

Review

THE RELATIONSHIP AMONG GUT MICROBIOTA, NUTRITION, AND THE DIGESTIVE SYSTEM IN BROILER CHICKENS

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ABSTRACT

Among the many factors that influence the population and composition of gastrointestinal microbiota in monogastric animals, diet is regarded as the most significant factor that animal nutritionists can manipulate to modify the microflora in the digestive systems of these animals. The aim of this review article is to synthesise existing information on the gastrointestinal microbiota of broiler chickens and how they interact with dietary elements to enhance nutrient digestibility and absorption, regulate the immune system, sustain gut health, and ultimately improve the growth performance of broiler chickens. The various microorganisms inhabiting different parts of the digestive tract of avian species and their mechanisms of action have been explored. Additionally, the use of feed additives in broiler diets to influence intestinal microflora, boost the immune response, lower mortality rates, enhance body weight gain, and increase the profitability of broiler production has also been discussed. Several challenges are associated with the use of antibiotics, prebiotics, probiotics, and organic acids as feed additives for disease prevention, growth enhancement, and improving feed conversion ratios in poultry. The incorrect use and excessive reliance on antibiotics can lead to the development of antimicrobial-resistant pathogenic microorganisms and to the accumulation of antibiotic residues in meat products. Organic acids possess astringent odours that can diminish feed palatability, and their excessive use may result in bacteria developing resistance to organic acids. Given these concerns, it is essential to investigate feed ingredients that have similar characteristics to feed additives but do not negatively impact the intestinal microbiome or the host animal in the formulation of broiler feed.

Key words: diet manipulation; growth performance; gut health; intestinal microflora; nutrient digestibility; poultry

INTRODUCTION

The relationship between the digestive system, diet, and intestinal microbiota in broiler chickens is interdependent (Fathima *et al.*, 2022). The digestive tract of a broiler chicken consists of the crop, proventriculus, gizzard, duodenum, jejunum, ileum, caeca, and colon (Scanes and Pierzchala-Koziec, 2014). It is populated by bacteria that exhibit symbiotic, commensal, or pathogenic relationships with the host organism (Pan and Yu, 2014). The gut of a broiler chicken contains

various types of microorganisms collectively referred to as the microbial community or microbiome (Khasanah *et al.*, 2024). This microbiome consists of bacteria, protozoa, fungi, and viruses from numerous species and genera (Aruwa *et al.*, 2021). The intestinal microbiota is primarily made up of bacteria, with their cells outnumbering the host's somatic cells by a factor of ten to one (Pan and Yu, 2014). It is estimated that a broiler's gastrointestinal tract (GIT) is home to 640 bacterial species from 140 different genera (Khasanah *et al.*, 2024). The composition and abundance of microbiota in various parts of the

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Received: May 11, 2025
Accepted: September 16, 2025



gastrointestinal tract are affected by several factors, including the genotype, sex, and age of the broiler chicken, as well as the environment in which it is raised and the composition of its diet (Al Hakeem *et al.*, 2023).

The crop is home to *Lactobacillus* spp., which play a significant role in fermenting monosaccharides and oligosaccharides to generate lactate (Aruwa *et al.*, 2021). The mucosal lining of the proventriculus secretes gastric juices and hydrochloric acid, lowering the pH in both the proventriculus and gizzard to levels that favour beneficial microorganisms like *Lactobacillus*, *Bacillus*, and *Bifidobacteria*, while being harmful to pathogenic bacteria such as *Escherichia coli*, *Clostridia*, *Salmonella*, and *Campylobacter* (Guluwa *et al.*, 2020). Beneficial microbes residing in the proximal gut (crop, proventriculus, and gizzard) ferment carbohydrates that are indigestible to endogenous enzymes thereby producing lactate and synthesising vitamins (Tolnai *et al.*, 2021). Lactic acid decreases the pH of the intestinal lumen. The acidic pH in the proventriculus aids in converting pepsinogen into pepsin, which is an enzyme essential for protein digestion (Fathima *et al.*, 2022). The caeca are populated by anaerobic bacteria, including *Streptococci*, *Eubacteria*, *Clostridium*, and *Bacteroides* (Borda-Molina *et al.*, 2021). These anaerobic microorganisms break down non-starch polysaccharides that cannot be digested by the animal's own enzymes, converting them into short-chain fatty acids (Pan and Yu, 2014). According to the same authors, these fatty acids utilised by the host for energy and promote the development of enterocytes on the intestinal lining, improving nutrient absorption in the small intestine. Conversely, the gastrointestinal microflora depends on the mucin produced by the gut's goblet cells as a nutrient source for their survival and reproduction (Clark and Mach, 2023). Furthermore, the intestinal microbiota uses dietary fibre as a food source (Yang *et al.*, 2023).

Beneficial microorganisms in the gastrointestinal tract protect the gut from infections caused by harmful microbes through various methods (Kabir, 2025). If the gut pH is low, beneficial microbes such as lactic acid bacteria and *Bifidobacteria* grow and compete with acid-sensitive pathogens for attachment points on the intestinal lining through competitive exclusion (Fathima *et al.*, 2022). These advantageous microbes can produce bacteriocins that eliminate pathogen cells and also modulate the immune response of the host (Ducatelle *et al.*, 2023). Gut microbiota enhance intestinal health and decrease mortality rates in broiler

chickens (Bailey, 2010; Clark and Mach, 2023). In summary, intestinal microbiota improve feed efficiency, enhance growth performance, and increase the profitability of broiler chickens (Calvijo *et al.*, 2023; Ducatelle *et al.*, 2023; Fathima *et al.*, 2023).

Feed makes up 70–80 % of the expenses involved in broiler chicken production (Mallick *et al.*, 2020). Hence, it is essential to explore strategies that improve feed digestibility and absorption in the gastrointestinal tract of broiler chickens, ultimately improving feed efficiency, lowering feed costs, and boosting the profitability of broiler operations (Roth *et al.*, 2019). The inclusion of feed additives such as antibiotic growth promoters, prebiotics, probiotics, and organic acids has been a long-standing practice in the poultry sector (Ridgeway, 2019). These additives help eliminate detrimental microflora in the digestive tract of broiler chickens, modify gut microbiota, support the host's immune system, and enhance nutrient digestibility and absorption in their gastrointestinal system (Lalev *et al.*, 2020; Alabi *et al.*, 2024; Halder *et al.*, 2024; Chukwudi *et al.*, 2025). However, feed additives have disadvantages of their own. For instance, antibiotics are "anti-life" since they destroy both good and bad bacteria in broiler chickens' guts (Bean-Hodgins and Kiarie, 2025). In addition to creating bacteria that are resistant to antibiotics, antibiotic misuse can leave residues in meat and eggs (Habida *et al.*, 2023). If organic acids are utilised extensively, they can potentially produce bacteria that are resistant to them (Waghmare *et al.*, 2025). If used excessively, prebiotics might decrease the digestion of nutrients, increase feed intake and reduce feed efficiency (Ricke, 2018).

Considering the challenges associated with feed additives, it is important to explore the use of alternative feed ingredients that provide comparable benefits. Insect-larvae meal is increasingly favoured by poultry nutritionists and animal scientists as a viable replacement for the limited and costly soybean meal in the formulation of broiler chicken feed (Lalev *et al.*, 2022). Insect-larvae meal contains chitosan, a polysaccharide known for its prebiotic properties, and has an amino acid composition similar to that of soybean meal (Sajid *et al.*, 2023). Given that hens tend to prefer consuming live insects when they have the opportunity to roam, insects serve as a natural feed source for poultry (Al-Qazzaz and Ismail, 2016). Insects are also abundantly available, underutilized, and can be employed to convert low-value organic waste into high-value chicken feed (Elahi *et al.*, 2022).

Replacing some of the soybean in the diets of broiler chickens with insect-larvae meal has been shown to enhance growth performance metrics and sensory qualities (Elahi *et al.*, 2022). However, the impact of housefly (*Musca domestica*) larvae meal on the composition and population of microbiota, gut health, and histomorphometric indices has yet to be thoroughly explored (Biasato *et al.*, 2018; Radulovic *et al.*, 2018).

EFFECT OF FEEDSTUFF CHANGE TO THE GIT MICROBIOTA

The microbial population and diversity within the gastrointestinal tract are influenced by various factors, including the source of protein, the structure of the feed, the presence of anti-nutritional elements, and the ratio of indigestible carbohydrates to proteins in the feed (Qaisrani *et al.*, 2019). A feed component with a high ratio of indigestible carbohydrates to proteins fosters the growth of saccharolytic microorganisms capable of fermenting non-starch polysaccharides (Singh and Kim, 2021) and oligosaccharides, generating energy that can be utilised by caecal microbiota, while also preserving protein for microbial protein synthesis. On the other hand, a feedstuff that contains a higher amount of indigestible protein compared to indigestible carbohydrates offers an optimal substrate for the growth of caecal microbiota, leading to the fermentation of indigestible protein by peptidolytic or proteolytic bacteria, which can produce harmful metabolites such as ammonia, amines, indoles, and hydrogen sulphide (Qaisrani *et al.*, 2019; Elling-Staats *et al.*, 2022). These fermentation by-products elevate the caecal pH, creating an environment conducive to the growth of pathogenic microorganisms such as *Escherichia coli*, *Clostridia*, and *Campylobacter* (Mondal *et al.*, 2020). The presence of anti-nutritional substances like tannins, trypsin inhibitors, and chymotrypsin inhibitors diminishes protein digestibility, resulting in the passage of indigestible proteins from the foregut to the hindgut, where protein fermentation rises due to the increased supply of a substrate favoured by proteolytic bacteria (Qaisrani *et al.*, 2019; Mondal *et al.*, 2020). Protein fermentation plays a vital role in the gastrointestinal tract of broilers, as it releases toxic substances (including ammonia, amines, and hydrogen sulphide) that can severely impact gut health and the growth performance of broiler chickens (Elling-Staats

et al., 2022). Numerous studies have been conducted to explore how different feed ingredients affect the quantity and makeup of the intestinal microbiome, showing that the type of feed significantly influences gut microbiota (Feye *et al.*, 2020; Aruwa *et al.*, 2021; Kogut, 2022). The gastrointestinal tracts of birds that were given diets based on soybean meal hosted *Lactobacillus* and *Bifidobacteria*, whereas those that consumed fishmeal-based diets had *Clostridium perfringens* bacteria present. Fish meal contains histamine and gizzerosine, which are positively associated with *C. perfringens*. Gizzerosine triggers the proventriculus to secrete additional hydrochloric acid, resulting in gizzard erosion and making the intestinal lining more susceptible to necrotic enteritis, thus adversely impacting gut health (Bailey, 2010). According to the same author, oligosaccharides and polysaccharides found in soybean meal, like those found in other vegetable feedstuffs, function as prebiotics and are preferred by good bacteria known as *Lactobacilli* and *Bifidobacteria*. Based on the findings of Jia *et al.* (2024), broiler chickens that received a diet rich in protein showed a higher abundance of microorganisms in their caeca in comparison to those fed a low protein diet. This situation is linked to the presence of increased amounts of indigestible protein in the gastrointestinal tract, which pathogenic microorganisms prefer for their growth and reproduction. (Qaisrani *et al.*, 2019; de Souza Vilela *et al.*, 2021). Peristalsis transports the undigested dietary protein into the caeca when broiler chickens fed high-protein diets or diets with more protein than they need, are unable to digest it in the anterior organs of the gut (Sugiharto *et al.*, 2017). The fermentation of endogenous, dietary, and microbial proteins that are momentarily kept in the caeca is facilitated by anaerobic bacteria that colonize the caeca (Brian *et al.*, 2014; Kogut, 2022). This process produces harmful chemical compounds such as phenols, amines, ammonia, and indoles (Bailey, 2010; Kers *et al.*, 2018). Phenols generated from the anaerobic breakdown of aromatic amino acids are associated with cancer, while hydrogen sulphide produced during the fermentation of sulphur-containing amino acids has detrimental effects on cellular respiration (Elling-Staats *et al.*, 2022). The same authors reported that caecal protein metabolites influence the gut mucosa, diminishing cellular respiration, harming the mucin barrier, and exposing the host animal to microbial penetration through the intestinal lining. By-products of protein fermentation also

impact the expression of genes related to transport and cell maintenance (Bryan *et al.*, 2020).

Research indicates that broiler chickens fed diets rich in soluble carbohydrates tend to have an increased presence of pathogenic microbes in their cranial gut organs, as these highly viscous carbohydrates have a prolonged transit time and serve as preferred substrates for harmful microorganisms (Jha and Mishra, 2021). Soluble fibre has a strong capacity to absorb water and expand, leading to greater viscosity and prolonged retention time, which decreases the speed of feed consumption and ultimately slows down growth (Jha and Mishra, 2021). According to Khasanah *et al.* (2024), soluble fibre in the small intestine inhibits the growth of probiotic-like beneficial microbiota (*Bifidobacteria* and *Lactobacilli*) which have the responsibility of competing with pathogenic germs for attachment sites on the intestinal mucosa.

Beneficial bacteria, on the other hand, colonize the small intestine (jejunum and ileum) of broiler chickens that eat a diet with insoluble carbohydrates (Khasanah *et al.*, 2024). Incorporating insoluble carbohydrates such as wheat bran, oat hulls, and cotton hulls into chicken diets can enhance feed efficiency, growth performance, and intestinal health, with recommended inclusion levels not exceeding 5 – 10 % (Jha and Mishra, 2021). These bacteria serve as the first line of defence against pathogens on the intestinal mucosa (Wickramasuriya *et al.*, 2022).

MODE OF ACTION AND ROLES OF THE GIT MICROBIOTA

The jejunum and ileum are typically populated by saccharolytic bacteria, such as *Lactobacilli* and *Bifidobacteria*, which metabolise carbohydrates into lactic acid, leading to a decrease in the pH of these gastrointestinal organs (Yadav and Jha, 2019; Jha and Mishra, 2021; Khasanah *et al.*, 2024). Pathogenic microbes are unable to tolerate acidic conditions and cannot survive in such environments (Kogut, 2022). Furthermore, lactic acid-producing bacteria, or saccharolytic bacteria, surpass pathogenic species in resource competition and produce antimicrobial substances such as bacteriocins that eliminate harmful microorganisms (Sugiharto *et al.*, 2019; Dana *et al.*, 2022). These beneficial microorganisms vie with pathogens for binding sites on the mucosal surface of the gastrointestinal tract, a phenomenon referred to as competitive exclusion (Bailey, 2010; Aruwa *et al.*, 2021). Key microbes also enhance

the secretion of hydrochloric acid from the goblet cells of the proventriculus, which further reduces the pH in the proventriculus and gizzard, creating conditions unsuitable for the growth and persistence of acid-sensitive bacteria (Shivayothi and Krishna, 2020). Microbiota such as *Clostridium* spp., *Campylobacter* spp., *Bacteroides* spp., *Salmonella* spp., and *Escherichia coli* found in the caeca of broiler chickens ferment non-starch polysaccharides (NSP) to generate short-chain fatty acids (SCFAs) that lower the pH of the hindgut and eliminate harmful microorganisms (Kers *et al.*, 2019; Elling-Staats *et al.*, 2022). Propionic acid supplies energy to the intestinal cells of the host animals, promoting the growth of gut cells and enhancing the absorptive capacity of these cells (Qaisrani *et al.*, 2019). Microorganisms produce vitamins A and K, which can be utilised by the host animal (Kogut, 2022; de Souza Vilela *et al.*, 2021). Additionally, microbes secrete digestive enzymes (amylase, lipase, and protease) that help in the digestion of nutrients (Bailey, 2010; Tonks, 2018; Dana *et al.*, 2022).

The presence of proteolytic and ureolytic microorganisms in the caeca poses a threat to the gastrointestinal tract of broiler chickens, as they degrade dietary, endogenous, and microbial proteins anaerobically, producing harmful by-products like ammonia, amines, indoles, and phenols (Qaisrani *et al.*, 2019). These toxic by-products can harm the intestinal walls of the digestive system, leading to damage and potential perforation of intestinal tissues by pathogens (Cardoso Dal Pont *et al.*, 2020; Han *et al.*, 2021). The quantity of protein that accumulates in the caeca is influenced by the source of the protein or its digestibility, as well as the level of inclusion (Qaisrani *et al.*, 2014). When the protein is poorly digestible, it passes through the upper digestive tract without being absorbed and ultimately reaches the caeca (Elling-Staats *et al.*, 2022). If the diet provides more protein than what broiler chickens require, the excess will accumulate in the caeca (Qaisrani *et al.*, 2014). Fermentation in the caeca tends to focus on the nutrients that are most available; for instance, when there is a higher level of dietary fibre in the caeca, the breakdown of carbohydrates generates short-chain fatty acids that can be utilised by both the host and microbes for energy, while the protein serves to support microbial protein production (Borda-Molina *et al.*, 2021). Conversely, if the caeca contains a greater amount of protein compared to carbohydrates, the fermentation of proteins will supply energy for the

microbes but can also produce harmful by-products that negatively affect the gut health of broilers (Qaisrani *et al.*, 2014). The amount of protein in broiler starter and finisher diets must not exceed 20 – 22.5 % and 19 – 20 % of the diet, respectively (Beski *et al.*, 2015). The protein found in the hindgut of broilers creates a conducive environment for pathogenic microorganisms, resulting in an increase in these harmful bacteria and ultimately causing an enteric disorder referred to as dysbacteriosis (Fathima *et al.*, 2022).

Dysbacteriosis

Dysbacteriosis is an intestinal disorder that occurs due to an overpopulation of harmful bacteria in the digestive systems of broiler chickens (Teirlynck *et al.*, 2011; Salahi *et al.*, 2025). It is marked by a smaller bursa of Fabricius compared to that of healthier broiler chicken (Bailey, 2010). Infected broiler chickens produce wet and orange-coloured droppings that dampen the bedding, leading to further complications such as hock burn and pododermatitis (Ducatelle *et al.*, 2023). When broilers develop foot-pad dermatitis, they may struggle to access feed and water, resulting in decreased feed intake (Ringseis and Eder, 2022). Broiler chickens that cannot move and often sit on their breasts, develop a condition known as breast blisters (Bailey, 2010; Salahi *et al.*, 2025). Broilers affected by dysbacteriosis exhibit poor growth and are unlikely to reach the desired slaughter weight in a timely manner, leading to lower market prices and financial losses for the producer (Teirlynck *et al.*, 2011; Shanmugasundaram *et al.*, 2023).

EFFECTS OF SUPPLEMENTING POULTRY DIETS WITH FEED ADDITIVES ON GIT MICROBIOTA, GROWTH PERFORMANCE PARAMETERS AND GUT HEALTH

"Feed additives" are a group of substances that are added to chicken feed in order to increase the nutrients' digestibility (Ricke *et al.*, 2020; Ayana *et al.*, 2024; Abd El-Ghany, 2024). The functions of feed additives, which include organic acids, probiotics, prebiotics, and antibiotics, are covered in this section (Pirgozliev *et al.*, 2019).

Antibiotics

Antibiotics are naturally occurring substances produced by fungi that are utilized for the prevention or treatment of illnesses (Alabi *et al.*, 2024). Additionally, they can enhance feed efficiency and promote growth in livestock (Bean-Hodgins and Odunsi, 2025). As stated by Idro *et al.* (2009), antibiotics can be classified into three categories: Penicillins (such as fluxcloxacillin and amoxicillin), Cephalosporins (including cefaclor and cephalixin), and Tetracyclines (like tetracycline and doxycycline). These antibiotics target pathogenic microorganisms in the digestive systems of poultry, facilitating improved digestion and nutrient absorption, which ultimately boosts feed efficiency and growth in birds (Bean-Hodgins and Kiarie, 2025). In general, antimicrobials promote better gut health in the host animal, leading to reduced mortality rates among broiler chickens, lower production costs, and increased profitability (Alabi *et al.*, 2024).

In accordance with the findings of Onifade and Odunsi (1998) as well as Bean-Hodgins and Kiarie (2025), antibiotics function through several mechanisms, including:

- Breaking down bacterial cell walls and interfering with cell metabolism in microorganisms.
- Preserving the equilibrium of gut microflora, thereby preventing enteric infections.
- Modifying the immune response of the host animal.
- Enhancing the digestion and absorption of nutrients.
- Counteracting enterotoxins.

Azeem *et al.* (2022) found through their research that the inclusion of flavomycin, lincomycin, or zinc bacitracin in broiler chicken diets did not lead to any notable differences in growth performance, immune organ morphometry, antibody response, or feed efficiency. The same authors pointed out that the use of antibiotic growth promoters increases the production costs of broilers compared to those that were not treated.

However, the careless use of antibiotics in poultry farming has been linked to the development of resistance among certain gastrointestinal microorganisms, and the overuse of these drugs can result in residues in meat and eggs, posing potential health risks to humans consuming products from affected chickens (Mehdi *et al.*, 2018). Roth *et al.* (2019) and Kariuki *et al.* (2023) revealed that antibiotic-resistant bacteria can be passed from poultry to humans through

food chains and through contact with contaminated environments, such as poultry manure, wastewater, soil, and migratory birds. Residues of antibiotics in meat and other animal products are noted for their high carcinogenic and cytotoxic properties, which can be extremely harmful to human health (Alabi *et al.*, 2024). A research study by Jammoul and El Darra (2019) found that 77.5 % of the 80 chicken meat samples tested for antibiotic residue contamination returned positive results. The same authors additionally asserted that the levels of ciprofloxacin, amoxicillin, and tetracyclines in chicken meat exceeded the Maximum Residue Limit established by the Codex Alimentarius Commission. The Codex Alimentarius Commission has defined the allowable maximum limits for contamination with tetracyclines, sulfamethazine, and tylosin at 100 µg/kg, while the limit for neomycin adulteration has been set at 500 µg/kg (Amine *et al.*, 2020).

Due to the adverse effects of antibiotics on both poultry and humans, several European nations have prohibited the use of antibiotics in poultry farming, with other countries that raise poultry likely to adopt similar measures (Amine *et al.*, 2020; Alabi *et al.*, 2024; Bean-Hodgins and Kiarie, 2025). Nevertheless, the move away from antibiotics as a method for preventing diseases and enhancing growth in poultry has led to challenges, including disease outbreaks, increased mortality rates, lower poultry production, and diminished income from poultry farming (Alabi *et al.*, 2024). Consequently, alternative dietary strategies should be investigated to address the difficulties that arise from the elimination of antibiotics in the poultry sector (Bean-Hodgins and Kiarie, 2025).

Probiotics

Probiotics are beneficial live microorganisms that are safe and non-toxic, often included in livestock feed to influence the gut microbial community, enhance immune function, and boost the growth performance of the host animal (Idrus *et al.*, 2009; Hashemitabar and Hosseinian, 2024). The poultry industry is increasingly favoring probiotics over antibiotics since probiotics do not contribute to antibiotic resistance or leave residues in meat and other animal products, alongside the rising consumer demand for organically sourced meat and animal products (Tsega *et al.*, 2024).

Probiotics can be classified as either single-strain or multi-strain formulations, with single-strain

probiotics containing only one type of bacteria and multi-strain probiotics composed of more than two types of microorganisms (Halder *et al.*, 2024; Naeem and Bourassa, 2025). According to Tsega *et al.* (2024), the selection of probiotics is based on their capacity to:

- compete with harmful microbes effectively
- endure high temperatures during the processing and storage of feed
- survive in the acidic environment of the cranial gut

Spore-forming probiotics are typically beneficial because they can withstand high temperatures during feed processing (thermoduric) and the low pH found in the proventriculus, gizzard, and small intestine of broiler chickens (acidophilic) (Halder *et al.*, 2024). Probiotics can be formulated using microorganisms that naturally inhabit the gastrointestinal tract, including but not limited to *Lactobacillus* spp, *Bacillus* spp, *Enterococcus* spp, *Bifidobacteria* spp, and *Saccharomyces* spp (Kabir, 2009; Ayana and Kamutambuko, 2024).

Benefits of probiotics in poultry production

Probiotics offer a range of advantages to the host animal. They encourage the proventriculus to produce gastric juice that is high in hydrochloric acid (HCl) (Ayana and Kamutambuko, 2024). This hydrochloric acid lowers the pH in the upper gut, creating an environment that is hostile to pathogenic microorganisms (Halder *et al.*, 2024). Certain probiotics, such as lactic acid-producing bacteria (including *Lactobacillus* spp, *Bacillus* spp, and *Bifidobacteria* spp), ferment non-starch polysaccharides (NSP) to generate lactic, propionic, butyric, and acetic acids, which further reduce the pH in the gastrointestinal tract and create conditions unfavorable for harmful bacteria (Qasim *et al.*, 2022). Direct-fed microbials (probiotics) can compete with pathogens for nutrients and for adhesion sites on the lining of the intestines (Kabir, 2009; Naeem and Bourassa, 2025). This process is technically known as "competitive exclusion".

Probiotics generate antimicrobial substances, including bacteriocins, which can eliminate or inhibit harmful microorganisms within the gastrointestinal tract of broiler chickens (Biswas *et al.*, 2021). Direct-fed microbials promote the release of immunity cells, such as antibodies, lymphocytes and immunoglobulins, in the host animal during disease outbreaks (Tsega *et al.*, 2024). Probiotics also influence the production of macrophages and natural killer cells. In a study conducted by Emami *et al.* (2020), broiler chickens afflicted with

necrotic enteritis were given diets enriched with probiotics or multi-component feed additives (which included probiotics, prebiotics, and essential oils), and it was found that these additives decreased the prevalence of pathogens and intestinal damage, lowered feed intake and feed conversion ratio (FCR), and enhanced nutrient absorption and growth performance. In order to conduct the above-mentioned study, Emami *et al.* (2020) divided 960 day-old Cobb 500 broiler chicks into four different dietary groups:

- 1) Negative control (NC): A basal diet comprising corn and soybean meal
- 2) Positive Control: NC supplemented with 20 g of Virginiamycin per ton of diet
- 3) Probiotic: NC combined with *Bacillus Licheniformis* Probiotic (227 g per ton of diet from 0 to 42 days of age)
- 4) Probiotic/prebiotic/essential oils supplement (PPEO): NC enriched with PPEO (435 g per ton of diet from 0 to 42 days of age).

The probiotic contained *Bacillus licheniformis* (3.2×10^9 colony forming units (CFU) per gram), while the PPEO featured 276 ppm of mannan-oligosaccharides and β -glucans, along with 8000 ppm of capsaicin and curcuma per kilogram.

Probiotics can also activate immune organs like the thymus to release stress hormones to manage stress in animals exposed to extreme temperatures (Halder *et al.*, 2024; Hashemitabar and Hosseinian, 2024). Hashemitabar and Hosseinian (2024) explored the effects of adding either *Bacillus* spp or *Lactobacillus* spp probiotics to the diets of heat-stressed broilers and discovered that these probiotics decreased harmful microbes in the caeca, enhanced the release of stress hormones, improved feed digestion and absorption, and boosted the growth performance of broiler chickens. Direct-fed microbials encourage the goblet cells in the gastrointestinal tract (GIT) to release mucin, which covers the intestinal lumen with mucus, thereby protecting the intestinal epithelial tissue from pathogens (Kabir, 2009; Biswas *et al.*, 2021). Probiotics also promote the proliferation of GIT enterocytes and the formation of tight junction proteins that help seal paracellular gaps between neighbouring cells, thereby obstructing the passage of pathogens from the intestinal lumen into the bloodstream (Emami *et al.*, 2020).

Ayana and Kamutambuko (2024) and Tsega *et al.* (2024) found that probiotics can modify the GIT

microbiota to prevent imbalances within intestinal microbiota (dysbacteriosis). Utilising the mechanisms mentioned above, probiotics can displace or eliminate pathogenic microbes, uphold gut integrity and health, and reduce mortality in broilers (Halder *et al.*, 2024; Naeem and Bourassa, 2025).

Prebiotics

According to Choudhari *et al.* (2008) and Lalev *et al.* (2020), prebiotics are classified as indigestible feed components that influence the intestinal microbiota, enhance growth performance, regulate the immune response, and positively impact the gastrointestinal tract morphology in animals. They also promote the production of eggs, and meat (Lalev *et al.*, 2020). Froebel *et al.* (2019) indicated that common ingredients used as prebiotics in poultry farming consist of oligosaccharides (including fructooligosaccharides, mannanoligosaccharides, and oligochitosan) and polysaccharides (such as non-starch polysaccharides). Sarangi *et al.* (2016) performed an experiment where broiler diets were supplemented with either fructooligosaccharide at a rate of 300 g per tonne of diet or oligochitosan at 100 g per tonne of diet, and they found that diets supplemented with prebiotics exhibited comparable growth-promoting effects to those treated with antibiotics. In a separate study by Lalev *et al.* (2020), the Actigen prebiotic notably enhanced live weight, feed efficiency, and the European poultry efficiency factor (EPEF) in broiler chickens when compared to the control group.

Prebiotics function in various ways within the digestive system of bird species (Choudhari *et al.*, 2008; Lalev *et al.*, 2020). As noted by Froebel *et al.* (2019), prebiotics generate lactic acid (LA) and short-chain fatty acids (such as acetate, propionate, and butyrate) as a result of fermentation by anaerobic microorganisms in the gastrointestinal tract of broiler chickens. Lactic acid and short-chain fatty acids reduce the pH of intestinal contents, ultimately leading to the death or suppression of pathogenic microbes (Rickie *et al.*, 2020). Prebiotics can also agglutinate pathogens, preventing them from attaching to the intestinal lining; for instance, mannose creates FimH-like adhesins that bind to *Salmonella* and *Escherichia coli*, obstructing their adherence to the intestinal walls of broiler chickens (Froebel *et al.*, 2020). Prebiotics enhance the proliferation of beneficial bacteria that, in turn,

compete with harmful pathogens for nutrients and attachment sites on the intestinal walls, a process commonly known as "competitive exclusion by antagonism" (Sarangi *et al.*, 2016; Froebel *et al.*, 2020). Additionally, prebiotics elevate the osmotic pressure of gut contents, creating an environment that is unfavourable for harmful microflora in the gastrointestinal tract (Froebel *et al.*, 2019). They also promote the growth of advantageous microbiota that produce exogenous enzymes, which assist endogenous enzymes in nutrient digestion, thus making nutrients accessible to the host animal (Lalev *et al.*, 2020). Furthermore, prebiotics help maintain a balanced microbial community and support the gastrointestinal health of poultry (Sarangi *et al.*, 2016). The efficacy of prebiotics in animal nutrition is affected by the type of prebiotic used, the dosage, and the method of administration (Lalev *et al.*, 2020). As reported by Aragon (2023), broiler chickens that were fed a diet with 2 % prebiotics exhibited a higher protein digestibility coefficient (77.49 %) compared to those diets with 0 %, 3 %, 4 %, and 5 % prebiotics, which ranged from 50.81 % to 76.38 %. However, the feed conversion ratio did not show any statistically significant differences ($P > 0.05$) among all the treatment groups. Additionally, the same authors noted that the feed cost per kg of meat produced was significantly lower for the group receiving the 2 % prebiotic diet in comparison to the other treatments.

Organic acids

According to Haq *et al.* (2017), organic acids refer to carboxylic acids, including fatty acids, characterised by the chemical formula "R-COOH." These organic acids can be categorized into three types: short-chain fatty acids (such as lactic, acetic, propionic, and butyric acids), medium-chain fatty acids, and long-chain fatty acids (Abd El-Ghany, 2024). Organic acids (acidifiers) are being utilised as alternatives to the increasingly discouraged antibiotics in poultry feed, as they do not leave residues in chicken meat and eggs and are environmentally sustainable, avoiding nitrogen pollution from poultry waste (Kim *et al.*, 2015; Waghmare *et al.*, 2025). In contrast to antibiotics, which are "anti-life" by eliminating both beneficial and harmful microflora in the gastrointestinal tract, acidifiers foster the growth of beneficial microorganisms while inhibiting or completely eradicating

pathogenic microbes in the digestive systems of poultry (Dittoe *et al.*, 2018).

According to Jong *et al.* (2015) and Chukwudi *et al.* (2025), organic acids enhance gut health through various mechanisms, including, but not limited to, the stimulation of goblet cells in the intestinal walls to secrete mucin, as well as promoting the development of immune organs and the release of immunoglobulins and lymphocytes into the bloodstream of animals (Kim *et al.*, 2015; Haq *et al.*, 2017; Abd El-Ghany, 2024). It has been noted that organic acids increase the size of the bursa of Fabricius, thymus, and spleen in broiler chickens (Abd El-Ghany, 2024), all of which are involved in immunity. Broiler chickens that are given diets enriched with organic acids have shown a higher count of lymphocytes and antibody titres in their blood (Khan and Iqbal, 2015; Waghmare *et al.*, 2025).

Organic acids also decrease the pH levels in poultry diets and gastrointestinal tract (GIT) contents (Kim *et al.*, 2015; Haq *et al.*, 2017), thereby promoting flourishing of beneficial microbes and suppression of the harmful microbes. Ndelekwute *et al.* (2019) found that adding 0.25 % of acetic acid, butyric acid, formic acid, or citric acid notably lowered the pH levels in the gastrointestinal tracts of broiler chickens in comparison to the control group. Abd El-Ghany (2024) stated that organic acids serve as effective feed preservatives by inhibiting the contamination of feed from environmental pathogens and extending the shelf life of food products. Acetic acid, benzoic acid, and their sodium salts help preserve meat and reduce the transmission of zoonotic pathogens (such as *Campylobacter jejuni*, *Clostridium perfringens*, *Salmonella*, and *Escherichia coli*) from meat to humans (Haq *et al.*, 2017). As noted by Kim *et al.* (2015), the pH in the upper part of the digestive tract (including the crop, proventriculus, and gizzard) is significantly lower compared to that in the lower GIT (like the small intestine, ceca, and colon). This lower pH in the proximal GIT effectively kills or restricts the growth of pathogens, while also encouraging the growth of beneficial bacteria (including *Lactobacillus*, *Bacillus*, and *Bifidobacteria*) (Haq *et al.*, 2017). Acidifiers have bactericidal effects, meaning they disrupt the functions of bacterial cells when they enter the cytoplasm of pathogenic microorganisms (Waghmare *et al.*, 2025). Bacterial cells feature both negatively charged anions and positively charged protons (Abd EL-Ghany, 2024). When organic acids infiltrate bacterial cells, they dissociate,

releasing protons within the cells and causing a condition where there are more protons than anions, leading to a decrease in the internal pH of the impacted cells (Dittoe *et al.*, 2018). The bacteria that are affected expend more energy to stabilize their cells, which results in slowed growth or potentially their death (Jong *et al.*, 2015).

Organic acids decrease feed consumption, decrease the feed conversion ratio, and lead to improved weight gain in broilers (Kim *et al.*, 2015; Haq *et al.*, 2017; Abd El-Ghany, 2024). The high solubility of organic acids contributes to better nutrient digestibility. Adding 0.25 % of acetic acid, citric acid, formic acid, or butyric acid to broiler diets significantly improved the digestibility of dietary dry matter, crude protein, crude fiber, and ether extracts (Ndelekwa *et al.*, 2019). Additionally, acidifiers slow down the transit time of feed through the gastrointestinal tract (GIT) by reducing pH and increasing digesta viscosity, allowing prolonged exposure to digestive enzymes and enhancing nutrient retention (Haq *et al.*, 2017). According to Chukwudi *et al.* (2025), organic acids encourage the stomach and pancreas of broiler chickens to produce hydrochloric acid and pancreatic enzymes (such as trypsin, chymotrypsin, carboxypeptidases, amylases, and lipases), which are crucial for nutrient digestion and availability for absorption in the GIT of broiler chickens. Hydrochloric acid in the proventriculus activates pepsinogen, converting it into pepsin, which effectively breaks down proteins into peptides and individual amino acids (Kim *et al.*, 2015). Dittoe *et al.* (2018) found that organic acids enhance the development of intestinal structures, including the height and depth of villi as well as the length of the intestines. A rise in the height and depth of villi, along with the ratio of villi height to crypt depth, subsequently boosts the nutrient absorption capability of these organs (Jong *et al.*, 2015; Chukwudi *et al.*, 2025).

CONCLUSION

Feed additives can be included in poultry feed to regulate gastrointestinal microflora, boost the immune response of the host animal, enhance nutrient digestibility and feed efficiency, as well as lower feed expenses and increase the profitability of a broiler operation. However, these feed additives can create unfavourable conditions for both host animals and

humans. Antibiotics can lead to the development of drug-resistant harmful intestinal microbiota and may result in residual antibiotics being present in poultry meat and eggs. Prebiotics have the potential to induce digestive issues such as bloat when consumed in excessive amounts, whereas probiotics may lower nutrient digestibility when added in high quantities. Incorporating organic acids into poultry diets at levels exceeding 1.5 % may lead to a decrease in feed consumption and impaired growth performance, as well as metabolic acidosis. Organic acids are also very corrosive to metal objects. Thus, investigating feed components with beneficial attributes akin to those of feed additives should be considered. The impact of adding insect meals to broiler diets on growth performance metrics has been thoroughly researched, but their influence on gut microbiota composition and diversity, as well as histomorphometric characteristics and overall gut health, is yet to be examined. Insect meals include chitosan, an oligosaccharide that can function similarly to prebiotics. Additionally, insect meals have antimicrobial and antioxidant qualities that can be compared to those of either antibiotics or organic acids. Consequently, the potential for insect meals to serve as a substitute for feed additives in poultry diets warrants investigation.

AUTHOR'S CONTRIBUTIONS

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Investigation: BUMHIRA, E., MUTIBVU, T., CHIGEDE, N.

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Project administration: not applicable

All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

CONFLICT OF INTEREST

No conflicts of interest are disclosed by the writers.

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