# Probiotics and Prebiotics: The Future of Antibiotic Free Poultry Industry

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## Abstract

The poultry industry is experiencing crucial change because eco-friendly, antibiotic-free meat production has become more important to demanding customers. Health risks related to antimicrobial resistance are the main reason behind this industry shift which compels producers to transform their practices toward more sustainable methods. Collaborative use of prebiotics together with probiotics offers a hopeful solution to increase poultry productivity and health status without depending on antibiotic medicines. The use of probiotic microorganisms, including *Lactobacillus* and *Bifidobacterium*, helps maintain healthy intestinal microorganisms in chickens, thus protecting them from diseases. The absorption of nutrients and immune system strength of broiler chicken benefit significantly from prebiotic substances including inulin together with mannan oligosaccharides. Probiotics form a strong symbiotic interplay with prebiotics because prebiotics provide nutrient-rich feed for probiotic microbes that probiotics introduce to an environment. The synbiotic interaction supports sustainable gut health because it creates improved defense mechanisms and optimal nutrient use while lowering antibiotic use. New knowledge about probiotic formulations made for specific animal species, along with prebiotic advancements, indicates that it will be possible to enhance both animal health and agricultural productivity. Building on the framework discussion this segment deepens the evaluation of regulatory patterns and production differences within chicken facilities while demonstrating how probiotics and prebiotics will establish major roles in antibiotic-free poultry advancement.

Keywords: Mannan Oligosaccharides, Lactobacillus, Feed Additives, Microbial population,

**Cite this Article as:** Munir ETR, Masood S, Ashraf S, Ijaz S, Masood A, Sarim M, Ateeq MK and Zehra A, 2025. Probiotics and prebiotics: the future of antibiotic free poultry industry. In: Aadil RM, Salman M, Mehmood K and Saeed Z (eds), Gut Microbiota and Holistic Health: The Role of Prebiotics and Probiotics. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 119-125. https://doi.org/10.47278/book.HH/2025.59



A Publication of Unique Scientific Publishers **Chapter No:** 25-016

Received: 13-Feb-2025 Revised: 21-March-2025 Accepted: 18-Apr-2025

## Introduction

The world's population is rapidly growing, making it clear that more secure food supply systems are required. The growth of Pakistan's poultry sector is helping to resolve the country's protein shortfall, ensuring a steady supply to meet growing demand. The early 1960s marked the beginning of commercial chicken farming in Pakistan and exhibited remarkable expansion throughout the decades. The poultry industry's early development was facilitated by the collaborative efforts of farmers and government agencies. To boost the food processing industry, the government granted preferential incentives to the poultry sector, recognizing its strategic importance; consequently, the government declared the poultry industry tax-free, waiving sales tax, income tax, and import taxes for a specific duration. Poultry production in Pakistan is a well-organized and active sector, accounting for 26.8%, 5.76%, and 1.26% of total meat production, agricultural sector, and GDP, respectively. In recent years, the poultry sector has grown significantly and now employs over 1.5 million people (Khan et al., 2023).

## The Transition toward Antibiotic-Free Poultry Industry

Due to its affordability and widespread appeal, poultry meat is a leading source of protein for many consumers. The use of preventive medicine has been a common practice in chicken farming for decades, aimed at optimizing growth and gut health. Yet, with the alarming rise of antibiotic resistance as a result of antibiotic misuse, these practices are presently facing intense regulatory and consumer pressure to be discontinued. New approaches are needed to replace antibiotics that have comparable positive results in terms of modifying the gastrointestinal microflora and boosting animal health and development. A range of innovative feed additives, such as prebiotics, probiotics, synbiotics, organic acids, essential oils, enzymes, and emerging chemicals, are being investigated for use in chicken producti on. Probiotics and prebiotics are becoming increasingly popular as potential alternatives to antibiotics. In addition to examining the usefulness of various non-antibiotic growth promoters in the chicken business, the projected eradication of antibiotics from livestock farming has progressed, so the emergence of innovative methods to maximize the effectiveness of various alternative options. The digestive system in all animals is characterized by a hollow, intricately structured organ with multiple functions. This chapter investigates the role of probiotics and prebiotics

as antibiotic replacements in the poultry industry, focusing on practical application strategies for enhancing their efficacy in broiler chickens (Adewole & Khomayezi, 2021).

## Probiotics

The term probiotic comes from the Greek language, with 'pro' (for) and 'bio' (life), to convey the idea of promoting life and well-being. Mechnikov most likely proposed the first probiotic notion in 1907. Bacteria may benefit the gut microbiota, according to one researcher (Markowiak & Śliżewska, 2017). The word "probiotic" was most likely coined by Ferdinand Vergin, who, in his 1954 publication "Anti- und Probiotika, highlights the stark contrast between the disruptive effects of antibiotics on gut microbiota and the restorative effects of probiotics and prebiotics (Markowiak & Śliżewska, 2018)

Probiotics are carefully selected, live microorganisms that provide health benefits to the host. (FAO/WHO, 2002). The International Scientific Association for Probiotics and Prebiotics (ISAPP) maintained this definition in 2013. The word "probiotic" refers to formulae or products that fulfill certain rigorous parameters. The most significant of these requirements are a sufficient quantity of live cells, a good influence on the well-being of the host (which may include promotion of growth), and a positive impact on gut integrity. The therapeutic benefits of probiotics are dependent on a number of variables. As a result, precise bacterial strain selection and administration of the appropriate dosage are critical. Probiotics are widely utilized in animal diets, particularly for poultry, since they improve health and stimulate development. Probiotic formulas, containing one or more carefully selected microorganisms, can be tailored to specific host animals based on their species and age, and are available in various forms, including powders, solutions, capsules, pastes and gels.

## **Criteria and Requirements for Probiotic Strains**

Ensuring the safety of health-promoting microbial strains is crucial for their optimal use, but it presents major problems. The precise processes by which probiotics operate as microbial additions in feed are not well known. Probiotics can connect to the digestive tract, helping them to withstand tough circumstances while also contributing to the intestinal environment's equilibrium. These helpful microbes help to regulate digestive and metabolic functions while also boosting the immune system. As a consequence, probiotics improve animal health, increase production, and boost immunity. Their capacity to influence the immune system is very crucial for sustaining health and treating illnesses since it involves both innate and adaptive immunity (Takahashi et al., 1998).

The gastrointestinal system is a constantly changing environment that contains nutrients, beneficial microorganisms, pathogens, poisons, and foreign substances. The gastrointestinal epithelial cells produce a selectively permeable barrier that protects against hazardous substances while allowing for nutrition absorption. This barrier serves as an essential defensive mechanism (gut innate immunity) against dangerous microorganisms. Yet, the integrity of this barrier can be compromised by stress or disease, emphasizing the need for therapies such as probiotics to maintain or restore its function (Liao & Nyachoti, 2017).

## **Probiotics Microorganisms**

Single-strain or multi-strain probiotic products are available. In the (EU) European Union, bacteria are the most prevalent microbes utilized as dietary additives. Gram-positive bacteria from the genera *Bacillus, Enterococcus, Lactobacillus, Pediococcus*, and *Streptococcus* are predominately used as probiotics. Additionally, certain fungi and yeast species, such as *Saccharomyces cerevisiae* and *Kluyveromyces*, are used as probiotics. Bacteria from the genera *Lactobacillus* and *Enterococcus* are naturally present in the gut microflora of animals, typically at concentrations of  $10^7-10^8$  CFU/g and  $10^5-10^6$  CFU/g, respectively. In contrast, yeast and bacteria from the *Bacillus* genus are not naturally abundant in the GIT tract.

While most of these microbes are considered safe for the host, some may present risks. For example, *Enterococcus* bacteria can contribute to the spread of (AMR) antimicrobial resistance, and certain strains of *Bacillus cereus* can produce harmful endotoxins and emetic toxins (Anadón et al., 2006). There has been a notable increase in the application of probiotics in chicken farming. This trend emphasizes the efficacy of numerous probiotic products in practical applications, public acceptability as an extensively utilized product, and the global movement toward alternative feed additives as a result of the reduction or restriction of antibiotic growth promoters. Probiotics, like AGPs, improve animal performance by lowering inflammation in the gastrointestinal tract (Shini & Bryden, 2022).

## **Gut Health in Poultry**

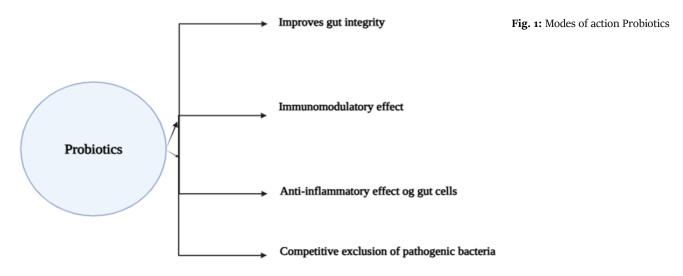
The phrase "gut health" is frequently used in both human and animal studies, particularly when discussing the effects of probiotics. However, its meaning is often wide and imprecise. Probiotics are well known in human health research for their function in maintaining gut homeostasis, which is achieved by influencing the gastrointestinal micro-flora (Jugder et al., 2021). The digestive tract is responsible for physiological processes, including digestion, absorption, metabolism, energy production, intestinal barrier maintenance, immune function, and supporting a balanced microbiome. A 'healthy gut' in poultry refers to the lack of unusual or impaired morphology and processes, allowing the bird to be protected from diseases while also digesting feed and absorbing nutrients effectively to attain peak performance. There is strong evidence which suggests that probiotics play a key part in gut health by preserving digestive balance (Sharma Bajagai et al., 2016; Zommiti et al., 2020).

## Mode of Action of Probiotics

Probiotics have been confirmed to be very beneficial, however, the actual ways in which these good bacteria yield their effects are not known yet. A fine web of factors derived from the field allows different mechanisms and pathways of action, proving the complexity of this issue (Abou-Kassem et al., 2021) Other therapeutic benefits of probiotics might be the result of a bunch of asynchronous mechanisms, so some of them are examined in this context (Fig. 1).

## Modulation of the Intestinal Microbiota

Farm animals have a specific composition of bacteria that develops at the epithelial and mucosal levels all along the gastrointestinal tract. A balanced gut microbiome and absorption of the metabolites produced by the gut biome are necessary parts of a healthy body. Probiotics have been considered among the treatments that are an effective substitute for antibiotics. It might be a much healthier body also. They help the interaction with beneficial bacteria like *Lactobacillus* and *Bifidobacteria* (Gagliardi et al., 2018) to establish an enriched gut flora that is highly diverse and stable.



By challenging harmful microorganisms for nutrients and attachment sites, these bacteria are the agents to maintain healthy gut equilibrium. These actions include the pursuit and competition for adhesion with mucus and nutrition, as well as the production of killing factors in the lumen when infections appear. The method has been found to be efficient in keeping *Salmonella, Shigella, Clostridium*, and *Listeria* out of the digestive system. However, the probiotic bacteria were able to attach to the intestine in chicken, thus giving them the advantage over the pathogens and promoting a healthy gut (Collado et al., 2005).

#### Maintaining the Functional Aspects of the Gut Barrier

The intestinal epithelium forms part of the dynamic, semi-impermeable barrier that regulates the passage of luminal contents (nutrient components), allowing or denying the cells and the basal lamina from accessing the microvascular network. The mechanisms may include tight junctions (TJ), gap junctions, adherens junctions (AJ), and desmosomes within the cell junctions by controlling the paracellular permeability, keeping the epithelial barrier in the belly wall intact or the integrity of the epithelial tight uniform junction of the barrier. The apical junctional complex, which controls the intestinal barrier, is made up of tight and adherens junctions. Tight junctions consist of four integral transmembrane proteins (occludin, claudin, junctional adhesion molecule, and tricellulin) that interact with the actin cytoskeleton (Ulluwishewa et al., 2011). Mucosal barrier integrity is crucial for ensuring effective digestion, absorption, and protection against infectious agents and toxic substances. Loss of mucosal barrier integrity is a major contributor to changes in physiological function and decreased feed intake, ultimately impacting overall health. The use of probiotics can enhance the integrity of the mucosal barrier by boosting mucus production, limiting bacterial movement, and reinforcing TJ, thereby replicating the benefits of a normal gut microbiome (La Fata et al., 2018).

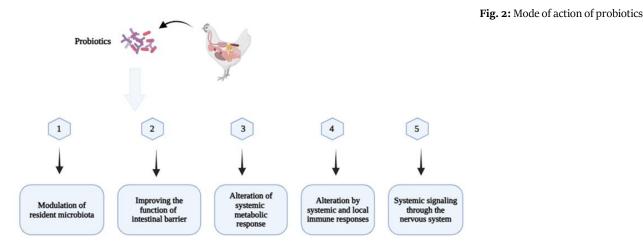
#### Modulation of Inflammatory and Immunological Responses

Probiotics strengthen host immunity through non-invasive mechanisms, avoiding tissue infection or colonization. The intestinal mucosa recognizes probiotics as foreign entities, activating both innate and acquired immune responses, which is similar to how it responds to other non-self-organs. The reaction to commensal and probiotic bacteria is a desirable outcome, as it contributes significantly to their immune-modulating properties and promotes overall health. The inflammatory response serves as the initial trigger, activating cells of the immune system, inflammatory mediators, proteins of acute phase reaction, and other signaling mediators to defend the body against potential threats (Abdulkhaleq et al., 2018). An unresolved inflammatory response can cause intestinal or systemic disorders, as well as increased energy expenditure required to maintain a prolonged immune response. This has an impact on the health and performance of birds. Pathogen exposure activates intestinal defense systems, with the innate (non-specific) response being the first, which includes the inflammatory response. Pattern recognition receptors on innate immune cells, including dendritic cells, recognize microbial fragments, initiating a potent response to eliminate the infection. The majority of probiotics exhibit a high degree of immuno-tolerance, suggesting they are well-accepted by immune cells (Plaza-Díaz et al., 2017). The protective and anti-inflammatory actions of probiotics in intestinal inflammation are attributed to multiple mechanisms, which have been proposed based on scientific evidence. Probiotics increase the formation of short-chain fatty acids (SCFAs) like butyrate, which have anti-inflammatory characteristics. They also enhance the manufacture of antimicrobial peptides, such as bacteriocins, which aid in the resolution of inflammation in mucosal tissue (Tarradas et al., 2020).

## Modification of Digestion and Metabolism

Certain commensal bacteria, such as probiotics, secrete enzymes that break down complex nutrients, not only supporting their own

metabolism but also enhancing the host's digestive efficiency (Sharma Bajagai et al., 2016; Rowland et al., 2018). Beyond enzyme production, probiotics release a variety of chemicals that might interact with the host's nutritional state, either directly or indirectly (Figure 2). Butyrate, a kind of SCFA, is a good illustration of how enterocytes may use it for energy. Probiotics promote a healthy and intact epithelial lining, leading to enhanced nutrient uptake and improved feed utilization. By facilitating nutrient digestion and ATP production, probiotics indirectly influence gut homeostasis by optimizing nutrient uptake through an activated transport system. Changes in excreta quality, as well as the presence of watery excreta or unabsorbed nutrients, are important indicators of poor digestion and absorption. The lack of a pasty vent in hens implies proper digestion and absorption (Shini & Bryden, 2022).



With the decrease or elimination of antibiotic growth promoters (AGPs) in chicken feed, there is a rising global interest in alternative feed additives, notably probiotics. A recent study has shown that probiotics improve full intestinal health and production. The increased demand for probiotics in poultry emphasizes their potential advantages to chicken production. However, despite much study on probiotics and gastrointestinal health, some issues persist. Advances in microbial and molecular research will help us better understand how nutrition, microbiota, and host metabolism interact to keep the gut healthy, particularly in the intestinal mucosa. These findings will aid in the clarification of probiotic action mechanisms and the identification of strain-specific characteristics. Understanding why probiotics may not always be effective in practice may help guide the development of next-generation probiotics and influence the usage of additional feed additives to promote.

## Prebiotics

Gibson and Roberfroid classified a prebiotic chemical as a fiber-rich dietary element used by gut microbes (Gibson & Roberfroid, 1995). It enhances the physiological processes of the host by specifically increasing the development and/or activity of one or a small number of bacteria in the digestive system, hence boosting gut health and microbial balance. Gibson et al. amended the definition and described prebiotics as specialized feed ingredients that selectively support the growth and activity of beneficial gut microbiota, leading to the benefit of the host's overall health. Some researchers limited prebiotics to indigestible oligosaccharides (Kolida et al., 2002; Gibson et al., 2017).

## **Ideal Characteristics of Prebiotics**

Patterson and Burkholder stated the most beneficial features,

- (1) Prebiotics should not be metabolized by enzymes in the gut.
- (2) Cells in the gut cannot absorb prebiotics directly.
- (3) Prebiotics selectively enhance one or a few helpful microorganisms.
- (4) Prebiotics influence the gut flora and its activity.
- (5) Prebiotics enhance luminal or systemic immunity to pathogen invasion (Patterson & Burkholder, 2003).

Beneficial bacteria in the colon digest prebiotics, resulting in the production of lactic acid, short-chain fatty acids, and antimicrobial peptides such as bacteriocins, which help to control pathogenic species (Bogusławska-Tryk et al., 2012). The beneficial effects of these substances extend beyond the intestinal microbiota, as they also enhance the integrity of intestinal epithelial cells, resulting in improved nutrient absorption and animal performance (Pourabedin & Zhao, 2015). The inclusion of prebiotics in broilers diets can modulate the gut microbiota, promoting the growth of beneficial Lactobacillus and Bifidobacteria while suppressing the proliferation of pathogenic coliform bacteria (Yang et al., 2008).

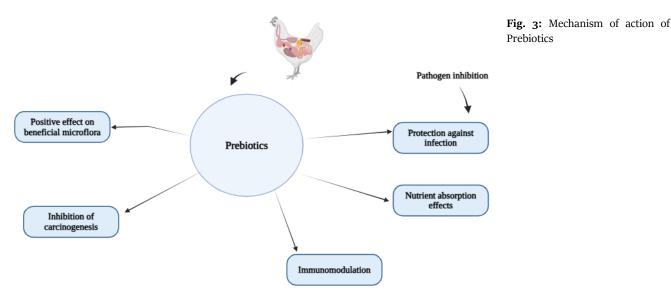
## **Mechanism of Action of Prebiotics**

Prebiotics are fiber-rich dietary substances that preferentially promote the proliferation and activity of beneficial microbes, hence enhancing gut health (Figure 3).

## Selective Stimulation of Beneficial Microbiota

Prebiotics, such as inulin and fructooligosaccharides, favorably support healthy gut flora, such as Bifidobacteria and Lactobacilli, while

limiting the growth of harmful pathogens (Gibson & Roberfroid, 1995). This selective stimulation promotes a healthy gut microbiota and prevents dysbiosis, which is frequently associated with gastrointestinal disorders.



## Fermentation and Production of Short-Chain Fatty Acids (SCFAs)

The fermentation of prebiotics by beneficial gut microbiota produces short chain fatty acids including acetate, propionate, butyrate, which energize colon cells, enhance gut barrier function, and exert anti-inflammatory effects (D'Argenio et al., 2014). In addition to their other benefits, SCAFs contribute to immune system regulation and gut balance, preventing the colonization of detrimental bacteria.

#### **Improved Gut Barrier Function**

The intake of prebiotics reinforces the intestinal barrier function by stimulating the production of mucus and tight junction proteins, which helps to prevent the translocation of harmful microbes (Bindels et al., 2015). It has been proven that through prebiotics, which work on both the gut-associated lymphoid tissue (GALT) and the systemic immune system, we can achieve a balanced, sustainable, and relatively strong immune system. By increasing the production of defensive cytokines and IgA antibodies, which are so-called IgA, prebiotics ameliorate the body's defense against infections and help to keep the immune system stable (Miquel et al., 2015).

## Pathogen Growth and Adhesion Inhibition

Prebiotics decrease attached pathogens on the intestinal wall by stimulating the production of good bacteria; hence, gut health is improved. The competition between good bacteria and pathogenic bacteria for food resources and binding sites of the gastrointestinal tract decreases the chances of infectious diseases. Hence, the vigorous and healthy gut ecosystem is maintained (Cummings et al., 2004). As a part of their role in strengthening the gut, prebiotics also prevent the occurrence of infections and enhance immunity; these facts are the important reasons for them being on a diet that is good for health.

#### Future of Antibiotic-Free Poultry Industry in Pakistan

The sustainability and success of Pakistan's Poultry Industry with special emphasis on antibiotic-free production, depend on the existing obstacles being removed and long-term thinking, as well as solutions application. The worldwide growing need for antibiotic-free chicken, continually boosted by health and health-conscious people and also a result of the resistance of bacteria to antimicrobials, presents Pakistan's Poultry with an opportunity to reshape and intensify its position on the world scene (Hussain *et al.*, 2015)

#### **Overcoming Challenges and Fostering Growth**

The chicken sector of Pakistan has been in crisis following the following hurdles- lack of proper energy storage, poor infrastructure facilities for the production of the poultry industry, and insufficient feed quality that have quite impacted the productivity and efficiency of the sector. Moreover, power storage due to harsh weather and local feed's mycotoxin contamination have disrupted Pakistan's livestock business, impacting animal health and productivity. Inadequate production of pathogen-free chickens has made the control of the sickness very difficult in the poultry sector of Pakistan. Thus, productiveness in the poultry sector of Pakistan has been declining for the reason that disease control measures are failing. In addition, the proper functioning of poultry farms and the industry, in general, is dependent on the health of the poultry. Herbicides being used on the rice paddies and the incineration of the old crops to prepare for the new crop to be planted are harmful to soil microorganisms and other beneficial invertebrates in the fields that keep the pests in check and render the fields more productive of foodstuffs and woody biomass (Mohsin, 2019).

## Moving Toward Sustainable Practices

Exploring alternatives to combat pathogen colonization not only aligns with global market trends but also helps address the pressing issue

of antimicrobial resistance. Local chicken producers will benefit greatly from training and support to understand how these methods can enhance disease prevention and promote gut health in their flocks. To successfully move towards antibiotic-free chicken farming, it's important to prioritize biosecurity measures. In the past, having chicken farms clustered together has led to more frequent disease outbreaks. Therefore, selecting appropriate sites for farms and creating biosecure environments will be essential for preventing these outbreaks and ensuring the overall health and well-being of the chickens (Mohsin, 2019)

## Value-Added Products and Consumer Awareness

To thrive in a world where antibiotics are no longer used, the poultry industry will need to focus on offering products that go beyond the basics. Some brands like K&N's, MENU, and Mann-o-Salwa Chicken are already taking steps in this direction, but there's still plenty of room for more brands to get involved. By producing value-added products, poultry farmers can enjoy steadier income and attract more customers. It's also essential to keep educating consumers about the safety, quality, and health benefits of antibiotic-free poultry to ensure these products succeed in the market (Cervantes, 2018).

## The Role of Research and Technological Advancements

Advancements in microbiological and molecular technologies are crucial for the future of chicken farming without antibiotics. The chicken industry has a real opportunity to lessen its dependence on antibiotics while still maintaining production levels and profitability. This can be achieved through the development of tailored probiotics and prebiotics, as well as refining alternative feeding strategies.

As we learn more about the gut microbiome, how chickens resist diseases, and how to modulate their immune systems, we're seeing exciting changes in the poultry industry. These insights will help us create innovative, antibiotic-free approaches to improve chicken health. Additionally, the creation of effective biomarkers to evaluate alternatives to antibiotics will be a game-changer. These tools will enable farmers to optimize these alternatives and ensure they work effectively. By using these biomarkers, we can accurately evaluate how well probiotic treatments and other methods are helping, leading to smarter, data-driven choices in agricultural practices (Cervantes, 2018).

## Conclusion

As the world moves towards more sustainable and safer food production, Pakistan's poultry industry has a fantastic opportunity for growth, especially in antibiotic-free production. By utilizing probiotics and prebiotics, the industry can tackle ongoing challenges like power outages, disease control, and inconsistent feed quality. These bioactive additives not only promote gut health but also boost productivity and reduce the reliance on antibiotics. This is crucial in addressing the global issue of antimicrobial resistance. To facilitate a smooth transition in the poultry sector, it's important to focus on improving biosecurity measures, farm management practices, and feed quality standards. Educating farmers about site selection and management will empower them to take proactive steps in disease prevention and gut health maintenance. Additionally, informing consumers about the benefits and safety of antibiotic-free chicken can help spark demand and support the continued growth of this sector. The latest advances in molecular and microbiological research will deepen our understanding of probiotics and prebiotics, leading to more effective and tailored solutions. By adopting precise formulations and alternative methods, producers can cut down on antibiotic use without sacrificing productivity. Moreover, using biomarkers will allow for accurate assessments of treatment success, ultimately enhancing poultry health outcomes. With smart investments, research, and consumer education, Pakistan has the potential to transform its chicken industry, creating a viable and sustainable antibiotic-free market. The strategic use of probiotics, prebiotics, and innovative techniques will not only help meet local and global demand but also ensure a healthier and more sustainable future for everyone involved.

## References

- Abdulkhaleq, L. A., Assi, M. A., Abdullah, R., Zamri-Saad, M., Taufiq-Yap, Y. H., & Hezmee, M. N. M. (2018). The crucial roles of inflammatory mediators in inflammation: A review. *Veterinary World*, 11(5), 627–635. https://doi.org/10.14202/vetworld.2018.627-635
- Adewole, D., & Khomayezi, R. (2021). Probiotics, prebiotics, and synbiotics: an overview of their delivery routes and effects on growth and health of broiler chickens. *World's Poultry Science Journal*. https://doi.org/10.1080/00439339.2022.1988804
- Anadón, A., Martínez-Larrañaga, M. R., & 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
- Bindels, L. B., Delzenne, N. M., Cani, P. D., & Walter, J. (2015). Towards a more comprehensive concept for prebiotics. *Nature Reviews*. *Gastroenterology & Hepatology*, 12(5), 303–310. https://doi.org/10.1038/nrgastro.2015.47
- Bogusławska-Tryk, M., Piotrowska, A., & Burlikowska, K. (2012). Dietary fructans and their potential beneficial influence on health and performance parametrs in broiler chickens. *Journal of Central European Agriculture*, *13*(2), 272–291. https://doi.org/10.5513/JCEA01/13.2.1045
- Collado, M., Gil, J., Efeyan, A., Guerra, C., Schuhmacher, A. J., Barradas, M., Benguría, A., Zaballos, A., Flores, J. M., Barbacid, M., Beach, D., & Serrano, M. (2005). Tumour biology: senescence in premalignant tumours. *Nature*, *436*(7051), 642. https://doi.org/10.1038/436642a
- Cummings, D. E., Overduin, J., & Foster-Schubert, K. E. (2004). Gastric bypass for obesity: mechanisms of weight loss and diabetes resolution. *The Journal of Clinical Endocrinology and Metabolism*, 89(6), 2608–2615. https://doi.org/10.1210/jc.2004-0433
- D'Argenio, V., Casaburi, G., Precone, V., & Salvatore, F. (2014). Comparative metagenomic analysis of human gut microbiome composition using two different bioinformatic pipelines. *BioMed Research International*, 2014. https://doi.org/10.1155/2014/325340
- FAO/WHO. (2002). In WHO working group report on drafting guidelines for the evaluation of probiotics in food (pp. 30).
- Gagliardi, A., Totino, V., Cacciotti, F., Iebba, V., Neroni, B., Bonfiglio, G., Trancassini, M., Passariello, C., Pantanella, F., & Schippa, S. (2018). Rebuilding the Gut Microbiota Ecosystem. *International Journal of Environmental Research and Public Health*, 15(8).

https://doi.org/10.3390/ijerph15081679

- Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., Verbeke, K., & 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 & Hepatology*, 14(8), 491–502. https://doi.org/10.1038/nrgastro.2017.75
- Gibson, G. R., & Roberfroid, M. B. (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
- Jugder, B.-E., Kamareddine, L., & Watnick, P. I. (2021). Microbiota-derived acetate activates intestinal innate immunity via the Tip6o histone acetyltransferase complex. *Immunity*, *54*(8), 1683-1697.e3. https://doi.org/https://doi.org/10.1016/j.immuni.2021.05.017
- Khan, S., Sattar, A., Lodhi, S. S., Abbas, A., Ali, F., Rahman, S. U., Ahmad, A., Abbas, M., Ahmad, N., Ullah, K., Shoukat, M. U., Ullah, Z., Tauseef, I., & Naveed, M. (2023). Individual and Combined Efficacy of Antibiotics and Probiotics on the Growth of Broiler Chicken. *Pakistan Journal of Medical and Health Sciences*, 17(2), 698–702. https://doi.org/10.53350/pjmhs2023172698
- Kolida, S., Tuohy, K., & Gibson, G. R. (2002). Prebiotic effects of inulin and oligofructose. *The British Journal of Nutrition*, 87(2), S193-S197. https://doi.org/10.1079/BJNBJN/2002537
- La Fata, G., Weber, P., & Mohajeri, M. H. (2018). Probiotics and the Gut Immune System: Indirect Regulation. *Probiotics and Antimicrobial Proteins*, *10*(1), 11–21. https://doi.org/10.1007/s12602-017-9322-6
- Liao, S. F., & Nyachoti, M. (2017). Using probiotics to improve swine gut health and nutrient utilization. *Animal Nutrition (Zhongguo Xu Mu Shou Yi Xue Hui)*, 3(4), 331–343. https://doi.org/10.1016/j.aninu.2017.06.007
- Markowiak, P., & Ślizewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathogens*, 10(1), 1-20. https://doi.org/10.1186/s13099-018-0250-0
- Markowiak, P., & Śliżewska, K. (2017). Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. *Nutrients*, *9*(9). https://doi.org/10.3390/nu9091021
- Miquel, S., Leclerc, M., Martin, R., Chain, F., Lenoir, M., Raguideau, S., Hudault, S., Bridonneau, C., Northen, T., Bowen, B., Bermúdez-Humarán, L. G., Sokol, H., Thomas, M., & Langella, P. (2015). Identification of metabolic signatures linked to anti-inflammatory effects of *Faecalibacterium prausnitzii*. *MBio*, 6(2). https://doi.org/10.1128/mBio.00300-15
- Abou-Kassem, D. E., Elsadek, M. F., Abdel-Moneim, A. E., Mahgoub, S. A., Elaraby, G. M., Taha, A. E., Elshafie, M. M., Alkhawtani, D. M., Abd El-Hack, M. E., & Ashour, E. A. (2021). Growth, carcass characteristics, meat quality, and microbial aspects of growing quail fed diets enriched with two different types of probiotics (Bacillus toyonensis and Bifidobacterium bifidum). *Poultry Science*, 100(1), 84–93. https://doi.org/10.1016/j.psj.2020.04.019
- Cervantes, H. M. (2018). Antibiotic-free poultry production: Is it sustainable? *Journal of Applied Poultry Research*, 24(1), 91-97. https://doi.org/10.3382/japr/pfv006
- Hussain, J., Rabbani, I., Aslam, S., Ahmad, H. A., Lahore, A. S., Lahore, A. S., & Sciences, A. (2015). *HHS Public Access*. 71(4), 689-700. https://doi.org/10.1017/S0043933915002366.An
- Shini, S., & Bryden, W. L. (2022). Probiotics and gut health: linking gut homeostasis and poultry productivity. *Animal Production Science*, 62(12), 1090–1112. https://doi.org/10.1071/AN20701
- Patterson, J. A., & Burkholder, K. M. (2003). Application of prebiotics and probiotics in poultry production. *Poultry Science*, 82(4), 627–631. https://doi.org/10.1093/ps/82.4.627
- Plaza-Díaz, J., Ruiz-Ojeda, F. J., Vilchez-Padial, L. M., & 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
- Pourabedin, M., & Zhao, X. (2015). Prebiotics and gut microbiota in chickens. *FEMS Microbiology Letters*, 362(15), fnv122. https://doi.org/10.1093/femsle/fnv122
- Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., & Tuohy, K. (2018). Gut microbiota functions: metabolism of nutrients and other food components. *European Journal of Nutrition*, *57*(1), 1–24. https://doi.org/10.1007/s00394-017-1445-8
- Sharma Bajagai, Y., Klieve, A., Dart, P., & Bryden, W. (2016). Probiotics in animal nutrition: production, impacts and regulation. FAO. Shini, S., & Bryden, W. L. (2022). Probiotics and gut health: linking gut homeostasis and poultry productivity. Animal Production Science, 62(12), 1090–1112. https://doi.org/10.1071/AN20701
- Takahashi, T., Nakagawa, E., Nara, T., Yajima, T., & Kuwata, T. (1998). Effects of orally ingested Bifidobacterium longum on the mucosal IgA response of mice to dietary antigens. *Bioscience, Biotechnology, and Biochemistry*, *62*(1), 10–15. https://doi.org/10.1271/bbb.62.10
- Tarradas, J., Tous, N., Esteve-Garcia, E., & Brufau, J. (2020). The control of intestinal inflammation: A major objective in the research of probiotic strains as alternatives to antibiotic growth promoters in poultry. *Microorganisms, 8*(2), 148.
- Ulluwishewa, D., Anderson, R. C., McNabb, W. C., Moughan, P. J., Wells, J. M., & Roy, N. C. (2011). Regulation of tight junction permeability by intestinal bacteria and dietary components. *The Journal of Nutrition*, *141*(5), 769–776. https://doi.org/10.3945/jn.110.135657
- Yang, Y., Iji, P. A., Kocher, A., Mikkelsen, L. L., & 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
- Zommiti, M., Feuilloley, M. G. J., & Connil, N. (2020). Update of Probiotics in Human World: A Nonstop Source of Benefactions till the End of Time. *Microorganisms*, 8(12). https://doi.org/10.3390/microorganisms8121907
- Mohsin, M. (2019). The under reported issue of antibiotic-resistance in food-producing animals in Pakistan. *Pakistan Veterinary Journal*, 39(3), 8318.