabilization. Yeast-induced declines in 2 hydroxypropanoic acid concentrations and the required ruminal pH regulation may be explained by the enhancement of lactic acid-utilizing bacteria. It has been demonstrated that lactic acid-consuming bacteria such as *S. ruminantium*, which uses mannitol, are promoted in the laboratory using saccharomyces cerevisiae in a blend of stomach fluid cultures (Newbold et al., 1998).

Additionally, *S. cerevisiae* can contend with *Streptococcus gallolyticus*, the primary rumen 2 Hydroxypropanoic acid generator, for the uptake of soluble carbohydrates (Chaucheyras et al., 1995). Girard (Girard, 1996) demonstrated that several yeast cell fractions contained both heat-stable (short-chain peptides) and heat-labile (likely lipidic) stimulating components. It has been demonstrated that yeast supplies vitamins, particularly vitamin B1, to promote the growth of rumen fungus (Chaucheyras et al., 1995). It was also proposed to remove oxygen, which would prevent the rumen's exclusively anaerobic bacteria from growing. Although the liquids that come out of the rumen are mainly oxygen-deprived, during the daily feeding cycle, a decreased amount of dissolved oxygen can be observed. While the animal is feeding, oxygen is taken up by the rumen from both the feed and the saliva. The availability of oxygen in the rumen during consumption of food, grinding, and water consumption is mostly responsible for the increase in redox potential seen in sheep following meals (Mathieu et al., 1996). Linoleic acid undergoes ruminal biohydrogenation, which turns it into rumenic acid. It then hydrogenates this acid to create trans-11 (vaccenic acid), which is then converted into octadecanoic acid (Harfoot, 1981; Kim et al., 2000). The hydrogenation by biosynthesis of cis- 9,12-Octadecadienoic and cis-9,12-Octadecadienoic acid in the rumen is facilitated by several bacterial species. Groups A and B can be created from these bacteria based on the products that they produce during fermentation. While group B is specialized in producing stearic acid (18:0), group A's bacteria are in charge of converting linoleic and linolenic fatty acids into trans-11 (vaccenic acid). Some strains of *Butyrivibrio fibrisolvens* are among the rumen bacteria in group A, while two strains of Fusocillus are found in group B (Harfoot, 1981). By promoting the growth of the bacteria engaged in the process, yeast can affect the rumen's biohydrogenation pathway. Since the enzyme Δ9-desaturase converts trans-11 to cis-9, trans-11 CLA in the tissue, an increase in the ruminal manufacturing of trans- 11 C18:1 indicates that beef and dairy products will contain more cis-9, trans-11 CLA (Figure 2) (Bauman et al., 1999; Bauman et al., 2020).

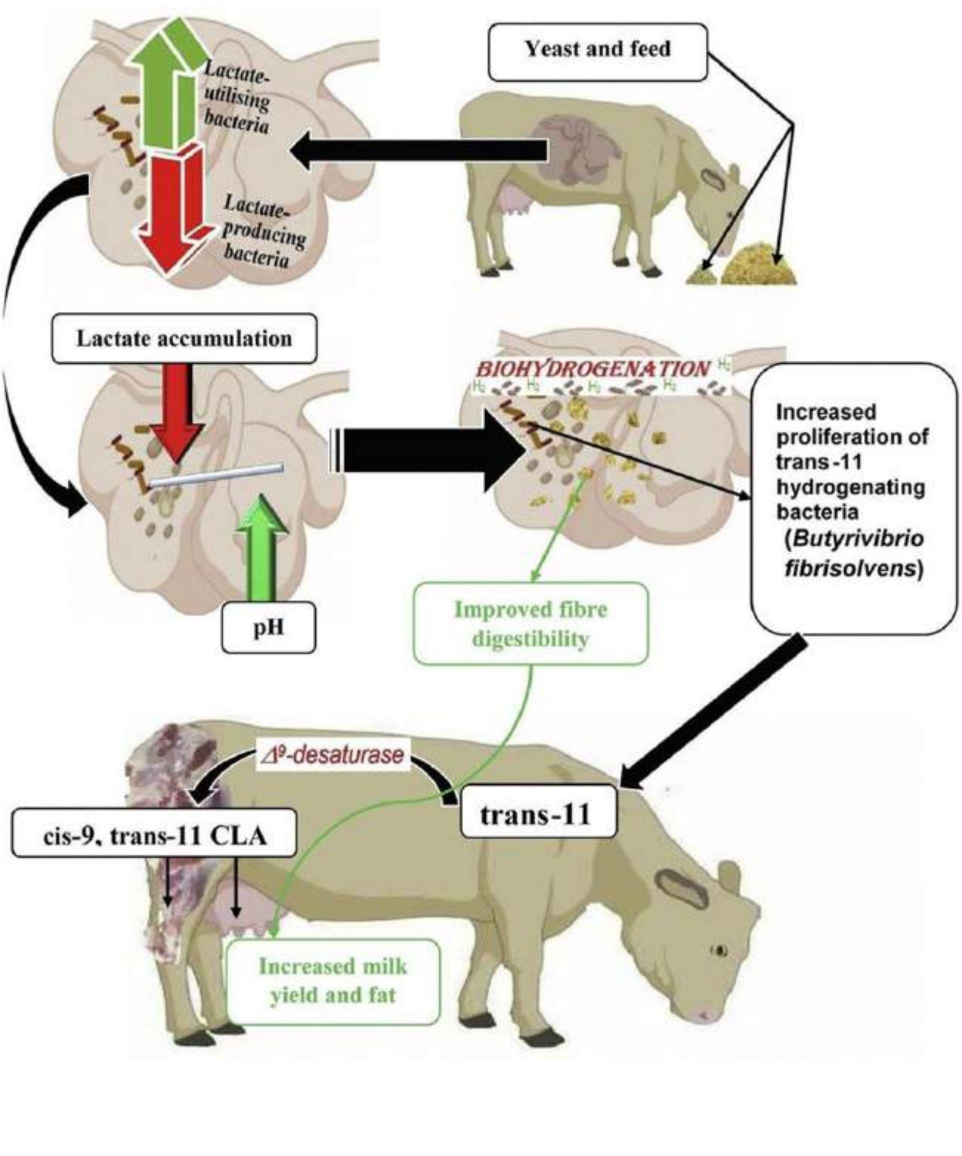

Fig. 2: The benefits of adding yeast culture to feeds with varying carbohydrate contents on cow milk production efficiency and eating behaviour.

Anti-adhesive Properties of Yeast

It is widely acknowledged that the initial stage of bacterial infection of mucous membranes is epithelial adherence. Similar to the link between antigens and antibodies, bacteria have attaching molecules on their surfaces that can interact stereospecifically with host-cell membranes. There is proof that some strains of salmonella or *E. Coli* have a fibroblast adherence that attaches to mannose residues on the membranes of epithelial cells (Ofek et al., 1977). According to Korhonen (1979), these bacteria or their separated fimbriae will also agglutinate yeast that has mannan in the outer layer of their cell wall. D- mannose solutions prevent this agglutination (Korhonen, 1979).

Pathogens that bind to the cell wall of yeast have an antimicrobial effect because the combined structure mix of *S. cerevisiae* and pathogen is quickly removed from the gastrointestinal tract (Gedek, 1989). Yeast's helpful activity may be explained by pathogens and yeast competing for intestinal cell adhesion, which is essential for the manifestation of the cytopathogenic effect (Oyofo et al., 1989; Line et al., 1998). Both mannose and yeast therapy dramatically decreased the frequency of *Salmonella typhimurium* colonization in broilers; however, the addition of yeast had little effect on *Campylobacter* colonization. It has also been demonstrated that *S. cerevisiae* inhibits the adherence of *Entamoeba histolytica* trophozoites and *Staphylococcus aureus* to human cells (Rigothier et al., 1990; Kvidera et al., 2017).

Yeast and Immunity Stimulation in Ruminants

Studies showing that when engaged, the defence systems of pigs and cattle utilize about 1 kg of glucose in 12 hours provide evidence for the energy need (Bontempo et al., 2006; Kvidera et al., 2016).When yeast is added to cattle and pigs, the immune system responds in different ways (Table 2). The non- specific response, which includes the first, is generally innate to an infective agent, and the natural immune response, which is an infection-specific response, comprises the two components of the immune system. Supplementing with yeast has been shown to influence both of these immunological responses.

WBC fluctuations may be a sign of an infection. Therefore, any alteration in WBC concentrations could indicate a better or more severe state of the illness. The most common WBC subtype in pigs and cattle is T cells, which are accompanied by monocytes. Steers' circulating neutrophils and lymphocytes significantly decreased in response to a vaccination challenge; however, this response was not observed in steers given a disintegrated yeast supplement (Kim et al., 2011).

Table 2: The outcome of *saccharomyces cerevisiae* and cerevisiae-based products ingestion on the immunological reaction in bovine and pigs.

No.	Classification	*Saccharomyces cerevisiae*	Effect on Immunological Response	Reference
1.	Beef Cattle	decomposed *Saccharomyces cerevisiae*	restrained decline in leukocyte 1	(Kim et al., 2011)
2.	Beef Cattle	YCW3	Diminished IL-6 concentrations	(Sanchez et al., 2013)
3.	Beef Cattle	YCW	Diminished acute phase proteins	(Lei et al., 2013)
4.	Dairy Calves	Yeast	Raised neutrophil ability	(Ryman et al., 2013; Fomenky et al., 2018)
5.	Sows	SCFP	Decreased leukocyte amounts	(Shen et al., 2009)
6.	Weaned pigs	YCW	Decreased leukocyte amounts	(Burdick Sanchez et al., 2020)
7.	Weaned pigs	*Saccharomyces cerevisiae*	Raised leukocyte; diminished inflammation causing lymphokines levels	(Collier et al., 2011)
8.	Weaned pigs	*Saccharomyces cerevisiae* culture	Reduced type II interferon level and TH cells	(Shen et al., 2009)
9.	Weaned pigs	β-glucan	Raised CD4+ T cells	(Hahn et al., 2006)
10.	Weaned pigs	SCFP	Raised amount of inflammation causing lymphokines	(Sanchez et al., 2018)
11.	Weaned pigs	MOS-4	Raised WBC; decreased cytokine concentrations	(Che et al., 2011)
12.	Weaned pigs	β-glucan	Raised cytokine amounts	(Li et al., 2006)
13.	Weaned pigs	β-glucan	Diminished cachetin and BSF2; Increased IL-10	(Li et al., 2005)
14.	Weaned pigs	Yeast	Unchanged lymphocyte amount	(Lessard et al., 2009)

SCFP: *S. cerevisiae* fermentation product; 2 YCW: Yeast cell wall; 3 MOS: mono oligosaccharide 4 B-cell stimulatory factor 2.

On the other hand, it was shown that beef cows treated with a Yeast fermented product (SCFP) before being challenged with LPS had greater quantities of thrombocyte and Leukocyte populations, but that after the immunological obstacle, their levels of cytokines that promote inflammation were lower (Burdick Sanchez et al., 2020).According to the authors, the higher WBC numbers before the LPS challenge primed the immune system, getting it ready for the obstacle and eventually leading to the lower mediator amounts seen after the test. However, supplementing heifers with a mixture of live yeast and YCW products did not alter the level of WBCs before or after an outbreak of breathing disorders caused by bacteria and viruses (Word et al., 2019).

Hence, WBC populations appear to respond differently to yeast supplementation in pigs, much like they do in cattle. Alterations in immune cell subtypes have also been examined in research. For instance, weaned pigs were given yeast culture supplementation and exhibited a decrease in CD4+ cells or T- helper cells (Shen et al., 2009). Nonetheless, it was discovered that weaned pigs given nutritional β- glucan derived from yeast had a higher number of CD4+ cells (Hahn et al., 2006). These results show that variations in particular leukocyte communities are also impacted by the difficult setting and saccharomyces food additive employed. Cytokine production is necessary for immune system activation, which includes WBC recruitment and the induction of illness behaviour. Cachectin, B-cell stimulatory factor 2 (BSF-2), and type II interferon are examples of cytokines associated with inflammation that induce fever, illness behaviour, and the synthesis of APP. They also promote the creation of other cytokines that aid in the immunological response. On the other hand, anti-inflammatory cytokines like cytokine synthesis inhibitory factor (CSIF) and Pitrakinraboth stimulate the adaptive immune system and lessen inflammation. As a result, mediator ratios may be a useful marker of stimulation of the immune system and systemic inflammation. When beef heifers are fed with two distinct YCW products, their BSF2 values in blood samples after an LPS defiance were lower than those of non-supplemented heifers in terms of both amplitude and durability (Sanchez et al., 2013). Decreased temperature of the bowel was also noted in the study, which may indicate a general decrease in the inflammatory response in addition to this TNF response. On the other hand, inflammation-causing lymphokines cachexin and BSF2 were shown to be enhanced in the serum for a comparable amount of time in weaned pigs supplemented with SCFP as opposed to non-supplemented pigs (Sanchez et al., 2018). Before an LPS challenge, supplementing young pigs with β-glucan led to decreased levels of BSF-2 and cachexin, but raised IL-10 during the acute (3–6 hours) period that followed LPS injection (Li et al., 2006). The authors hypothesized that this cytokine response could explain the better pig performance seen in the study by indicating a reduction in an allergic reaction that promotes swelling and an elevation in the inhibition of inflammation. In a similar vein, it was discovered that weaned pigs given MOS supplements and confronted by the virus that causes pig fertility and lung infections had lower TNF-α but higher IL-10 concentrations after infection, as well as higher WBC concentrations in the early stages (Che et al., 2011).Thus, depending on the product and challenge model, there is a variance in serum lymphokine amount, which is comparable to changes in WBC populations. These variations are probably responsible for variations in cytokine duration as well as amplitude. The APP binds pathogens, initiates complements, and binds cellular debris, among other actions, that assist the immune response. Furthermore, while cytokines that cause inflammation have significance for the invulnerable reaction excess stimulation of these inflammatory mediators for a longer duration can lead to an inflammatory hypercondition that could be harmful to the animal's well-being and healing (Gruys et al., 2005).

Impact of Yeast on Metabolic Activity and Expected Mechanisms of Action

When yeast products are added to cow and swine feed, significant variations in the immunological response have been noted; however, the metabolic process seems to be more stable (Table 3). Weaned beef cows supplemented with yeast cell walls had different metabolic responses to LPS challenge; higher glucose and insulin responses were seen, while non-esterified fatty acid (NEFA) concentrations were decreased (Sanchez et al., 2014). Steers supplemented with SCFP after an LPS exposure similarly showed increased glucose responses (Burdick Sanchez et al., 2020). According to these two studies, adding yeast products to an immunological challenge may increase its energy availability, which could help hasten its resolution. Likewise, higher sucrose amounts were noted in Holstein calves that did not exhibit passive transfer after being supplemented with yeast (Galvão et al., 2005). Furthermore, contrasted to untreated cows, nursing milking cows treated using *S. cerevisiae* cultures or living yeast had higher serum glucose and lower nitrogen in their blood (Doležal et al., 2011; Dehghan-Banadaky et al., 2013).

Table 3: Impact of supplementing with saccharomyces and saccharomyces-based commodities on pigs and bovine metabolic metrics. YCW: yeast cell wall; NEFA: non-esterified fatty acid; SCFP: *Saccharomyces cerevisiae* fermentation product.

No.	Family	*Saccharomyces cerevisiae* derivatives	Digestion Domain	Reference
1.	Beef calves	YCW 1	Raised glucose and insulin decreased NEFA 2	(Sanchez et al., 2014)
2.	Beef calves	SCFP 3	Accentuated glucose	(Burdick Sanchez et al., 2020)
3.	Feeder calves	Live yeast and YCW	Kindred glucose; decreased urea nitrogen	(Word et al., 2019)
4.	Dairy calves	Yeast	Raised glucose	(Galvão et al., 2005)
5.	Milking cows	Live yeast or Yeast culture	Raised glucose; diminished urea nitrogen	(Galvão et al., 2005; Doležal et al., 2011)
6.	Milking cows	Live yeast	No effect on glucose	(Dehghan-Banadaky et al., 2013)
7.	Weaned pigs	YCW	Diminished NEFA levels	(Burdick Sanchez et al., 2020)
8.	Sows	SCFP	Ability for decreased urea nitrogen	(Shen et al., 2009)

Living yeast-fed cows showed increased numbers of fibrolytic and lactate-utilizing bacteria (Pinloche et al., 2013). As previously mentioned, this is one of the possible ways that yeast works and could lead to a more stable pH. Elevations in both fibrolytic and cellulolytic bacteria facilitate the breakdown of fibre- based livestock feed, hence stabilizing the pH of the rumen and increasing the amount of swallowed tubular animal feed that is utilized. Furthermore, it has been shown that yeast products, like SCFP, raise rumen propionate concentrations, which may contribute to an increase in cattle's serum glucose levels (Zhu et al., 2017). Dairy cows treated with live yeast showed higher levels of total VFA and acetate, indicating increased substrate availability for microbial glucose synthesis (Kumprechtová et al., 2019). Dairy cows treated with live yeast showed higher levels of total VFA and acetate, indicating increased substrate availability for microbial glucose synthesis (Kumprechtová et al., 2019). Furthermore, compared to non-supplemented cows, glucose was higher and NEFA was less at the height of lactation, indicating a potential role for yeast in reducing the stress associated with an imbalance in energy. All the same, it seems that yeast contributes to higher blood sugar levels which might offer more energy for the immune system to use and possibly hasten the healing process following an infection.

Conclusion

The field of probiotic research has experienced rapid growth in the last several years, with a growing interest in the use of probiotic yeasts, which has been used sparingly until now. When yeast is added to cattle and pigs, the immune system responds in different ways WBC fluctuations may be a sign of an infection. Therefore, any alteration in WBC concentrations could indicate a better or more severe state of the illness. Compared to *S. cerevisiae*, *S. cerevisiae var. boulardii* has more copies of the genes involved in response to stressed conditions and translation These genes might be involved in pseudo-hyphal transitioning, rapid development rates, and enhanced resilience to raise pH. It might be feasible in future that probiotics may be recommended as drugs either in addition to or as substitute of antibiotics for certain medical conditions.

REFERENCES

Altmann, M. (2017). The Benefits of Saccharomyces boulardii. In *The Yeast Role in Medical Applications*. IntechOpen.

Bach, A., Guasch, I., Elcoso, G., Chaucheyras-Durand, F., Castex, M., Fàbregas, F., and Aris, A. (2018). Changes in gene expression in the rumen and colon epithelia during the dry period through lactation of dairy cows and effects of live yeast supplementation. *Journal of Dairy Science*, *101*(3), 2631-2640.

Bauman, D., Baumgard, L., Corl, B., and Griinari, D. J. (1999). Biosynthesis of conjugated linoleic acid in ruminants. American Society of Animal Science,

Bauman, D. E., Corl, B. A., and Peterson, D. G. (2020). The biology of conjugated linoleic acids in ruminants. In J.-L. Sebedio, W. W. Christie, and R. Adlof (Eds.), *Advances in Conjugated Linoleic Acid Research* (1st ed., pp. 146-173). AOCS Publishing.

Bontempo, V., Di Giancamillo, A., Savoini, G., Dell'Orto, V., and Domeneghini, C. (2006). Live yeast dietary supplementation acts upon intestinal morpho-functional aspects and growth in weanling piglets. *Animal Feed Science and Technology*, *129*(3-4), 224-236.

Bradford, B., Yuan, K., Farney, J., Mamedova, L., and Carpenter, A. (2015). Invited review: Inflammation during the transition to lactation: New adventures with an old flame. *Journal of Dairy Science*, *98*(10), 6631-6650.

Burdick Sanchez, N. C., Carroll, J. A., Broadway, P. R., Edrington, T. S., Yoon, I., and Belknap, C. R. (2020). Some aspects of the acute phase immune response to a lipopolysaccharide (LPS) challenge are mitigated by supplementation with a Saccharomyces cerevisiae fermentation product in weaned beef calves. *Translational Animal Science*, *4*(3), txaa156.

Chaucheyras-Durand, F., Walker, N., and Bach, A. (2008). Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. *Animal Feed Science and Technology*, *145*(1-4), 5-26.

Chaucheyras, F., Fonty, G., Bertin, G., and Gouet, P. (1995). Effects of live Saccharomyces cerevisiae cells on zoospore germination, growth, and cellulolytic activity of the rumen anaerobic fungus, Neocallimastix frontalis MCH3. *Current Microbiology*, *31*, 201-205.

Che, T., Johnson, R., Kelley, K., Van Alstine, W., Dawson, K., Moran, C., and Pettigrew, J. (2011). Mannan oligosaccharide improves immune responses and growth efficiency of nursery pigs experimentally infected with porcine reproductive and respiratory syndrome virus. *Journal of Animal Science*, *89*(8), 2592-2602.

Collier, C., Carroll, J., Ballou, M., Starkey, J., and Sparks, J. (2011). Oral administration of Saccharomyces cerevisiae boulardii reduces mortality associated with immune and cortisol responses to Escherichia coli endotoxin in pigs. *Journal of Animal Science*, *89*(1), 52-58.

Czerucka, D., Piche, T., and Rampal, P. (2007). yeast as probiotics–Saccharomyces boulardii. *Alimentary Pharmacology and Therapeutics*, *26*(6), 767-778.

Dehghan-Banadaky, M., Ebrahimi, M., Motameny, R., and Heidari, S. (2013). Effects of live yeast supplementation on mid-lactation dairy cows performances, milk composition, rumen digestion and plasma metabolites during hot season. *Journal of Applied Animal Research*, *41*(2), 137-142.

Di Giancamillo, A., Bontempo, V., Savoini, G., Dell'Orto, V., Vitari, F., and Domeneghini, C. (2007). Effects of live yeast dietary supplementation to lactating sows and weaning piglets. *International Journal of Probiotics and Prebiotics*, *2*(1), 55-66.

Doležal, P., Dvořáček, J., Doležal, J., Čermáková, J., Zeman, L., and Szwedziak, K. (2011). Effect of feeding yeast culture on ruminal fermentation and blood indicators of Holstein dairy cows. *Acta Veterinaria Brno*, *80*(2), 139-145.

Durand-Chaucheyras, F., Fonty, G., and Bertin, G. (1997). L'utilisation de levures vivantes, additifs microbiens chez le ruminant: effets sur la microflore et les fermentations ruminales, effets zootechniques. *Bull. Group. Technology Véterinary*, *5*, 35-52.

Fiems, L., De Campeneere, S., Cottyn, B., Vanacker, J., and Boucqué, C. (1999). Effect of amount and degradability of dietary starch on animal performance and meat quality in beef bulls. *Journal of Animal Physiology and Animal Nutrition*, *82*(5), 217-226.

Fomenky, B. E., Chiquette, J., Lessard, M., Bissonnette, N., Talbot, G., Chouinard, Y. P., and Ibeagha-Awemu, E. M. (2018). Saccharomyces cerevisiae var. boulardii CNCM I-1079 and Lactobacillus acidophilus BT1386 influence innate immune response and serum levels of acute-phase proteins during weaning in Holstein calves. *Canadian Journal of Animal Science*, *98*(3), 576-588.

Galvão, K. N., Santos, J. E., Coscioni, A., Villaseñor, M., Sischo, W. M., and Berge, A. C. B. (2005). Effect of feeding live yeast products to calves with failure of passive transfer on performance and patterns of antibiotic resistance in fecal Escherichia coli. *Reproduction Nutrition Development*, *45*(4), 427-440.

Gedek, B. (1989). Interaktionen zwischen lebenden Hefezellen und darmpathogenen Escherichia-coli-Keimen. Ökosystem Darm: Morphologie, Mikrobiologie, Immunologie Klinik und Therapie akuter und chronischer entzündlicher Darmerkrankungen,

Girard, I. (1996). The mode of action of yeast culture in stimulating ruminal fermentation. *Feed Compounder*, *16*(11), 16-17.

Gruys, E., Toussaint, M., Niewold, T., and Koopmans, S. (2005). Acute phase reaction and acute phase proteins. *Journal of Zhejiang University-Science B*, *6*(11), 1045-1056.

Habeeb, A. A. M. (2017). Importance of yeast in ruminants feeding on production and reproduction. *Ecology and Evolutionary Biology*, *2*(4), 49.

Hahn, T.-W., Lohakare, J., Lee, S., Moon, W., and Chae, B. (2006). Effects of supplementation of β-glucans on growth performance, nutrient digestibility, and immunity in weanling pigs. *Journal of Animal Science*, *84*(6), 1422-1428.

Harfoot, C. (1981). Lipid metabolism in the rumen. *Lipid Metabolism in Ruminant Animals*, 21-55.

Kim, M., Seo, J., Yun, C., Kang, S., Ko, J., and Ha, J. (2011). Effects of hydrolyzed yeast supplementation in calf starter on immune responses to vaccine challenge in neonatal calves. *Animal*, *5*(6), 953-960.

Kim, Y. J., Liu, R. H., Bond, D. R., and Russell, J. B. (2000). Effect of linoleic acid concentration on conjugated linoleic acid production by Butyrivibrio fibrisolvens A38. *Applied and Environmental Microbiology*, *66*(12), 5226-5230.

Korhonen, T. K. (1979). Yeast cell agglutination by purified enterobacterial pili. *FEMS Microbiology Letters*, *6*(6), 421-425.

Kumprechtová, D., Illek, J., Julien, C., Homolka, P., Jančík, F., and Auclair, E. (2019). Effect of live yeast (Saccharomyces cerevisiae) supplementation on rumen fermentation and metabolic profile of dairy cows in early lactation. *Journal of Animal Physiology and Animal Nutrition*, *103*(2), 447-455.

Kvidera, S., Horst, E., Abuajamieh, M., Mayorga, E., Fernandez, M. S., and Baumgard, L. (2017). Glucose requirements of an activated immune system in lactating Holstein cows. *Journal of Dairy Science*, *100*(3), 2360-2374.

Kvidera, S., Horst, E., Abuajamieh, M., Mayorga, E., Sanz Fernandez, M., and Baumgard, L. (2016). A procedure to estimate glucose requirements of an activated immune system in steers. *Journal of Animal Science*, *94*(11), 4591-4599.

Lei, C., Dong, G., Jin, L., Zhang, S., and Zhou, J. (2013). Effects of dietary supplementation of montmorillonite and yeast cell wall on lipopolysaccharide adsorption, nutrient digestibility and growth performance in beef cattle. *Livestock Science*, *158*(1-3), 57-63.

Lessard, M., Dupuis, M., Gagnon, N., Nadeau, E., Matte, J., Goulet, J., and Fairbrother, J. (2009). Administration of Pediococcus acidilactici or Saccharomyces cerevisiae boulardii modulates development of porcine mucosal immunity and reduces intestinal bacterial translocation after Escherichia coli challenge. *Journal of Animal Science*, *87*(3), 922-934.

Li, J., Li, D., Xing, J., Cheng, Z., and Lai, C. (2006). Effects of β-glucan extracted from Saccharomyces cerevisiae on growth performance, and immunological and somatotropic responses of pigs challenged with Escherichia coli lipopolysaccharide. *Journal of Animal Science*, *84*(9), 2374-2381.

Li, J., Xing, J., Li, D., Wang, X., Zhao, L., Lv, S., and Huang, D. (2005). Effects of β-glucan extracted from Saccharomyces cerevisiae on humoral and cellular immunity in weaned piglets. *Archives of Animal Nutrition*, *59*(5), 303-312.

Line, J. E., Bailey, J. S., Cox, N. A., Stern, N. J., and Tompkins, T. (1998). Effect of yeast-supplemented feed on Salmonella and Campylobacter populations in broilers. *Poultry Science*, *77*(3), 405-410.

Ma, J., Shah, A. M., Shao, Y., Wang, Z., Zou, H., and Kang, K. (2020). Dietary supplementation of yeast cell wall improves the gastrointestinal development of weaned calves. *Animal Nutrition*, *6*(4), 507-512.

Mathieu, F., Jouany, J., Senaud, J., Bohatier, J., Bertin, G., and Mercier, M. (1996). The effect of Saccharomyces cerevisiae and Aspergillus oryzae on fermentations in the rumen of faunated and defaunated sheep; protozoal and probiotic interactions. *Reproduction Nutrition Development*, *36*(3), 271-287.

McFarland, L. V. (1996). Saccharomyces boulardii is not Saccharomyces cerevisiae. *Clinical Infectious Diseases*, *22*(1), 200-201.

Newbold, C., McIntosh, F., and Wallace, R. (1998). Changes in the microbial population of a rumen-simulating fermenter in response to yeast culture. *Canadian Journal of Animal Science*, *78*(2), 241-244.

Ofek, I., Mirelman, D., and Sharon, N. (1977). Adherence of Escherichia coli to human mucosal cells mediated by mannose receptors. *Nature*, *265*(5595), 623-625.

Oyofo, B., DeLoach, J., Corrier, D., Norman, J., Ziprin, R., and Mollenhauer, H. (1989). Prevention of Salmonella typhimurium colonization of broilers with D-mannose. *Poultry Science*, *68*(10), 1357-1360.

Pinloche, E., McEwan, N., Marden, J.-P., Bayourthe, C., Auclair, E., and Newbold, C. J. (2013). The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle. *PLOS one*, *8*(7), e67824.

Rigothier, M., Maccario, J., Vuong, P., and Gayral, P. (1990). Effets des levures Saccharomyces boulardii sur les trophozoïtes d'Entamoeba histolytica in vitro et dans l'amibiase caecale du jeune rat. *Annales de Parasitologie Humaine et Comparée*, *65*(2), 51-60.

Ryman, V., Nickerson, S., Kautz, F., Hurley, D., Ely, L., Wang, Y., and Forsberg, N. (2013). Effect of dietary supplementation on the antimicrobial activity of blood leukocytes isolated from Holstein heifers. *Research in Veterinary Science*, *95*(3), 969-974.

Sanchez, N. C. B., Carroll, J. A., Broadway, P. R., Bass, B. E., and Frank, J. W. (2018). Modulation of the acute phase response following a lipopolysaccharide challenge in pigs supplemented with an all-natural Saccharomyces cerevisiae fermentation product. *Livestock Science*, *208*, 1-4.

Sanchez, N. C. B., Young, T. R., Carroll, J. A., Corley, J. R., Rathmann, R. J., and Johnson, B. J. (2013). Yeast cell wall supplementation alters aspects of the physiological and acute phase responses of crossbred heifers to an endotoxin challenge. *Innate Immunity*, *19*(4), 411-419.

Sanchez, N. C. B., Young, T. R., Carroll, J. A., Corley, J. R., Rathmann, R. J., and Johnson, B. J. (2014). Yeast cell wall supplementation alters the metabolic responses of crossbred heifers to an endotoxin challenge. *Innate Immunity*, *20*(1), 104-112.

Shen, Y., Piao, X., Kim, S., Wang, L., Liu, P., Yoon, I., and Zhen, Y. (2009). Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. *Journal of Animal Science*, *87*(8), 2614-2624.

Word, A. B., Broadway, P. R., Burdick Sanchez, N. C., Roberts, S. L., Richeson, J. T., Liang, Y. L., and Ballou, M. A. (2019). Immune and metabolic responses of beef heifers supplemented with Saccharomyces cerevisiae to a combined viral-bacterial respiratory disease challenge. *Translational Animal Science*, *3*(1), 135-148.

Zhu, W., Wei, Z., Xu, N., Yang, F., Yoon, I., Chung, Y., and Wang, J. (2017). Effects of Saccharomyces cerevisiae fermentation products on performance and rumen fermentation and microbiota in dairy cows fed a diet containing low quality forage. *Journal of Animal Science and Biotechnology*, *8*, 1-9.

Chapter 19

Research Progress on the Relationship between Avian trichomonosis and the Microbiota of the Oral Cavity and Intestine

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ABSTRACT

Avian trichomonosis is a parasitic protozoan infection that can affect various avian species, including poultry, and frequently results in high mortality rates among juvenile birds, thereby posing a significant threat to the pigeon farming industry. The oral and intestinal microbiota represent crucial components of animal health; they are complex microbial communities that establish long-term residence within the host and exhibit mutual dependence with it. There exists an intricate interplay between parasites and microbiota: the latter can influence the processes of invasion, colonization, and pathogenicity of parasites, while parasites have the capacity to modify the composition of microbiota. In-depth study of the interaction between *Trichomonas gallinae* and the host's oral and intestinal microbiota is not only of great significance for the prevention and control of avian trichomonosis but also has potential value for understanding broader host-parasite interaction mechanisms, developing new prevention and control strategies, and promoting human health. Through these studies, we can better protect the health of pigeons and other birds, while also providing valuable insights for the field of human medicine.

KEYWORDS
Avian trichomonosis, Microbiota, Oral Cavity, Intestines

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INTRODUCTION

Avian trichomonosis is a contagious disease caused by the protozoan *Trichomonas gallinae (T. gallinae*) which primarily affects pigeons and other birds. This disease is distributed worldwide and poses a serious threat to the pigeon breeding industry. The rock pigeon (*Columba livia*), as the main host of *T. gallinae*, is widely distributed and migratory in nature, which is considered a key factor in the global spread of the disease (Harmon et al., 1987). In pigeon populations, squabs have not fully developed immune systems, hence the infection rate in squabs is usually higher than in adolescent and breeding pigeons (Stabler, 1954). Pigeons infected with *T. gallinae* often develop yellowish-white caseous lesions in the oropharyngeal area, which not only affect the birds' feeding and breathing but may also impact the balance of the oral microbiota (Rogers et al., 2018). An imbalance in the oral microbiota can lead to other oral diseases, further affecting the health of pigeons. The intestinal microbiota is one of the most complex and important ecosystem in an animal, playing a key role in the health and disease of the host. Under normal conditions, the intestinal microbiota is generally in a state of dynamic equilibrium, which is crucial for the host's health, however, this balance can be disrupted by various factors, such as age, diet, pathogens, etc. Studies have shown that parasites colonizing the host can directly or indirectly affect the dynamic balance of the microbiota (Mehlhorn et al., 2009). For example, parasites may affect the composition and function of the intestinal microbiota by consuming nutrients within the host, secreting toxins or altering the host's physiological environment.

Currently, there is no effective vaccine available for avian trichomonosis. Therefore, a deep understanding of the interaction between *Trichomonas gallinae* and the host's oral and intestinal microbiota is of great significance for developing new prevention and control strategies. Studying these interactions can better understand how *Trichomonas gallinae* survives and reproduces within the host and how they affect the host's health. This knowledge can provide a reference for the research and development of new therapeutic drugs, vaccines, or management strategies to reduce the harm of avian trichomonosis to pigeons and other birds.

Overview of *T. gallinae*

The *T. gallinae* belongs to the Phylum Protozoa, Class Mastigophora, Subclass Zoomastigophorea, Order Trichomonadida, Family Trichomonadidae, and Genus Trichomonas, has a pear-shaped body with numerous wrinkles and indentations on its surface. It measures 5-9µm in length and 2-9µm in width. Under the microscope, the live organism moves rapidly with a spiral swimming pattern. It is characterized by having four free flagella of varying sizes, which are typical features and serve as its primary locomotive organs. The four anterior flagella are closely packed together, emerging from the flagellar pocket. The wall of the pocket having crescent-shaped membranous structures is visible to the cell's anterior end, and the flagella extends from a single axostyle on one side (Mehlhorn et al., 2009). The organism possesses an undulating membrane that starts at the front end and ends just in front of the rear end, resembling a fish fin, with several distinct undulations and sometimes fewer and shallower ones. The outer edge of the undulating membrane is composed of paraxial rods and attached recurrent flagella, which are not free and are located within the grooves of the undulating membrane, extending beyond the end of the membrane. Under the optical microscope, a single axostyle can be seen, often extending beyond the rear edge of the cell, running from the anterior end to the caudate posterior end of the trichomonad, positioned near the longitudinal axis of the cell. The terminal part of the axostyle protrudes about one-third of the cell length beyond the posterior surface of the organism. *T. gallinae* has a simple morphology, being a single-celled structure without mitochondria, but it does have hydrogenosomes. The organism is semi-transparent and relatively bright. With no complex life cycle, the organism exists only in the hape of the rophozoite stage and reproduces by binary fission.It can reproduce a generation approximately every 4 hours.

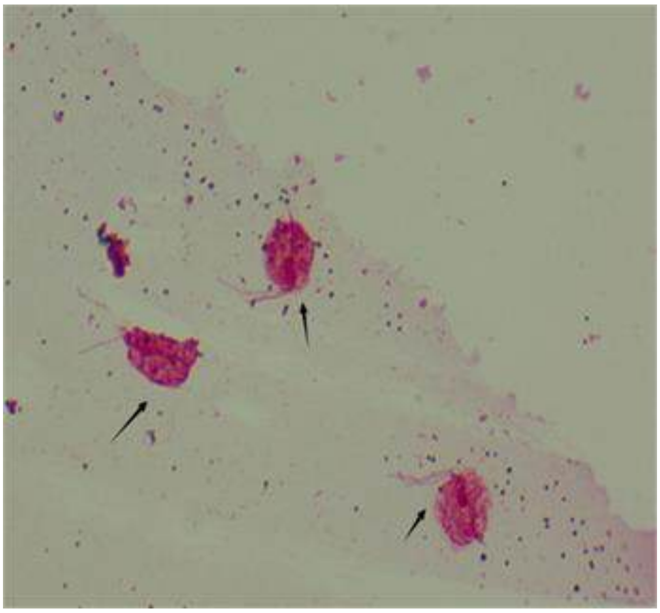

Fig.1: Microscopic image of *Trichomonas gallinae* stained with Gram stain (1000X).

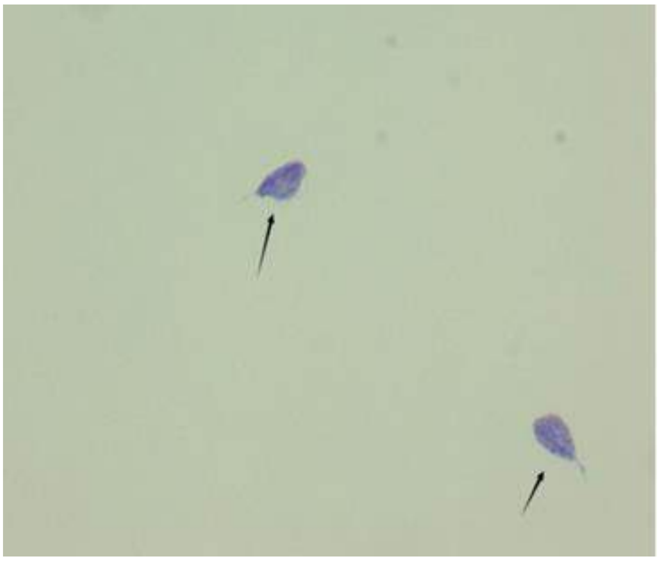

Fig. 2: Microscopic image of *Trichomonas gallinae* stained with Giemsa stain (1000X).

Overview of Avian Trichomonosis

Avian trichomonosis predominantly affects birds' upper respiratory and digestive tracts, with a common occurrence in the pharynx, esophagus, and liver. It can cause severe ulceration and yellow-white caseous necrotic lesions in the digestive tract of pigeons. In severe cases, it may lead to systemic infection. In 2022, it was observed that *T. gallinae* can invade the heads of avian species, parasitizing the brain and eyes. The most important route of transmission between birds is through the ingestion of trophozoites via shared water and food sources facilitated by saliva (Villanúa et al., 2006; Gerhold et al., 2007). It has been suggested that the transmission of parasites via crop milk from infected parent birds to squabs seems most efficient for establishing an infection (Harmon et al., 1987). Infected adult pigeons often do not show significant symptoms, becoming asymptomatic carriers and a new source of infection. Additionally, birds of prey can become infected by consuming infected prey, as *T. gallinae* can survive in carcasses for at least 48 hours. The parasite can persist in various water sources for up to an hour (Purple et al., 2015), such as drainage ditches and water drinkers. However, higher temperatures (30–35°C) can extend its survival time (Kocan,1969). Although it can form pseudocysts under adverse conditions, a moist environment is crucial for maintaining its infectivity (Stabler, 1954). Clinical manifestations of infection vary from mild to severe, with severe, potentially fatal inflammation causing birds to die from starvation or asphyxiation by obstructing the esophagus and respiratory tract. *T. gallinae* are generally considered normal residents (symbionts) on the mucosal surface of the upper digestive tract. However, by causing inflammation in the underlying tissues or when entering the more distal parts of the avian digestive tract, this protozoan parasite can cause mild to severe lesions, depending on the strain's virulence and the host's susceptibility. Infection with highly pathogenic strains can lead to death. Laboratory diagnosis typically includes direct smear microscopic observation and microscopic observation after in vitro culture to observe the living *T. gallinae* as the key to confirmation. In addition, molecular biological techniques such as PCR, RAPD, RFLP, and high-throughput sequencing can also be used for more sensitive diagnosis (Turner et al., 2023). In terms of prevention and treatment, there is currently no effective vaccine, and nitroimidazole drugs are usually used for prevention and treatment. The method of drug use needs attention because some drugs, such as metronidazole, have certain toxic side effects and should not be used arbitrarily in increased dosage or extended course. Generally, a course of treatment is preferable for five days (Gómez-Muñoz et al., 2022). In addition to using drugs, it is also necessary to strengthen breeding management, reduce breeding density, supplement nutrients, maintain a dry and sanitary environment, regularly check health conditions, and timely detect and treat infections. At the same time, for dead pigeons, they should also be treated harmlessly in a timely manner, and effective disinfection work should be carried out to avoid causing widespread transmission.

Fig. 2: Pictorial dissection of the head in pigeons with avian trichomonosis.

The Interaction between Avian Trichomonosis Infection and the Oral Microbiota

It is generally understood that the intricate interactions between microbiomes and host-parasite microorganisms can be bidirectional and significantly impact animal health. Dietary changes, host morphology and phylogeny, captivity, antibiotic treatment, age, gender, and the presence of certain diseases can all affect oral microbiota composition. There is little evidence available about how trichomonosis infection affects the composition, structure, and dynamics of avian oral microbial communities. Previous research indicates that Cooper's hawks' susceptibility to trichomonosis is substantially connected to the age-specific pH of the oral cavity (Urban et al., 2014).

The mean pH of fluid in the oral cavity of nestling Cooper's hawks is 6.8, whereas that of fledglings and adults is 6.0–6.1, which is at least seven times more acidic. Trichomonas gallinae thrives when pH is between 6.5 and 7.5 (optimum 7.2). Fluid in the oral cavity of Cooper's hawks becomes more acidic after birds have fledged and are nearing independence (≥50 days of age), but the reason for the change is unknown (Urban et al., 2014). Many animals undergo similar changes in body chemistry (i.e., a change in acidity) during maturation, which are often associated with changes in their bacterial communities. Therefore, Taylor et al. (2019) hypothesized that oral microbiota composition may be related to age-specific differences in susceptibility to *T. gallinae* by comparing the oral microbiota of nestling and adult Cooper's hawks. The study found significant differences in the oral microbiome composition between nestling and juvenile/adult Cooper's hawks, which is surprising given the feeding behavior of the species. Breeding adults consume the same prey as that fed to the nestlings, so dietary changes at different ages are unlikely to play a role in the observed changes in the oral microbiome.

There is little study on the relationship of *T. gallinae* with the oral microbiome. Claudio Alba et al. (2023) investigated how captive breeding and trichomonad infection affected the oral microbiome of Bonelli's eagle nestlings. Bonelli's eagle's core oral microbiota comprises Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria, with Megamonas and Bacteroides being the most numerous taxa. The study discovered that trichomonad infection had a minor impact on the microbiota composition, with a considerable increase in the relative abundance of the Gemella genus in eaglets with trichomonosis lesions. This genus lives on the oral mucosal surface and is an opportunistic pathogen known to cause human abscess problems, inflammation, and abscesses in various places. Abscesses, comparable to those seen in human endocarditis, meningitis, and orbital or maxillary abscesses (Maraki et al., 2019; McQuinn et al., 2019) were found in highly infected birds. *T. gallinae*-infected eaglets also contained a larger proportion of planktonic bacteria. This phylum is found in a variety of environments, including marine, freshwater, and wastewater treatment plants, and its presence in water sources aids in the spread of *T. gallinae*, which may explain its link with the parasite.

The Interaction between Avian Trichomonosis Infection and the Intestinal Microbiota

The intestinal microbiota, a complex community of microorganisms living in the animal's gut, plays a crucial role in the function of the intestinal barrier, including promoting nutrient absorption and digestion, maintaining intestinal physiological functions, and regulating the body's immune system. Under normal conditions, the population density and composition of the animal gut microbiota are in a state of balance, which can be altered by various factors such as age, diet, and pathogens. The interaction between parasites and gut microbiota is significant for maintaining intestinal homeostasis. Parasitic infections can lead to changes in the gut microbiota, dysbiosis, and inflammatory diseases (Faivre et al., 2019). Gaining a deep understanding of how parasites affect the composition and function of the host's intestinal flora may, in turn, affect the infection and pathogenicity of the parasites through these changes. Research into the interplay between parasites and the host's intestinal microbiota can also provide references for other areas of study. For example, understanding how parasites affect the host's immune system can help develop new immune modulation strategies to enhance the host's resistance to other pathogens. At the same time, these studies can also provide insights for the field of human medicine, as many human diseases are also related to the imbalance of the gut flora, such as inflammatory bowel disease (Sultan et al., 2021) ,obesity, and diabetes, etc (Ortega et al.,2020; Madhogaria et al., 2022). However, recent research had found that helminths had potential therapeutic effects on inflammatory bowel diseases, challenging the conventional notion that parasites are generally harmful to humans (Wang et al., 2020).

Infection with *T. gallinae* can affect the diversity and composition of the pigeon's gut microbiota. When comparing the richness of different microbial communities in the small intestine and rectum of healthy pigeons, it was found that the *Lactobacillus* genus was dominant. However, after infection with *T. gallinae*, the richness of *Firmicutes* and *Lactobacillus* decreased, while the richness of *Proteobacteria*, *Enterococcus*, *Atopobium*, *Roseomonas*, *Bifidobacterium*, and *Peptostreptococcus* increased (Ji et al., 2020). When 14-day-old pigeons were infected with *T. gallinae*, the richness of the crop microbiota significantly decreased, with the proportion of *Lactobacillus* reducing by at least 90%. *Lactobacillus* is a beneficial microorganism that competes with pathogens through adhesion and replication, produces pathogen-resistant complex substances, and regulates immune functions, playing an important role in maintaining intestinal health (Sengupta et al., 2013). The reduction in the abundance of *Lactobacillus* may lead to drastic changes in the intestinal environment, for example, pH, followed by an apparent increase in the abundance of harmful bacteria, such as *Enterococcus* and *Atopobium*. *Enterococcus* is an important pathogen for chickens, ducks, and pigeons, and it exhibits intrinsic or acquired resistance to many antibiotics (Osman et al., 2019). *Atopobium vaginae* may be a marker for bacterial vaginosis in women (Marconi et al., 2012). Besides, *T. vaginalis* was reported to be associated with vaginal microbiota consisting of low proportion of *lactobacilli* and high proportions of *Mycoplasma, Parvimonas, Sneathia*, and *Atopobium* (Brotman et al., 2012).

The growth and development status of pigeons is closely linked to their ability to resist invasion by foreign pathogens. Squabs have weaker immunity, and the normal bacterial composition of the intestinal barrier is not fully established. Several experimental studies have confirmed that probiotics can promote the growth and development of the body (Chen et al., 2017). Before infection with *T. gallinae* in the early age of pigeons, administering *Lactobacillus* can help beneficial bacteria form a dominant population in the pigeon's gut, thereby preventing infection with *T. gallinae* (Ji et al., 2020). Administering *Lactobacillus* after infection can improve the body's immunity to a certain extent, stimulate the body's immune response, inhibit the growth and reproduction of *T. gallinae*, reduce the infection rate of *T. gallinae*, and promote the body's clearance of *T. gallinae*.

Summary and Outlook

With the development of high-throughput sequencing technology, especially the second-generation sequencing technology (Next-Generation Sequencing, NGS), the impact of parasitic infections on the species diversity and community structure of the host microbiota is increasingly receiving attention, and the understanding of the complex interactions between parasites and host microbiota is also becoming more in-depth. In the host's oral and intestinal tracts, microbial communities are closely related to the host's health status. These microbial communities participate in a variety of physiological processes, including nutrient metabolism, the development and regulation of the immune system, and defense against pathogens. Parasitic infections may disrupt the balance of these microbial communities, leading to a decrease or increase in species diversity and changes in community structure (Berrilli et al., 2012). This imbalance may affect the host's health and increase the risk of disease. Avian trichomonosis, as a global parasitic disease, poses a serious threat to the pigeon breeding industry and wild bird populations. Currently, the interaction between *Trichomonas gallinae* and the host's oral and intestinal microbiota is still in the initial stage of exploration. Studies have shown that *Trichomonas gallinae* may affect the microbial composition in the host's oral and intestinal tracts, thereby affecting the host's health. For example, infection may increase the number of certain pathogenic bacteria or reduce the number of beneficial microbes. These changes may affect the host's immune response and disease progression, making the host more susceptible to other diseases.

Probiotics are a class of beneficial microorganisms that can affect the ability of intestinal microbes to participate in the regulation of various biological processes in the host through multiple mechanisms. This includes interacting with host sex hormones, regulating stress responses and cognitive conditions, and affecting central nervous system-related functions through the microbiota-gut-brain axis (Le Morvan de Sequeira et al,.2022; Ashique et al,.2024). Probiotic interventions have shown great potential in combating parasitic infections and have been proven to reduce the pathogenicity of parasitic infections, providing new strategies for the treatment and prevention of avian trichomonosis. They can enhance the host's immune system, inhibit the growth of pathogens, or improve the structure and function of the microbial community through interactions with the host's microbiota. In addition, the antiviral effects of probiotics have also attracted attention. Probiotics may function by enhancing the intestinal mucosal barrier , stimulating the body's immune system, inducing anti-inflammatory cytokines, downregulating pro-inflammatory cytokines, and inhibiting signaling pathways (Petrariu et al,.2024).

Based on the understanding of the interaction between *Trichomonas gallinae* and the host microbiota, it also lays the foundation for the development of new targeted antiparasitic products, which may have higher selectivity and lower side effects. In the research and development of targeted antiparasitic products, innovative antiparasitic drugs and treatment methods are continuously being introduced. For example, the canine antiparasitic drug BRAVECTO QUANTUM launched by Merck, which is administered by injection, can provide protection against fleas and ticks for up to one year, showing new progress in antiparasitic product innovation (Fisara et al., 2023) .

By delving into the interplay between *Trichomonas gallinae* and the host's oral and intestinal microbiota, we can provide more effective strategies for the treatment and prevention of avian trichomonosis, laying a scientific foundation for the development of new targeted antiparasitic products, and also provide references for the study of other parasitic diseases. These studies not only help to improve the benefits of poultry farming but may also have a positive impact on human health, as many principles of parasitic disease prevention and treatment are common between birds and humans. With the application of NGS technology and further research on probiotic interventions, we hope to achieve more precise and personalized prevention and treatment of parasitic diseases in the future.

REFERENCES

Alba, C., Sansano-Maestre, J., Cid Vázquez, M. D., Martínez-Herrero, M. D. C., Garijo-Toledo, M. M., Azami-Conesa, I., Moraleda Fernández, V., Gómez-Muñoz, M. T., and Rodríguez, J. M. (2023). Captive Breeding and Trichomonas gallinae Alter the Oral Microbiome of Bonelli's Eagle Chicks. *Microbial Ecology*, *85*(4), 1541–1551.

Ashique, S., Mohanto, S., Ahmed, M. G., Mishra, N., Garg, A., Chellappan, D. K., Omara, T., Iqbal, S., and Kahwa, I. (2024). Gut-brain axis: A cutting-edge approach to target neurological disorders and potential synbiotic application. *Heliyon*, *10*(13), e34092.

Berrilli, F., Di Cave, D., Cavallero, S., and D'Amelio, S. (2012). Interactions between parasites and microbial communities in the human gut. *Frontiers in Cellular and Infection Microbiology*, *2*, 141.

Brotman, R. M., Bradford, L. L., Conrad, M., Gajer, P., Ault, K., Peralta, L., Forney, L. J., Carlton, J. M., Abdo, Z., and Ravel, J. (2012). Association between Trichomonas vaginalis and vaginal bacterial community composition among reproductive-age women. *Sexually Transmitted Diseases*, *39*(10), 807–812.

Chen, X., Yang, H., Meng, J., Wang, Z., and Wang, Y. (2017).The effect of probiotics on the growth performance, slaughter performance, immune organ index, and serum biochemical indicators of pigeon squabs. *China Feed*, 26-29, 34.

Faivre, B., Bellenger, J., Rieu, A., Guivier, E., Galan, M., Ollivier, A., Poloni, L., and Sorci, G. (2019). Disentangling the effect of host genetics and gut microbiota on resistance to an intestinal parasite. *International Journal for Parasitology*, *49*(11), Fisara, P., and Guerino, F. (2023). Year-round efficacy of a single treatment of fluralaner injectable suspension (Bravecto Quantum™) against repeated infestations with Ixodes holocyclus in dogs. *Parasites and Vectors*, *16*(1), 375.

Gerhold, R. W., Tate, C. M., Gibbs, S. E., Mead, D. G., Allison, A. B., and Fischer, J. R. (2007). Necropsy findings and arbovirus surveillance in mourning doves from the southeastern United States. *Journal of Wildlife Diseases*, *43*(1), 129–135.

Gómez-Muñoz, M. T., Gómez-Molinero, M. Á., González, F., Azami-Conesa, I., Bailén, M., García Piqueras, M., and Sansano-Maestre, J. (2022). Avian Oropharyngeal Trichomonosis: Treatment, Failures and Alternatives, a Systematic Review. *Microorganisms*, *10*(11), 2297.

Harmon, W. M., Clark, W. A., Hawbecker, A. C., and Stafford, M. (1987). Trichomonas gallinae in columbiform birds from the Galapagos Islands. *Journal of Wildlife Diseases*, *23*(3), 492–494.

Ji, F., Zhang, D., Shao, Y., Yu, X., Liu, X., Shan, D., and Wang, Z. (2020). Changes in the diversity and composition of gut microbiota in pigeon squabs infected with Trichomonas gallinae. *Scientific Reports*, *10*(1), 19978.

Kocan R. M. (1969). Various grains and liquid as potential vehicles of transmission for Trichomonas gallinae. *Wildlife Disease*, *5*(3), 148–149.

Le Morvan de Sequeira, C., Hengstberger, C., Enck, P., and Mack, I. (2022). Effect of Probiotics on Psychiatric Symptoms and Central Nervous System Functions in Human Health and Disease: A Systematic Review and Meta-Analysis. *Nutrients*, *14*(3), 621.

Madhogaria, B., Bhowmik, P., and Kundu, A. (2022). Correlation between human gut microbiome and diseases. *Infectious Medicine*, *1*(3), 180–191.

Maraki, S., Plevritaki, A., Kofteridis, D., Scoulica, E., Eskitzis, A., Gikas, A., and Panagiotakis, S. H. (2019). Bicuspid aortic valve endocarditis caused by Gemella sanguinis: Case report and literature review. *Journal of Infection and Public Health*, *12*(3), 304–308.

Marconi, C., Cruciani, F., Vitali, B., and Donders, G. G. (2012). Correlation of Atopobium vaginae Amount With Bacterial Vaginosis Markers. *Journal of Lower Genital Tract Disease*, *16*(2), 127–132.

McQuinn, M., and Horswell, B. B. (2019). First Case of Cutaneous Orbital Abscess Caused by Gemella: A Case Report and Review of the Literature. *Journal of oral and maxillofacial surgery: official Journal of the American Association of Oral and Maxillofacial Surgeons*, *77*(7), 1414–1417.

Mehlhorn, H., Al-Quraishy, S., Aziza, A., and Hess, M. (2009). Fine structure of the bird parasites Trichomonas gallinae and Tetratrichomonas gallinarum from cultures. *Parasitology Research*, *105*(3), 751–756.

Ortega, M. A., Fraile-Martínez, O., Naya, I., García-Honduvilla, N., Álvarez-Mon, M., Buján, J., Asúnsolo, Á., and de la Torre, B. (2020). Type 2 Diabetes Mellitus Associated with Obesity (Diabesity). The Central Role of Gut Microbiota and Its Translational Applications. *Nutrients*, *12*(9), 2749.

Osman, K. M., Badr, J., Orabi, A., Elbehiry, A., Saad, A., Ibrahim, M. D. S., and Hanafy, M. H. (2019). Poultry as a vector for emerging multidrug resistant Enterococcus spp.: First report of vancomycin (van) and the chloramphenicol-florfenicol (cat-fex-cfr) resistance genes from pigeon and duck faeces. *Microbial Pathogenesis*, *128*, 195–205.

Petrariu, O. A., Barbu, I. C., Niculescu, A. G., Constantin, M., Grigore, G. A., Cristian, R. E., Mihaescu, G., and Vrancianu, C. O. (2024). Role of probiotics in managing various human diseases, from oral pathology to cancer and gastrointestinal diseases. *Frontiers in Microbiology*, *14*, 1296447.

Purple, K. E., Humm, J. M., Kirby, R. B., Saidak, C. G., and Gerhold, R. (2015). Trichomonas gallinae Persistence in Four Water Treatments. *Journal of Wildlife Diseases*, *51*(3), 739–742.

Rogers, K. H., Girard, Y. A., Woods, L. W., and Johnson, C. K. (2018). Avian trichomonosis mortality events in band-tailed pigeons (*Patagioenas fasciata*) in California during winter 2014-2015. *International Journal for Parasitology. Parasites and Wildlife*, *7*(3), 261–267.

Sengupta, R., Altermann, E., Anderson, R. C., McNabb, W. C., Moughan, P. J., and Roy, N. C. (2013). The role of cell surface architecture of lactobacilli in host-microbe interactions in the gastrointestinal tract. *Mediators of Inflammation*, *2013*, 237921.

STABLER R. M. (1954). Trichomonas gallinae:a review. *Experimental Parasitology*, *3*(4), 368–402.

Sultan, S., El-Mowafy, M., Elgaml, A., Ahmed, T. A. E., Hassan, H., and Mottawea, W. (2021). Metabolic Influences of Gut Microbiota Dysbiosis on Inflammatory Bowel Disease. *Frontiers in Physiology*, *12*, 715506.

Taylor, M. J., Mannan, R. W., U'Ren, J. M., Garber, N. P., Gallery, R. E., and Arnold, A. E. (2019). Age-related variation in the oral microbiome of urban Cooper's hawks (Accipiter cooperii). *BMC Microbiology*, *19*(1), 47.

Turner, S. A., Abou Shaar, R., and Yang, Z. (2023). The basics of commonly used molecular techniques for diagnosis, and application of molecular testing in cytology. *Diagnostic Cytopathology*, *51*(1), 83–94.

Urban, E. H., and Mannan, R. W. (2014). The potential role of oral pH in the persistence of Trichomonas gallinae in Cooper's Hawks (Accipiter cooperii). *Journal of Wildlife Diseases*, *50*(1), 50–55.

Villanúa, D., Höfle, U., PÉREZ-RODRÍGUEZ, L. O. R. E. N. Z. O., and Gortázar, C. (2006). Trichomonas gallinae in wintering common wood pigeons Columba palumbus in Spain. *Ibis*, *148*(4), 641-648.

Wang, Z., Hao, C., Zhuang, Q., Zhan, B., Sun, X., Huang, J., Cheng, Y., and Zhu, X. (2020). Excretory/Secretory Products from *Trichinella spiralis* Adult Worms Attenuated DSS-Induced Colitis in Mice by Driving PD-1-Mediated M2 Macrophage Polarization. *Frontiers in Immunology*, *11*, 563784

Chapter 20

The Interactive Effects between Toxoplasma Gondii and the Gut Microbiota

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ABSTRACT

Parasites, gut microbiota, and the host exist in a complex and intricate interplay. Infection with *Toxoplasma gondii* can lead to alterations, dysbiosis, and inflammatory diseases in the gut microbiome; concurrently, the gut microbiota influences the colonization, proliferation, and virulence of *T. gondii* within the host. Recent research on the interactions between intestinal parasites and the microbiota has revealed that the gut microbiota can either promote or inhibit the pathogenic effects of *T. gondii* on the host, and probiotics have shown specific preventive or therapeutic effects on intestinal *T. gondii* infections. Current research on the interaction between *T. gondii* and the microbiota is still in its infancy, and the mechanisms of their interaction are not yet fully understood. Elucidating the mutual influences and mechanisms of action between *T. gondii* and the gut microbiota is crucial for a deeper understanding of the relationships among *T. gondii*, the gut microbiota, and the host, as well as for developing effective anti-*T. gondii* microbiome preparations and the safeguarding of public health. This article reviews recent advances in research on the interaction between *T. gondii* and the microbiota. It provides perspectives on future research directions in this field, aiming to offer new strategies and theoretical foundations for preventing and controlling Toxoplasmosis.

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Toxoplasma gondii; Gut microbiota; Public health; Prevention and control	Revised: 12-Sep-24 Accepted: 15-Sep-24	Unique Scientific Publishers

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INTRODUCTION

Toxoplasma gondii and Toxoplasmosis

Toxoplasma gondii, a protozoan parasite belonging to the phylum Apicomplexa, is recognized as one of the most successful parasites on Earth. It is an important zoonotic pathogen capable of infecting all warm-blooded vertebrates, leading to abortions and stillbirths (Sanchez et al., 2021). This parasite significantly threatens livestock and public health safety, resulting in substantial economic losses. *T. gondii* exhibits a complex life cycle with five distinct stages of development: as tachyzoites and cysts within intermediate hosts, which include a range of mammals and birds, and as schizonts, gametocytes, and oocysts within definitive hosts, such as cats and other members of the Felidae family (Matta et al., 2021). Tachyzoites, known for their rapid replication within the host, are widespread throughout the body and are commonly found in acute cases. In contrast, cysts are often detected in chronic or asymptomatic cases within tissues such as the brain, skeletal muscle, heart, lungs, liver, and kidneys. Schizonts and gametocytes are typically found within the intestinal epithelial cells of definitive hosts. At the same time, oocysts are formed in the intestinal epithelial cells and released into the environment with the feces of the definitive host, exhibiting strong infectivity (Freppel et al., 2018). Although pyrimethamine and sulfonamide drugs are significantly effective in treating Toxoplasmosis, their hepato-renal toxicity and the issue of drug resistance cannot be overlooked (Montazeri et al., 2018; ShanShan Hu et al., 2024).

Recent research has highlighted the crucial role of the gut microbiota in various infectious diseases. However, studies on the impact of *T. gondii* infection on the gut microbiota are relatively limited. Understanding the link between Toxoplasmosis and the gut microbiota can aid in developing new strategies for preventing and treating *T. gondii* infection, safeguarding public health and animal welfare.

Gut Microbiota

The gut microbiota is vertebrate animals' most abundant and complex microbial ecosystem. It is an integral part of the immune system, closely related to the host's health status. Thousands of microbial communities form a dynamic and stable gut microbiome structure, including over 1500 species across more than 50 different phyla, with bacteria accounting for 90% of the total gut microbial population. Recent research advancements have rapidly expanded our understanding of the gut microbiota, encompassing disease pathogenesis, immune modulation, and parasite interactions. The complex interplay between gut microbiota and health implicates multiple pathways, including immune function, energy metabolism, lipid, and glucose metabolism (de Vos et al., 2022). Studies have demonstrated a link between gut microbiota dysbiosis and conditions such as obesity, type 2 diabetes, hepatic steatosis, inflammatory bowel diseases (IBDs), and certain types of cancer (Sepich-Poore et al., 2021). Parasitic infections can alter the composition and diversity of the gut microbiota, impacting the host's health (Méthot and Alizon, 2014; Hauck, 2017; Benson et al., 2009). Concurrently, the gut microbiota influences parasites' colonization, proliferation, and virulence (Tierney et al., 2004; Pérez et al., 2001; Foster et al., 2003; Matthew et al., 2004). Research has revealed that microbial agents such as probiotics, prebiotics, and synbiotics can modulate gut microbiota balance, enhance intestinal defense mechanisms, and positively affect the treatment of diseases like diarrhea, constipation, and indigestion (Sorbara et al., 2022). In recent years, breakthroughs in technologies such as high-throughput metagenomics analysis and artificial intelligence have facilitated a deeper understanding of the complexity of gut microbial communities and the exploration of their mechanisms of action, providing a foundation for developing new therapies. Modulating the balance of the gut microbiota is of significant importance for preventing and treating related diseases.

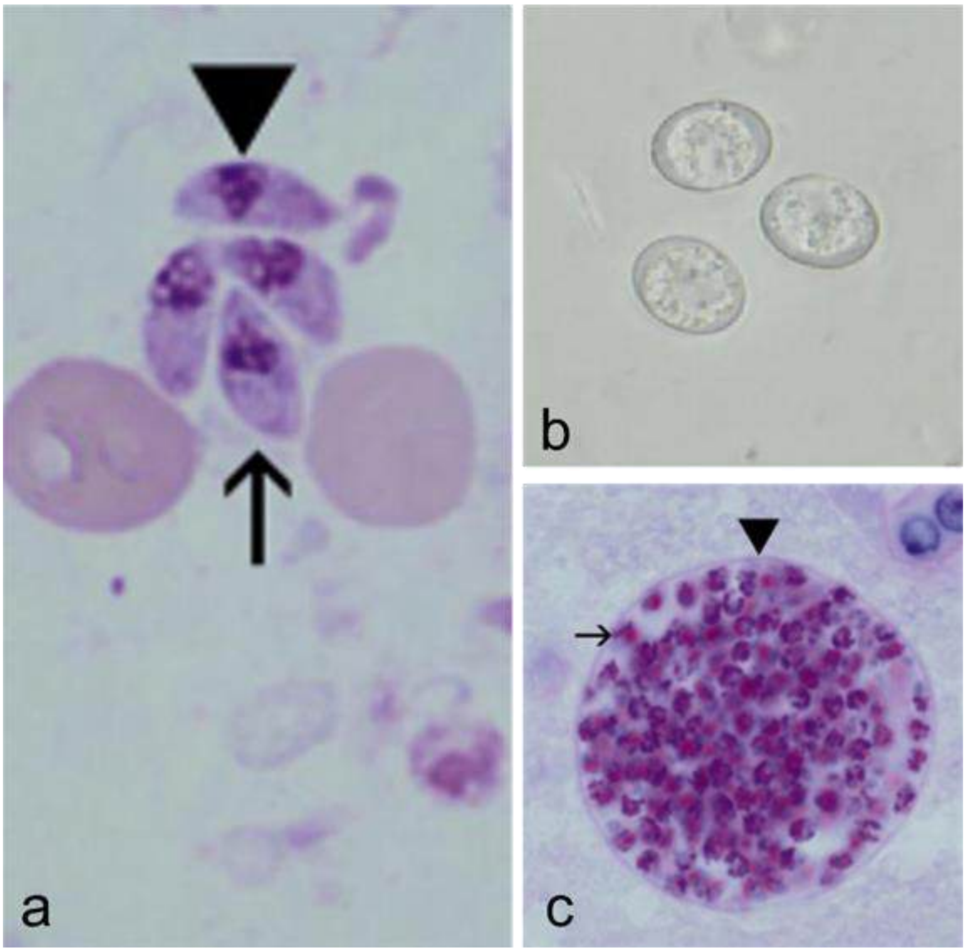

Fig. 1: The different developmental stages of *Toxoplasma gondii* (a. Tachyzoites, b. Oocysts, c. Tissue Cysts).

Interaction between Toxoplasma Infection and Gut Microbiota

Disruption of Intestinal Homeostasis by Toxoplasma Infection

Toxoplasma infection triggers intestinal inflammatory responses associated with changes in the gut microbiota composition. This study elucidates the shifts in gut microbiota composition during Toxoplasma infection and their implications for intestinal health. In healthy mice, the ileum is predominantly occupied by *Firmicutes* and *Bacteroidetes*, constituting 67% and 28% of the total microbiota, respectively. *Lactobacillus*, a probiotic, is essential for maintaining gut microbiota balance, preventing diseases, and promoting health. However, during Toxoplasma infection, there is a significant decrease in probiotics like *Lactobacillus*, while the numbers of *Enterobacteriaceae* from *Proteobacteria* and *Enterococcus* from *Firmicutes* increase, exhibiting a trend of initial rise followed by a decline. Conversely, the overall number of *Bacteroidetes* slightly increases compared to uninfected controls (Heimesaat et al., 2018; 2019). Molecular analyses reveal that the development of immunopathology is accompanied by profound changes in bacterial flora, with *Escherichia coli*, *Bacteroides*, and *Prevotella* dominating in acute ileitis. The total bacterial load in the ileum increases dramatically during inflammation, with significant increases in aerobic and anaerobic bacteria (Markus et al., 2006). Acute Toxoplasma infection activates the Th1 immune response, secreting many cytokines with anti-parasitic effects. However, an overactive immune response can decrease Paneth cells, which are vital for the intestinal epithelial barrier. The depletion of

Paneth cells and their antimicrobial secretions results in diminished expression and secretion of α-defensins, loss of gut microbiota diversity, and a pronounced expansion of *E. coli,* thereby exacerbating intestinal dysbiosis (Eriguchi et al., 2012; Raetz et al., 2013; Lu et al., 2018). In our experiments, high-throughput sequencing of the 16S rDNA of the gut microbiota in chickens yielded the following results, as shown in Fig. 2. After analyzing the sequencing results of the control group and the Toxoplasma gondii-inoculated group (from now on referred to as the infected group) in the 7-day-old group, the control group had 16 unique operational taxonomic units (OTUs). In contrast, the infected group had 69 unique OTUs. In the analysis of the sequencing results for the 28-day-old group, the control group had 26 unique OTUs, and the infected group had 50 unique OTUs. This indicates that the shared bacterial communities in the gut of the infected group are generally more diverse than those in the control group. At the phylum level (Fig. 3), the abundance of *Proteobacteria* and *Actinobacteria* was slightly lower in the infected group. In comparison, the abundance of *Bacteroidetes* and *Firmicutes* was slightly higher, with the most significant increase in abundance observed in *Bacteroidetes*.

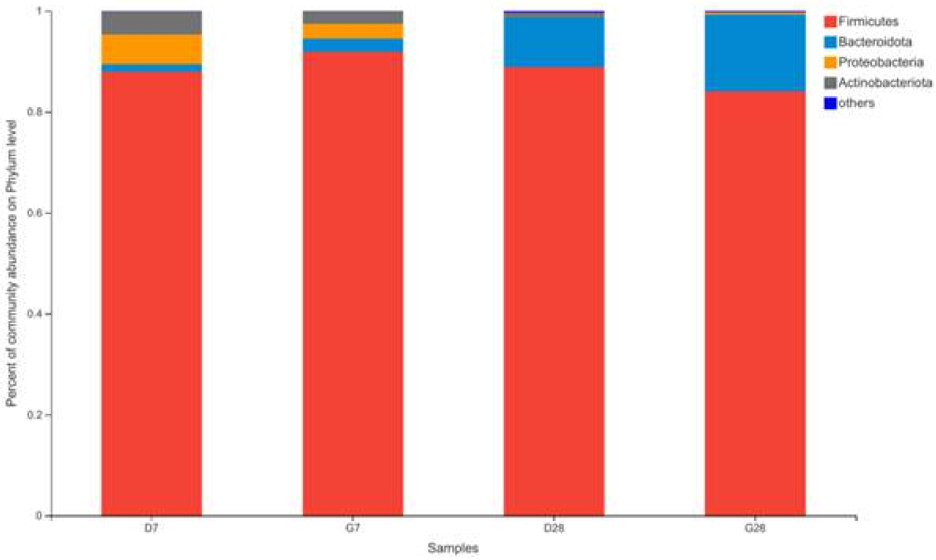

Fig. 2: Differences in species composition among samples. A) shows the comparison between the 7-day-old infected group and the control group; B) compares the 28-day-old infected group with the control group; and C) compares the 7-day-old infected group with the 28-day-old infected group.

Intestinal Dysbiosis and the Co-occurrence of *Toxoplasma gondii* Enteritis

The gut microbiota is an integral part of the immune system in humans and animals, contributing to the proper functioning of the host's immune defenses. The gut microbiota plays a crucial role in the immune system of humans and animals, influencing the development and function of both innate and adaptive immunity. The presence of gut microbiota facilitates the development of delicate structures in gut-associated lymphoid tissues and peripheral lymphoid organs, such as Peyer's patches and the spleen (Macpherson et al., 2006). The gut microbiota protects the host from pathogenic infections through various mechanisms, including competition for adhesion receptors and nutrients and stimulation of mucosal production of mucus and antimicrobial substances (Stecher et al., 2008). Gut microbiota expresses pathogen-associated molecular patterns (PAMPs) antigens that directly stimulate innate immune receptors, activating surface receptors on intestinal epithelial cells, dendritic cells, and macrophages. This leads to the production of antimicrobial substances such as COX-2, KGF-1, KGF-2, and angiogenin-4, and modifiable TGF-β1 through MyD88-dependent and TLR-dependent immune stimulation pathways, thereby maintaining intestinal homeostasis (Rakoff-Nahoum et al., 2004; Iwasaki et al., 2007). Under normal conditions, the gut maintains immune tolerance to commensal bacteria. However, during oral infection with *T. gondii*, the host loses immune tolerance to intestinal commensal bacteria (Hand et al. 2012), which facilitates the invasion of *T. gondii.*

Under normal conditions, the intestinal commensal bacteria can work with the mucosal immune system to produce beneficial effects for the host. However, in the case of dysbiosis, the overgrown gut microbiota can translocate to organs such as the mesenteric lymph nodes, liver, lungs, and blood, leading to secondary inflammation. An excessive increase in pro-inflammatory cytokines in the serum, including IFN-γ, TNF, MCP-1, IL-12, IL-6, and IL-10, triggers a cytokine storm. The host then exhibits symptoms such as weight loss, bloody diarrhea, and acute transmural enteritis, including apoptosis and necrosis of the small intestinal epithelial cells (Von Klitzing et al., 2017; Dos Santos et al., 2020). Current research suggests that Toxoplasmosis involves complex tripartite interactions between *T. gondii,* the mucosal immune system, and the host's gut microbiota (Cohen et al., 2014).

Toxoplasmosis can induce the formation of inflammatory bowel disease (IBD), and the intestinal state during acute *T. gondii* infection is similar to that of Crohn's disease (CD) patients (Egan et al., 2012). By orally infecting mice, the pathological process of CD can be simulated. The results show that germ-free (GF) mice have lower levels of IFN, IL-22, TIMP1, KC, and MPO in the intestine and higher levels of IL-1 compared to specific pathogen-free (SPF) mice, indicating that GF mice have better resistance to *T. gondii*-induced mucosal inflammation (Nascimento et al., 2017)). At the same time, the parasite load in GF mice is also significantly lower than that in SPF mice. Therefore, in normal gut microbiota, the microbiota can exacerbate the small intestinal inflammation caused by *T. gondii.*

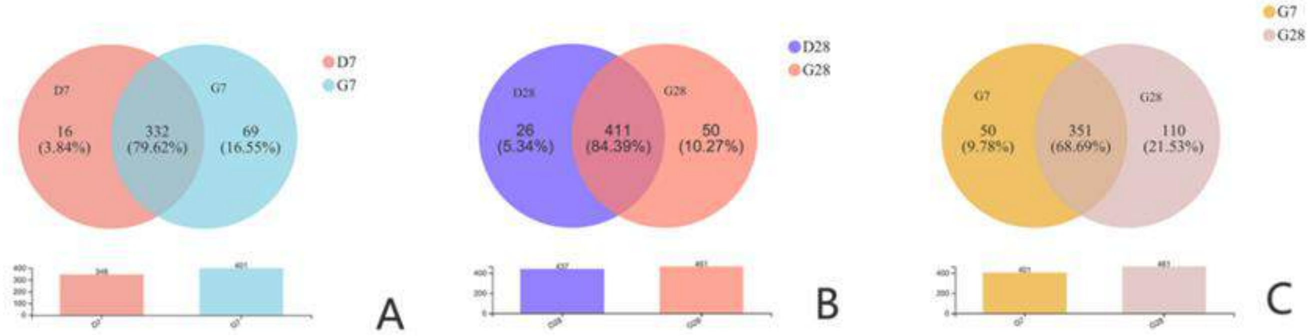

Fig. 3: Gut microbiota relative abundance at the phylum level.

The Treatment Effect of Probiotic Metabolites on *Toxoplasma Gondii* Infection

Indole-3-Propionic Acid (ILA) is a metabolite produced by bacteria of the *Lactobacillus genus*, which has a potential positive role in treating *Toxoplasma gondii* infection. Following infection with *T. gondii*, there is a significant decrease in the levels of ILA in the serum. ILA can activate the aryl hydrocarbon receptor signaling pathway in intestinal epithelial cells, promoting the activation of CD8+ T cells and the secretion of interferon-γ, thereby helping to suppress inflammation caused by *T. gondii* infection (Chen et al., 2024). This suggests that ILA, as a gut microbiota-related metabolite, may positively impact the treatment of *T. gondii* infection by regulating the host's immune response. According to existing studies, *T. gondii* infection leads to an imbalance in the gut microbiota, particularly reducing the abundance of probiotics, such as *lactobacilli* (Meng et al., 2023). This imbalance exacerbates damage to the intestinal and brain barriers. Research has found that the administration of *Lactobacillus murinus* and *Lactobacillus gasseri* can restore the balance of the gut microbiota, significantly inhibit the burden of *T. gondii* in the intestines, liver, and brain, and improve intestinal barrier damage, reducing central nervous system inflammation and neuronal apoptosis (Chen et al., 2024). Therefore, the supplementation of ILA and its producing bacteria, *Lactobacillus*, may become a potential strategy for treating *T. gondii* infection.

In addition, Alpha-Linolenic Acid (ALA), a gut microbiota-related metabolite, has been found to alleviate intestinal inflammation caused by *T. gondii* (Yang et al., 2023). Specifically, the study pointed out that oral administration of ALA and fecal microbiota transplantation (FMT) can both reduce the expression of pro-inflammatory cytokines and inhibit the MyD88/NF-κB signaling pathway, which helps to alleviate colitis and improve the survival rate of the host. Furthermore, the microbiota in the feces of mice treated with ALA can restructure the colonization of beneficial bacterial groups, such as *Enterobacteriaceae*, *Proteobacteria*, *Shigella*, *Lactobacillus*, and *Enterococcus*.

Summary and Prospects

Current research indicates a tripartite interaction between *Toxoplasma gondii*, the mucosal immune system, and the host's gut microbiota during *T. gondii* infection. During acute infection, the mucosal immune system is compromised, and the homeostasis of the gut microbiota is disrupted, exacerbating the occurrence of *T. gondii* enteritis. Previous studies have elucidated the molecular mechanisms related to *T. gondii*, gut microbiota, and immunity. These findings have revealed the connection between *T. gondii*-induced apoptosis and inflammatory responses and the gut microbiota, providing new directions for preventing and treating Toxoplasmosis.

High-throughput sequencing technology currently provides a platform for studying the interactions between parasites and the gut microbiota. The sequencing results can reveal extensive taxonomic changes in the gut microbiota, providing a basis for further exploration of the mechanisms of parasite-microbiota interactions. Metabolites of the gut microbiota, such as indole-3-lactic acid (ILA) and alpha-linolenic acid (ALA), have been found to inhibit the occurrence of *T. gondii* enteritis by regulating the host's immune system, providing a basis for the development and application of anti-*T—gondii* microbial preparations.

Furthermore, research on the interactions between intestinal parasites and microbiota in their natural state will help translate laboratory findings into clinical applications. Since the gut microbiota can affect the survival and infection outcomes of various parasites, understanding the interactions and mechanisms between parasites and the gut microbiota can lead to the design of prebiotics that stimulate the growth of specific microbes, suppress or reduce the virulence, colonization, or reproduction of parasites. This approach is expected to provide new directions and technologies for preventing and controlling parasitic diseases, which is of great significance for reducing the use of anti-parasitic drugs, promoting the development of animal husbandry, and ensuring public health security.

REFERENCES

Benson, A., Pifer, R., Behrendt, C. L., Hooper, L. V., and Yarovinsky, F. (2009). Gut commensal bacteria direct a protective immune response against Toxoplasma gondii. *Cell Host Microbe,* 6(2), 187-196.

Chen, J., Zhang, C., Yang, Z., Wu, W., Zou, W., Xin, Z., Zheng, S., Liu, R., Yang, L., and Peng, H. (2024). Intestinal microbiota imbalance resulted by anti-Toxoplasma gondii immune responses aggravate gut and brain injury. *Parasite and Vectors,* 17(1), 284.

Cohen, S. B., and Denkers, E. Y. (2014). Border maneuvers: deployment of mucosal immune defenses against Toxoplasma gondii. *Mucosal Immunolology,* 7(4), 744-752.

de Vos, W. M., Tilg, H., Van Hul, M., and Cani, P. D. (2022). Gut microbiome and health: mechanistic insights. *Gut,* 71(5), 1020-1032.

Dos Santos, L. M., Commodaro, A. G., Vasquez, A. R. R., Kohlhoff, M., de Paula Guerra, D. A., Coimbra, R. S., Martins-Filho, O. A., Teixeira-Carvalho, A., Rizzo, L. V., Vieira, L. Q., and Serra, H. M. (2020). Intestinal microbiota regulates tryptophan metabolism following oral infection with Toxoplasma gondii. *Parasite Immunolology,* 42(9), e12720.

Egan, C. E., Cohen, S. B., and Denkers, E. Y. (2012). Insights into inflammatory bowel disease using Toxoplasma gondii as an infectious trigger. *Immunology, and Cell Biolology*, 90(7), 668-675.

Eriguchi, Y., Takashima, S., Oka, H., Shimoji, S., Nakamura, K., Uryu, H., Shimoda, S., Iwasaki, H., Shimono, N., Ayabe, T., Akashi, K., and Teshima, T. (2012). Graft-versus-host disease disrupts intestinal microbial ecology by inhibiting Paneth cell production of α-defensins. *Blood,* 120(1), 223-231.

Foster, J. C., Glass, M. D., Courtney, P. D., and Ward, L. A. (2003). Effect of Lactobacillus and Bifidobacterium on Cryptosporidium parvum oocyst viability. *Food Microbiology*, 20(3), 351-357.

Freppel, W., Ferguson, D. J. P., Shapiro, K., Dubey, J. P., Puech, P. H., and Dumètre, A. (2019). Structure, composition, and roles of the Toxoplasma gondii oocyst and sporocyst walls. *Cell Surf,* 5, 100016.

Glass, M. D., Courtney, P. D., LeJeune, J. T., and Ward, L. A. (2004). Effects of Lactobacillus acidophilus and Lactobacillus reuteri cell-free supernatants on Cryptosporidium viability and infectivity in vitro. *Food Microbiology,* 21(4), 423-429.

Hand, T. W., Dos Santos, L. M., Bouladoux, N., Molloy, M. J., Pagán, A. J., Pepper, M., Maynard, C. L., Elson, C. O., 3rd, and Belkaid, Y. (2012). Acute gastrointestinal infection induces long-lived microbiota-specific T cell responses. *Science,* 337(6101), 1553-1556.

Hauck, R. (2017). Interactions Between Parasites and the Bacterial Microbiota of Chickens. *Avian Diseases,* 61(4), 428-436.

Heimesaat, M. M., Bereswill, S., Fischer, A., Fuchs, D., Struck, D., Niebergall, J., Jahn, H. K., Dunay, I. R., Moter, A., Gescher, D. M., Schumann, R. R., Göbel, U. B., and Liesenfeld, O. (2006). Gram-negative bacteria aggravate murine small intestinal Th1-type immunopathology following oral infection with Toxoplasma gondii. *Journal of Immunology,* 177(12), 8785-8795.

Heimesaat, M. M., Dunay, I. R., and Bereswill, S. (2019). Comprehensive Kinetic Survey of Intestinal, Extra-Intestinal and Systemic Sequelae of Murine Ileitis Following Peroral Low-Dose Toxoplasma gondii Infection. *Frontiers in Cell Infection and Microbiology*, 9, 98.

Heimesaat, M. M., Escher, U., Grunau, A., Fiebiger, U., and Bereswill, S. (2018). Peroral Low-Dose Toxoplasma gondii Infection of Human Microbiota-Associated Mice - A Subacute Ileitis Model to Unravel Pathogen-Host Interactions. *European Journal of Microbiology and Immunology*, 8(2), 53-61.

Hu, S., Batool, Z., Zheng, X., Yang, Y., Ullah, A., and Shen, B. (2024). Exploration of innovative drug repurposing strategies for combating human protozoan diseases: Advances, challenges, and opportunities. *Journal of Pharmaceutical Analysis,* 2024, 101084.

Iwasaki, A. (2007). Mucosal dendritic cells. *Annual Review Immunology*, 25, 381-418.

Lu, Y. Y., Dong, H., Feng, Y. J., Wang, K., Jiang, Y. B., Zhang, L. X., and Yang, Y. R. (2018). Avirulence and lysozyme secretion in Paneth cells after infection of BALB/c mice with oocysts of Toxoplasma gondii strains TgCatCHn2 (ToxoDB#17) and TgCatCHn4 (ToxoDB#9). *Veterinary Parasitology*, 252, 1-8.

Macpherson, A. J. (2006). IgA adaptation to the presence of commensal bacteria in the intestine. *Current Topics in Microbiology and Immunology,* 308, 117-136.

Matta, S. K., Rinkenberger, N., Dunay, I. R., and Sibley, L. D. (2021). Toxoplasma gondii infection and its implications within the central nervous system. *Nature Reviews in Microbiology*, 19(7), 467-480.

Meng, J.-X., Wei, X.-Y., Guo, H., Chen, Y., Wang, W., Geng, H.-L., Yang, X., Jiang, J., and Zhang, X.-X. (2023). Metagenomic insights into the composition and function of the gut microbiota of mice infected with Toxoplasma gondii. *Frontiers in Immunology,* 14.

Méthot, P. O., and Alizon, S. (2014). What is a pathogen? Toward a process view of host-parasite interactions. *Virulence,* 5(8), 775-785.

Montazeri, M., Mehrzadi, S., Sharif, M., Sarvi, S., Tanzifi, A., Aghayan, S. A., and Daryani, A. (2018). Drug Resistance in Toxoplasma gondii [Review]. *Frontiers in Microbiology*, 9.

Nascimento, B. B., Cartelle, C. T., Noviello, M. L., Pinheiro, B. V., de Almeida Vitor, R. W., Souza, D. D. G., de Vasconcelos Generoso, S., Cardoso, V. N., Martins, F. D. S., Nicoli, J. R., and Arantes, R. M. E. (2017). Influence of indigenous microbiota on experimental toxoplasmosis in conventional and germ-free mice. *International Journal of Experimental Pathology,* 98(4), 191-202.

Pérez, P. F., Minnaard, J., Rouvet, M., Knabenhans, C., Brassart, D., De Antoni, G. L., and Schiffrin, E. J. (2001). Inhibition of Giardia intestinalis by extracellular factors from Lactobacilli: an in vitro study. *Applied and Environmental Microbiology*, 67(11), 5037-5042.

Raetz, M., Hwang, S. H., Wilhelm, C. L., Kirkland, D., Benson, A., Sturge, C. R., Mirpuri, J., Vaishnava, S., Hou, B., Defranco, A.

L., Gilpin, C. J., Hooper, L. V., and Yarovinsky, F. (2013). Parasite-induced TH1 cells and intestinal dysbiosis cooperate in IFN-γ-dependent elimination of Paneth cells. *Nature Immunology,* 14(2), 136-142.

Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S., and Medzhitov, R. (2004). Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell,* 118(2), 229-241.

Sanchez, S. G., and Besteiro, S. (2021). The pathogenicity and virulence of Toxoplasma gondii. *Virulence,* 12(1), 3095-3114.

Sepich-Poore, G. D., Zitvogel, L., Straussman, R., Hasty, J., Wargo, J. A., and Knight, R. (2021). The microbiome and human cancer. Science, 371(6536).

Sorbara, M. T., and Pamer, E. G. (2022). Microbiome-based therapeutics. *Nature Reviews in Microbiology*, 20(6), 365-380.

Stecher, B., and Hardt, W. D. (2008). The role of microbiota in infectious disease. *Trends in Microbiology*, 16(3), 107-114.

Tierney, J., Gowing, H., Van Sinderen, D., Flynn, S., Stanley, L., McHardy, N., Hallahan, S., and Mulcahy, G. (2004). In vitro inhibition of Eimeria tenella invasion by indigenous chicken Lactobacillus species. *Veterinary Parasitology*, 122(3), 171-182.

Von Klitzing, E., Ekmekciu, I., Kühl, A. A., Bereswill, S., and Heimesaat, M. M. (2017). Intestinal, extra-intestinal and systemic sequelae of Toxoplasma gondii induced acute ileitis in mice harboring a human gut microbiota. *PLoS One,* 12(4), e0176144.

Yang, J., Liu, S., Zhao, Q., Li, X., and Jiang, K. (2023). Gut microbiota-related metabolite alpha-linolenic acid mitigates intestinal inflammation induced by oral infection with Toxoplasma gondii. *Microbiome,* 11(1), 273

Chapter 21

Summary of Common Intestinal Diseases in Pigeons and Related Pharmacological Prevention

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ABSTRACT

Pigeons are relatively easy and profitable to raise compared to other exotic birds. However, the large-scale development of the pigeon breeding industry has led to the emergence of various diseases. The intestine plays a crucial role in the digestion and absorption of nutrients in animals. The specific methods employed in pigeon rearing make them particularly susceptible to intestinal diseases such as colibacillosis, salmonella, and Newcastle disease. These diseases pose a threat not only to the pigeon industry but also to human health. While feed additives have been utilized in animal nutrition, the use of antibiotics as feed additives raises concerns regarding antimicrobial resistance, making the search for suitable alternatives essential for pigeon farming. Therefore, this article aims to summarize the intestinal-related diseases affecting pigeons, enhance the understanding of the occurrence and development of their intestinal functions, explore natural and novel antibiotic alternatives, and provide recommendations for the prevention and treatment of pigeon diseases.

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Pigeons; Intestinal diseases; Intestinal function and microorganisms; Drug prevention	Revised: 28-Jul-2024 Accepted: 25-Aug-2024	

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INTRODUCTION

As one of the earliest birds to be domesticated, pigeons not only possess ornamental and competitive value but also hold significant economic importance, making them a crucial type of commercial poultry (Wang et al., 2023). In many countries, pigeons have emerged as an important economic poultry species due to their nutritional value and meat quality, which are comparable to those of broiler chickens. Furthermore, they exhibit faster growth rates with minimal inputs, rendering their breeding profitable (Xu et al., 2020; Rani et al., 2021). Reports indicate that racing pigeons have become the fourth largest poultry product in my country, following chickens, ducks, and geese (Feng et al., 2022). According to relevant data, in 2021, China produced over 1.11 million pairs of pigeons, with approximately 1.6 billion pigeons slaughtered for food (Gao, 2022). However, unlike other poultry, the growth of young pigeons relies on exclusive feeding with milk secreted from the parental pigeon's crop. Following this initial phase, their diet gradually incorporates cereals and is eventually replaced by fodder, suggesting that this unique rearing method contributes to a faster growth rate (Xu et al., 2020a; Peng et al., 2023; Jin et al., 2023).Thus, the development of the pigeon industry has become a vital sector for maintaining the quality of life for the population and supporting economic growth.

Pigeon farming is a profitable business with low investment and labour, however there are challenges to the development of the pigeon industry, one of the major threats is the impact of intestinal diseases (Kim et al., 2010). The gut plays a crucial role in the digestion and absorption of nutrients, so gut health has a significant impact on poultry productivity (Yang et al., 2022). The pigeon's specific feeding regime also results in a significant impact on the nutrient and microflora in the gut at different stages (Guzman et al., 2013; Wang et al., 2023). Studies have shown that pigeon diarrhoea poses a major health risk at all ages, with a prevalence of up to 50%, and is accompanied by symptoms that lead to weight loss, crop stagnation, vomiting, anorexia and, in severe cases, death (Wang et al., 2024). Pigeons can also transmit these diseases (such as colibacillosis and salmonellosis) to humans and other mammals, and can also become a reservoir for certain infectious diseases in poultry, seriously affecting the safety of pigeon breeding and other animal industries (Grande et al., 2016; Ranjbar et al., 2020).Therefore an understanding of pigeon intestinal diseases and their functional development is essential for the prevention and control of this type of disease.

With the rapid advancement of poultry farming, animal food safety has emerged as a significant concern. The misuse of feed additives and the rise of antimicrobial drug resistance pose critical threats to animal health. Studies reveal that 80% of animals used in food production have received drug treatments at some point in their lives, and products derived from these animals (e.g., meat, milk, and eggs) may contain drug residues, contributing to the development of resistance (Chiesa et al., 2020; Silveira et al., 2021). Therefore more and more feed additives and supplements such as probiotics, prebiotics, organic acids and exogenous enzymes are being used as alternatives to antibiotics to regulate the gut microbiota in order to maintain normal life activities in poultry (Yadav et al., 2019).

Etiology

The intestinal tract is not only the centre of nutrient digestion and absorption, but also an important place for immune function (Xu et al., 2020; Li et al., 2020). Bacteria, viruses and parasites can cause intestinal diseases, and in severe cases, can cause the death of the animal, so understanding the causes of intestinal diseases is of great significance to the prevention and control of this type of disease.

Bacteria

Among the bacterial causes of pigeon diarrhea, *Escherichia coli* (*E. coli*), *Salmonella enterica*, *Campylobacter jejuni* in the phylum Aspergillus and *Clostridium difficile* in the phylum Thick-walled Bacteria are the main common pathogens (Wang et al., 2024). Research by Rosa Capita et al. shows that pigeon meat is an important reservoir of *E. coli* with genes for antibiotic resistance and virulence having the potential to cause disease in humans (Capita et al., 2019). Studies have shown that human infection with VT2f-producing *E. coli* is a zoonotic disease transmitted from pigeons. Pigeons can directly transmit VTEC strains that cause diarrhea, and can also indirectly release VT2f phages in the environment, thereby infecting humans(Grande et al., 2016). *E. coli,* as an opportunistic pathogen, can be widely present in the pigeon's intestinal tract, causing diarrhoea, enteritis and other diseases (Wang et al., 2022) (Fig. 1). Salmonellosis is a serious problem for all birds and is an important cause of high mortality from bacterial diseases in pigeons, mainly caused by *Salmonella enterica* subsp. enterica serovar Typhimurium (*S. Typhimurium*) and *Salmonella enterica* serovar *Enteritidis* (*S. Enteritidis*) (Ranjbar et al., 2020). Pigeons are common carriers of Salmonella and infection can be intestinal, parenteral (abscesses, pneumonia, osteomyelitis, septic arthritis, endocarditis and meningitis) and systemic (bacteremia), manifesting as Symptoms include enteritis, diarrhea and sepsis, and the bacteria can multiply in contaminated feces and remain viable for more than a month to continue to infect pigeons(Badr et al., 2022). At present, bacterial diarrhea in pigeons is mainly treated through standard microbiological examination and antibiotics. Ampicillin, streptomycin and tetracycline are classified as antibacterial in veterinary medicine (Stetsko et al., 2018).

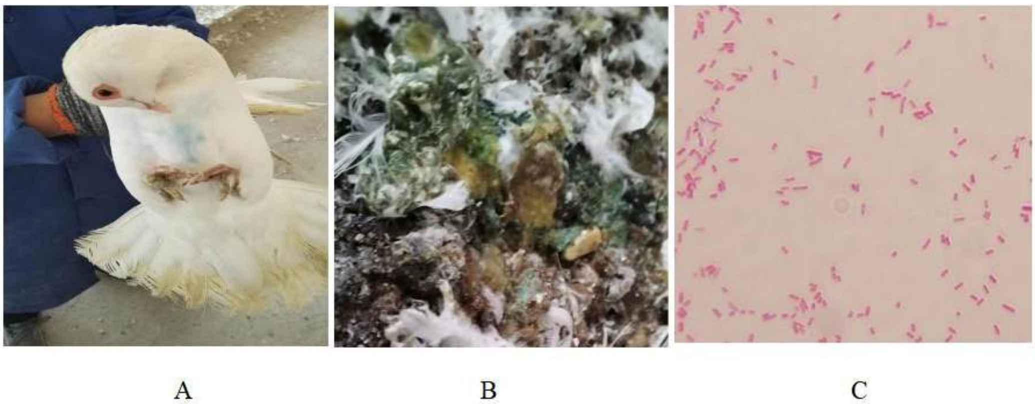

Fig. 1: Pigeons infected with *E. coli*. A) Symptoms of mental depression in pigeons; B) Symptoms of pigeon diarrhea ; C) *Escherichia coli* isolated from sick pigeons.

Virus

Viral diseases are common in both humans and animals and most viral infections severe enough to kill birds .In a study on pigeon diseases, the percentage of viral diseases in pigeons was found to be as high as 66.06%, with a high prevalence of Newcastle Disease and pigeon pox (Islam, 2020). Newcastle Disease in pigeons is a serious infectious disease caused by the Newcastle Disease Virus or Paramyxovirus type 1 in pigeons (Fig. 2; Liu et al., 2015). It infects young pigeons and adult pigeons and causes severe neurological and digestive symptoms such as bilateral or unilateral wing or leg movement disorders, diarrhoea and greenish loose stools (Zhang et al., 2022; Qian et al., 2022). Other viruses such as Pigeon Circovirus (PiCV) and Pigeon Rotavirus can also cause digestive disorders in pigeons. Pigeons of all ages may develop the disease affecting meat and racing pigeons. PiCV-infected pigeons exhibit symptoms such as ruffled feathers, depression, anorexia, weight loss, regurgitation, poor racing performance, diarrhea, and polydipsia.,while those infected with rotavirus show severe vomiting and diarrhea (McCowan et al., 2018; Abdulrasool et al., 2022). For this type of disease virus isolation and diagnosis of pigeons using PCR and immunofluorescence are crucial.The prevention and treatment of viral diseases is mainly achieved by vaccination, but it is more important to do a good job of pigeon husbandry management to reduce contact with all kinds of wild birds, free range poultry and contaminated environments, so as to avoid the infection of the disease (Abolnik, 2014; Liu et al., 2015).

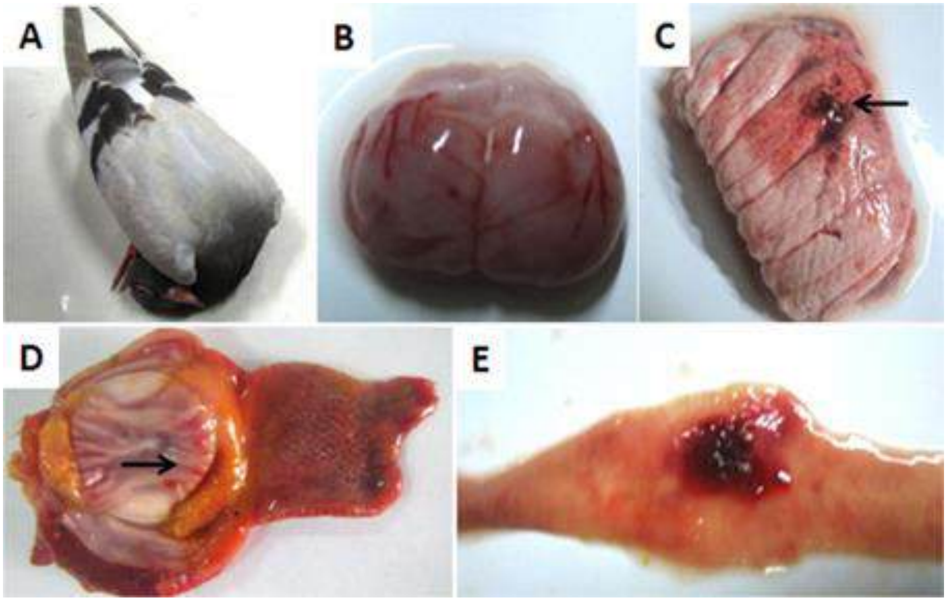

Fig. 2: Photos of pigeons infected with Newcastle Disease (Liu et al., 2015).

A) Pigeons infected exhibited severe nervous signs; B) Brain hyperemia and hemorrhage; C) Severe hemorrhage in the lung; D) Multifocal hemorrhages in the mucosa of muscular stomach; E) Multifocal hemorrhages in the mucosa of small intestine.

Parasites

Parasites is recognised as a major impediment to animal health and product performance, and the presence of internal and external parasites affects the growth, development and productivity of poultry, and in severe cases can lead to death (Mtd et al., 2020). It has been shown that coccidia (El-Seify et al., 2018), roundworms (Mtd et al., 2020) and trichinella (Cai et al., 2024) are the main parasites that infect pigeons with intestinal diseases.In the study of Gadelhaq et al. it was shown that globally the number of pigeons infected with coccidiosis is about 50-100% and the mortality rate reaches 70% (Gadelhaq et al., 2019). Pigeons infected with coccidia suffer from loss of weight and appetite, low immunity and wet feces, and bloody diarrhea in severe infections (Raś-Noryńska et al., 2011; Santos et al., 2020) and oocysts discharged in the fecal matter are non-pathogenic, but in the loft with appropriate temperature and humidity, they tend to grow and develop, thus, ultimately become invasive and able to infect pigeons (Balicka-Ramisz et al., 2014; Santos et al., 2020). Several studies have shown that the infection of pigeons with Trichinella is widespread in pigeons worldwide (Santos et al., 2020; Cai et al., 2024).Trichinella, as a parasite present in the respiratory (pharynx, oesophagus) and upper gastrointestinal (mouth) tracts, is mainly transmitted by feeding on the crop milk of infected pigeons, and the infected pigeons show clinical signs such as anorexia, weight loss, dyspnoea, dysphagia and diarrhoea, which in severe cases can lead to oesophageal luminal obstruction and death due to severe starvation. In severe cases, the oesophagus may be blocked and the pigeons may die due to severe starvation (Feng et al., 2020; Santos et al., 2020; Brunthaler et al., 2022). Heavy infections with roundworms can cause mild catarrhal enteritis, obstruction, dilatation and mild to necrotising ulceration of the small intestine in pigeons (Mtd et al., 2020). For this type of disease diagnosis is still mainly through clinical symptoms, microscopy to carry out, its treatment is still through the use of antiparasitic drugs plus symptomatic treatment of drugs, but also need to pay attention to the impact of drug resistance, the most fundamental is still the standardization of animal husbandry and management and maintain a clean environment in order to minimize the incidence of the disease and its spread.

Association between Intestinal Function and Intestinal Microorganisms in Pigeons

The intestinal tract, as a digestive organ, is responsible for the digestion and absorption of essential nutrients from poultry feed, and it also serves as a critical immune site with physical, chemical, immune, and microbial barriers that work synergistically against external stimuli (Liu et al., 2023). Pigeons are one of the few birds that can regurgitate pigeon milk to nourish their brood, and the feeding process is shown in Fig. 3 (Jin et al., 2023). Due to their delayed maturity, these pigeons cannot feed independently like other poultry and are entirely dependent on the milk secreted by the parental pigeon crop for a period following hatching. This milk, which is similar in composition to mammalian milk, is rich in microorganisms that facilitate microbiota establishment and metabolism, as well as promote the development of the immune system in the offspring (Dong et al., 2012; Ding et al., 2020; Jin et al., 2023).Intestinal function is usually reflected by intestinal morphology and structure, such as villus height, villus area, and crypt depth (Li et al., 2019). Studies have demonstrated that early weaning impairs intestinal development and health in pigeon squabs during artificial rearing periods (Wen et al., 2022).When pigeons are weaned at 7 days of age, their gastrointestinal tracts are often too immature to handle the physiological and environmental stresses associated with the transition from parental to captive feeding. This immaturity predisposes them to intestinal disorders and adversely affects their survival and disease resistance after weaning (Wen and Zhao et al., 2022).

Gut colonization by microbial communities is one of the most critical events in an animal's life, and the gut microbiota promotes poultry health by maintaining intestinal homeostasis, enhancing mucosal maturation, fostering immune system development, and inhibiting colonization by intestinal pathogens (Maynard et al., 2012; Wen et al., 2022). The gut microbiota can be categorised into probiotics, opportunistic pathogens and pathogens, where probiotics are beneficial to the host animal by inhibiting pathogens and balancing the gut microbiota, whereas opportunistic pathogens can be transformed into pathogens under specific conditions, which may threaten the health of the animal (Wang et al., 2014).The digestive system of newly hatched pigeons is sterile, immature, and highly susceptible to infection, with various microorganisms from the pigeon's milk and the environment briefly colonizing the gut (Wen et al., 2022). As the pigeon grows older its intestinal flora is gradually replaced from the Ascomycetes to the Thick-walled phylum due to the presence in the pigeon's milk of a rich microflora dominated by the representatives of the Thick-walled phylum, which are able to spread to the scales and participate in the process of bacterial colonization of the intestinal tract (Xi et al., 2019; Ding et al., 2020a). Many members of the Thick-walled phylum are associated with digestion and fermentation during starch metabolism, playing a crucial role in energy production, and eventually becoming the dominant flora in the intestinal tract (Ding et al., 2020a). Studies have shown that squab milk is rich in Lactobacillus and Bifidobacterium, with Lactobacillus being particularly beneficial to health due to its ability to inhibit the growth of pathogens through lactic acid production. Certain strains of Lactobacillus have been demonstrated to alleviate diarrhea in mammals by modulating the microbial community and enhancing immune system function in the small intestine, suggesting that Lactobacillus may play an important role in protecting the intestinal health of pigeon squabs (Bian et al., 2016; Ding et al., 2020a; Xu et al., 2022).

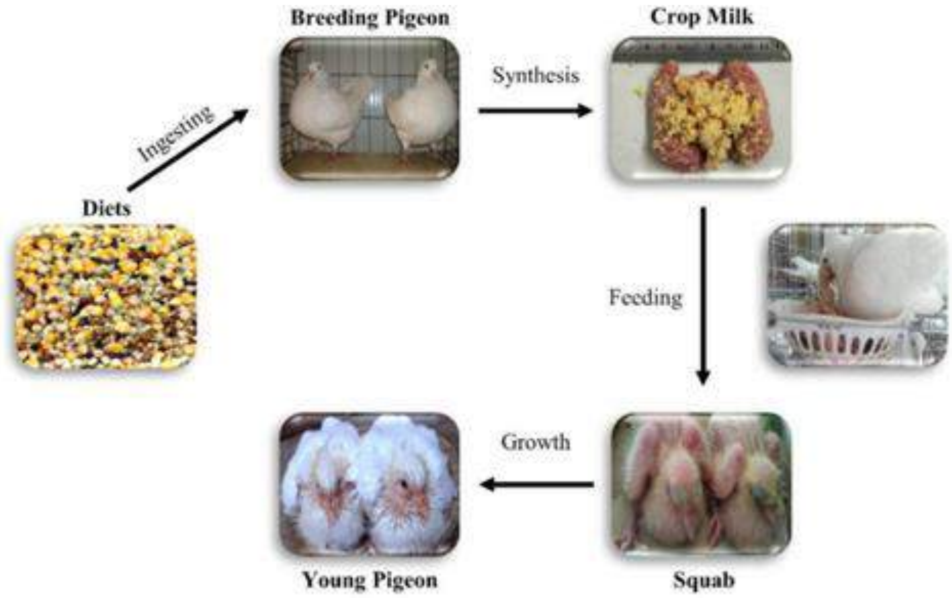

Fig. 3: Squab growth process (Jin et al., 2023).

Animal Feed Additive Alternatives for Intestinal Protection in Pigeons

Various feed ingredients and additives have been reported to modulate the gut microbiota and immune system of the host (Wang et al., 2023). However, with the rapid development of animal husbandry, issues related to bacterial resistance and drug residues resulting from antibiotic use have become increasingly prominent. Consequently, many countries and regions are gradually banning the addition of antibiotics to animal feed, prompting a heightened interest in the search for new medicinal drugs and alternative substances to antibiotics in animal feed (Cheng et al., 2014; Silveira et al., 2021; Hernando-Amado et al., 2020; Kim et al., 2022).Tea polyphenols, derived from tea, have antioxidant properties and promote the production of beneficial intestinal bacteria such as bifidobacteria and short-chain fatty acids (Su et al., 2019; Shao et al., 2022). Tea polyphenol supplementation in pigeon feed has been shown to improve growth performance, serum biochemicals, antioxidants and immunity, as well as enhance intestinal function to maintain intestinal health and improve the ability to digest and absorb nutrients (Chen et al., 2024). Astragalus, Epimedium, and Ligustrum lucidum (AEF) can enhance host immunity and improve animal growth performance. Supplementation of 0.1g/mL AEF in water enhances the pigeon's stress resistance, improves pigeon productivity (laying rate, egg quality, fertilization rate, weight gain, etc.), and strengthens pigeon's intestinal health and growth performance (Zhang et al., 2023).

Studies have shown that herbs and probiotics function extremely well as an alternative to antibiotics (Attia et al., 2023; Alagawany et al., 2023). Antibacterial peptide (ABP) is a broad-spectrum antibacterial and biologically active small molecule peptide, which exists in all kinds of animals, plants, bacteria, viruses and human beings, and can effectively kill bacteria, and not easy to produce drug resistance, which can enhance the immune function of the animal and improve the composition of the intestinal microflora, to maintain the health of the intestine, and to improve the production performance of the animal (Gadde et al., 2017; Mookherjee et al., 2020; Patyra et al., 2023). It has been demonstrated in the literature that supplementation of feed with ABP MccJ25 improves the serum antioxidant capacity of pigeons, enhances the intestinal barrier function and antioxidant capacity of pigeons, and promotes intestinal health (Cao et al., 2024). ABP 200 increased the abundance of beneficial bacteria and decreased the abundance of harmful bacteria in the pigeon's intestine, improved intestinal morphology, facilitated digestion and assimilation of nutrients, and promoted the growth and development of pigeon squabs(H et al., 2024).It has been found that supplementing the diet of pigeon mothers with appropriate amounts of linoleic acid (LA), is essential for poultry, and that deficiencies in LA can lead to a reduction in inflammation in the offspring and promote the growth of beneficial bacteria to strengthen the intestinal immune and luminal microbiological environment (Xu et al., 2020).The progress of animal production is also accompanied by the abuse of various drugs and the side effects of antibiotic resistance, so the use of natural feed ingredients and plant extracts has become an optimistic and strong candidate for the replacement of traditional drugs, and such a measure provides assistance in the growth and development of animals and the prevention of diseases, and also makes an important contribution to the sustainable development of the livestock and poultry industry.

Conclusion

With the expansion of pigeon breeding scale and the improvement of production level, people have also begun to pay attention to the occurrence and development of pigeon-related diseases. Understanding pigeon intestinal-related diseases and selecting appropriate intestinal protective agents play an important role in the production and development of pigeons.

REFERENCES

Abdulrasool, M. T. A., Khaleefah, A., Najem, H. A. (2022). A brief review of pigeon circovirus infection worldwide. *Multidisciplinary Reviews*, 5(4), 1-4. https://doi.org/10.31893/multirev.2022018

Abolnik, C. (2014). A current review of avian influenza in pigeons and doves (columbidae). *Veterinary Microbiology,* 170(0378-1135), 181-196. https://doi.org/10.1016/j.vetmic.2014.02.042

Alagawany, M., Farag, M. R., Harthi, M. A. A., Asiry, K. A., Bovera, F., and Attia, Y. A. (2023). The use of astragalus membranaceus as an eco-friendly alternative for antibiotics in diets of japanese quail breeders. *Poultry Science*, 102, 102909. https://doi.org/10.1016/j.psj.2023.102909

Attia, Y. A., Basiouni, S., Abdulsalam, N. M., Bovera, F., Aboshok, A. A., and Shehata, A. A., (2023). Alternative to antibiotic growth promoters: beneficial effects of saccharomyces cerevisiae and/or lactobacillus acidophilus supplementation on the growth performance and sustainability of broilers' production. *Frontier Veterinary Science,* 10, 1259426. https://doi.org/10.3389/fvets.2023.1259426

Badr, H., Shosha, E., Roshdy, H., Mohammed, A. A. E., Saad, N., and Aboelenin, S. M., (2022). Investigation of many bacterial and viral infections circulating in pigeons showing nervous symptoms. *Saudi Journal Biology Science,* 29, 2911-2920. https://doi.org/10.1016/j.sjbs.2022.01.023

Balicka-Ramisz, A., and Pilarczyk, B. (2014). Occurrence of coccidia infection in pigeons in amateur husbandry. Diagnosis and prevention. *PubMedicine*, 60, 93-97. https://doi.org/null

Bian, X., Wang, T., Xu, M., Evivie, S. E., Luo, G., and Liang, H., (2016). Effect of lactobacillus strains on intestinal microflora and mucosa immunity in escherichia coli o157:h7-induced diarrhea in mice. *Current Microbiology,* 73, 65-70. https://doi.org/10.1007/s00284-016-1010-3

Brunthaler, R., Teufelbauer, N., Seaman, B., Nedorost, N., Bittermann, K., and Matt, J., (2022). Trichomonosis in austrian songbirds—geographic distribution, pathological lesions and genetic characterization over nine years. *Animals*, 12, 1306. https://doi.org/10.3390/ani12101306

Cai, H., Liu, Y., Zhu, Y., Fang, S. C., Wang, D., and Zhang, Y., (2024). Drug resistance patterns and genotype associations of trichomonas gallinae in meat pigeons (columba livia): insights from guangdong province, china. *Frontier Veterinary Science,* 10, 0-0. https://doi.org/10.3389/fvets.2023.1343321

Cao, H., Lu, Y., Zhang, X., Zhou, J., Li, F., and Pan, Z., (2024). Effects of antibacterial peptide microcin j25 on growth performance, antioxidant capacity, intestinal barrier function and intestinal microbiota in pigeon squabs. *Ital. Journal Animal Science,* 23, 427-439. https://doi.org/10.1080/1828051x.2024.2326119

Capita, R., Cordero, J., Molina-González, D., Igrejas, G., Poeta, P., and Calleja, C. A. (2019). Phylogenetic diversity, antimicrobial susceptibility and virulence characteristics of escherichia coli isolates from pigeon meat. *Antibiotics*, 8, 259. https://doi.org/10.3390/antibiotics8040259

Chen, A., Ma, T., Zhong, Y., Deng, S., Zhu, S., and Fu, Z., (2024). Effect of tea polyphenols supplement on growth performance, antioxidation, and gut microbiota in squabs. *Frontier Microbiology,* 14. https://doi.org/10.3389/fmicb.2023.1329036

Cheng, G., Hao, H., Xie, S., Wu, X., Dai, M., and Huang, L., (2014). Antibiotic alternatives: the substitution of antibiotics in animal husbandry? *Frontier Microbiology,* 5. https://doi.org/10.3389/fmicb.2014.00217

Chiesa, L. M., Decastelli, L., Nobile, M., Martucci, F., Mosconi, G., and Fontana, M., (2020). Analysis of antibiotic residues in raw bovine milk and their impact toward food safety and on milk starter cultures in cheese-making process. *LWT,* 131, 109783. https://doi.org/10.1016/j.lwt.2020.109783

Ding, J., Liao, N. Q., Zheng, Y., Yang, L., Zhou, H., and Xu, K., (2020a). The composition and function of pigeon milk microbiota transmitted from parent pigeons to squabs. *Frontier Microbiology,* 11.

https://doi.org/10.3389/fmicb.2020.01789

Dong, X., Wang, Y. M., Dai, L., Azzam, M. M., Wang, C., and Zou, X. (2012). Posthatch development of intestinal morphology and digestive enzyme activities in domestic pigeons (columba livia). *Poultry Science,* 91, 1886-1892. https://doi.org/10.3382/ps.2011-02091

El-Seify, M. A., Metwally, A. M., Mahmoud, S. Z., and Abdelrheem, E. H. (2018). Prevalence of coccidia infection among domestic pigeon (columba livia domestica) and quails (coturnix ypsilophora) in qena province, southern egypt. *Kafrelsheikh Veterinary Medical Journal*, 16, 1-21. https://doi.org/10.21608/kvmj.2018.110173

Feng, J., Zhang, D., Shao, Y., Yu, X., Liu, X., and Shan, D., (2020). Changes in the diversity and composition of gut microbiota in pigeon squabs infected with trichomonas gallinae. *Science Reproduction,* 10. https://doi.org/10.1038/s41598-020-76821-9

Feng, J., Zhang, S., An, Y. S., Wang, Z., Shao, Y., and Du, S., (2022). Influence of dietary phosphorus concentrations on the performance of rearing pigeons (columba livia), and bone properties of squabs. *Poultry Science,* 101, 101744. https://doi.org/10.1016/j.psj.2022.101744

Gadde, U. D., Kim, W. H., Oh, S., and Lillehoj, H. S. (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. *Animal Health Research Review,* 18, 26-45. https://doi.org/10.1017/s1466252316000207

Gadelhaq, S. M., and HAbdelaty, A. (2019). The occurrence and distribution pattern of eimeria species among domestic pigeons in minia, egypt. *Journal of Veterinary Medical Research,* 26, 164-173. https://doi.org/10.21608/jvmr.2019.66098

Grande, L., Michelacci, V., Bondì, R., Gigliucci, F., Franz, E., and Badouei, M. A., (2016). Whole-genome characterization and strain comparison of vt2f-producingescherichiacolicausing hemolytic uremic syndrome. *Emergency Infection Disease,* 22, 2078-2086. https://doi.org/10.3201/eid2212.160017

Guzman, J. R., Conlin, V. S., and Jobin, C. (2013). Diet, microbiome, and the intestinal epithelium: an essential triumvirate? *Biomed Research International,* 2013, 1-12. https://doi.org/10.1155/2013/425146

Hernando-Amado, S., Coque, T. M., Baquero, F., and Martínez, J. L. (2020). Antibiotic resistance: moving from individual health norms to social norms in one health and global health. *Frontier Microbiology,* 11. https://doi.org/10.3389/fmicb.2020.01914

Islam, S. (2020). Prevalence of pigeon diseases in sylhet district, bangladesh. *Archives of Animal Husbandry and Dairy Science*, 2. https://doi.org/10.33552/aahds.2020.02.000532

Jin, C., He, Y., Jiang, S., Wang, X., Yan, H., and Tan, H., (2023). Chemical composition of pigeon crop milk and factors affecting its production: a review. *Poultry Science,* 102, 102681. https://doi.org/10.1016/j.psj.2023.102681

Kim, J., and Ahn, J. (2022). Emergence and spread of antibiotic-resistant foodborne pathogens from farm to table. *Food Science Biotechnology,* 31, 1481-1499. https://doi.org/10.1007/s10068-022-01157-1

Kim, Y. S., and Ho, S. B. (2010). Intestinal goblet cells and mucins in health and disease: recent insights and progress. *Current Gastroenterology Reports*, 12, 319-330. https://doi.org/10.1007/s11894-010-0131-2

Li, J., Yin, L., Wang, L., Li, J., Huang, P., and Yang, H., (2019). Effects of vitamin b6 on growth, diarrhea rate, intestinal morphology, function, and inflammatory factors expression in a high-protein diet fed to weaned piglets1. *Journal Animal Science,* 97, 4865-4874. https://doi.org/10.1093/jas/skz338

Li, X., Liu, L., Cao, Z., Li, W., Li, H., and Lü, C., (2020). Gut microbiota as an “invisible organ” that modulates the function of drugs. *Biomedicine Pharmacother,* 121, 109653. https://doi.org/10.1016/j.biopha.2019.109653

Liu, G., and Kim, W. K. (2023). The functional roles of methionine and arginine in intestinal and bone health of poultry: review. *Animals,* 13, 2949. https://doi.org/10.3390/ani13182949

Liu, M., Qu, Y., Wang, F., Liu, S., and Sun, H. (2015). Genotypic and pathotypic characterization of newcastle disease virus isolated from racing pigeons in china. *Poultry Science,* 94, 1476-1482. https://doi.org/10.3382/ps/pev106

Maynard, C. L., Elson, C. O., Hatton, R. D., and Weaver, C. T. (2012). Reciprocal interactions of the intestinal microbiota and immune system. *Nature*, 489, 231-241. https://doi.org/10.1038/nature11551

McCowan, C., Crameri, S., Kocak, A., Shan, S., Fegan, M., and Forshaw, D., (2018). A novel group a rotavirus associated with acute illness and hepatic necrosis in pigeons (columba livia), in australia. *Plos One,* 13, e203853. https://doi.org/10.1371/journal.pone.0203853

Mookherjee, N., Anderson, M. A., Haagsman, H. P., and Davidson, D. J. (2020). Antimicrobial host defence peptides: functions and clinical potential. *National Review Drug Discovery,* 19, 311-332. https://doi.org/10.1038/s41573-019-0058-8

Mtd, A., Mju, S., Islam, M. M., Mr, I., Nh, P., and Mn, A. (2020). Study on diseases prevalence in the selected area's emphasis on parasitic diseases of pigeon. *PrePrint*. https://doi.org/10.20944/preprints202012.0408.v1

Patyra, E., and Kwiatek, K. (2023). Insect meals and insect antimicrobial peptides as an alternative for antibiotics and growth promoters in livestock production. *Pathogens,* 12, 854. https://doi.org/10.3390/pathogens12060854

Peng, J., Huang, W., Zhang, W., Zhang, Y., Yang, M., and Zheng, S., (2023). Effect of different dietary energy/protein ratios on growth performance, reproductive performance of breeding pigeons and slaughter performance, meat quality of squabs in summer. *Poultry Science,* 102, 102577. https://doi.org/10.1016/j.psj.2023.102577

Qian, J., Li, X., Tang, Z., Wang, J., Wang, Y., and Wang, W., (2022). Genetic characterization and pathotypical analysis of two strains of pigeon newcastle disease virus of jiangsu in china. *Research Square (Research Square)*.

https://doi.org/10.21203/rs.3.rs-1529999/v1

Rani, P. B. R., and Rajakumari, S. (2021). Survey on prevalence of common diseases in pigeon (columba livia domestica) in tuticorin district, tamil nadu, india. *International Journal of Zoology and Applied Biosciences*, 6, 203-207. https://doi.org/10.55126/ijzab.2021.v06.i04.018

Ranjbar, V. R., Basiri, S., and Abbasi-Kali, R. (2020). Paratyphoid infection caused by salmonella typhimurium in a pigeon flock (columbia livia) in iran. *DOAJ.* https://doi.org/10.22034/jzd.2020.10587

Raś-Noryńska, M., Michalczyk, M., and Sokół, R. (2011). Coccidia infections in homing pigeons of various age during the racing season. *PubMedicine.,*57, 165-168.

Santos, H. M., Tsai, C. F., Catulin, G. E. M., Trangia, K. C. G., Tayo, L. L., and Liu, H., (2020). Common bacterial, viral, and parasitic diseases in pigeons (columba livia): a review of diagnostic and treatment strategies. *Veterinary Microbiology,* 247, 108779. https://doi.org/10.1016/j.vetmic.2020.108779

Shao, J., Wang, C., Shen, Y., Shi, J., and Ding, D. (2022). Electrochemical sensors and biosensors for the analysis of tea components: a bibliometric review. *Frontier Chemistry,* 9. https://doi.org/10.3389/fchem.2021.818461

Silveira, R. F., Borda, C. A. R., and Vicente, E. F. (2021). Antimicrobial peptides as a feed additive alternative to animal production, food safety and public health implications: an overview. *Animal Nutrition,* 7, 896-904. https://doi.org/10.1016/j.aninu.2021.01.004

Stanley, D., and Bajagai, Y. S. (2022). Feed safety and the development of poultry intestinal microbiota. *Animals,* 12, 2890. https://doi.org/10.3390/ani12202890

Stetsko, T. I., Muzyka, V., and Hunchak, V. M. (2018). Critically important antimicrobial preparations for veterinary medicine. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnology,* 20, 19-26. https://doi.org/10.15421/nvlvet8704

Su, S., Li, X., Xu, G., Zhou, R., Li, M., and Ming, P., (2019). Tea polyphenols reducing lipopolysaccharide-induced inflammatory responses in raw264.7 macrophages via nf-κb pathway. *Chemistry Research Chinical University,* 35, 1105-1110. https://doi.org/10.1007/s40242-019-8376-2

Wang, A., and Hu, C. (2022). Antimicrobial resistance analysis of escherichia coli isolated from pigeons in qingdao, shandong province, china. *Genes,* 13, 1510. https://doi.org/10.3390/genes13091510

Wang, F., Yun, Z., Hou, H., Yao, J., Zhu, L., and Liu, H., (2024). Treatment and prevention of pigeon diarrhea through the application of lactobacillus snk-6. *Poultry Science*. https://doi.org/10.1016/j.psj.2024.103476

Wang, L. X., Zhu, J., Xie, P., and Gong, D. (2023). Pigeon during the breeding cycle: behaviors, composition and formation of crop milk, and physiological adaptation. *Life*, 13, 1866. https://doi.org/10.3390/life13091866

Wang, Y., Yi, L., Zhao, M., Wu, J., Wang, M. Y., and Xiao, C. (2014). Effects of zinc–methionine on growth performance, intestinal flora and immune function in pigeon squabs. *Br. Poultry Science,* 55, 403-408. https://doi.org/10.1080/00071668.2014.919375

Wen, J., Xu, Q., Zhao, W., Hu, C., Zou, X., and Dong, X. (2022). Effects of early weaning on intestinal morphology, digestive enzyme activity, antioxidant status, and cytokine status in domestic pigeon squabs (columba livia). *Poultry Science*, 101, 101613. https://doi.org/10.1016/j.psj.2021.101613

Wen, J., Zhao, W., Li, J., Hu, C., Zou, X., and Dong, X. (2022). Dietary supplementation of chitosan oligosaccharide–clostridium butyricum synbiotic relieved early-weaned stress by improving intestinal health on pigeon squabs (columba livia). *Frontier Immunology,* 13. https://doi.org/10.3389/fimmu.2022.926162

Xi, Y., Niu, S., Tie, K., Zhang, Q., Deng, H., and Gao, C., (2019). Characteristics of the intestinal flora of specific pathogen free chickens with age. *Microbiology Pathogens,* 132, 325-334. https://doi.org/10.1016/j.micpath.2019.05.014

Xu, Q., Wen, J., Wang, X., Zou, X., and Dong, X. (2020). Maternal dietary linoleic acid altered intestinal barrier function in domestic pigeons (columba livia). *Br. Journal Nutrition,* 126, 1003-1016. https://doi.org/10.1017/s0007114520004973

Xu, Q., Zhao, W., Li, Y., Zou, X., and Dong, X. (2022). Intestinal immune development is accompanied by temporal deviation in microbiota composition of newly hatched pigeon squabs. *Microbiology Spectr,* 10. https://doi.org/10.1128/spectrum.01892-21

Xu, Q., Zou, X., and Dong, X. (2020). Maternal dietary linoleic acid altered intestinal barrier function in domestic pigeons (columba livia). *Research Square (Research Square)*. https://doi.org/10.21203/rs.3.rs-60857/v1

Yadav, S., and Jha, R. (2019). Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. *Journal Animal Science Biotechnology,* 10. https://doi.org/10.1186/s40104-018-0310-9

Yang, Q., Stewart, S., and Zhang, G. (2022). Gut microbiome and poultry health. *The Microbiomes of Humans, Animals, Plants, and the Environment*. https://doi.org/10.1007/978-3-030-90303-9_5

Zhang, X., Zhou, S., Liang, Y., Xie, G., Zhu, M., and Wang, Z., (2023). Effects of astragalus, epimedium, and fructus ligustri lucidi extractive on antioxidant capacity, production performance, and immune mechanism of breeding pigeons under stress. *Poultry Science*, 102, 102350. https://doi.org/10.1016/j.psj.2022.102350

Zhang, Y., Wang, W., Li, Y., Liu, J., Wang, W., and Bai, J., (2022). A pigeon paramyxovirus type 1 isolated from racing pigeon as an inactivated vaccine candidate provides effective protection. *Poultry Science,* 101, 102097. https://doi.org/10.1016/j.psj.2022.102097