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Feed additives and immunity boosting for chickens – a review

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FEED ADDITIVES AND IMMUNITY BOOSTING FOR CHICKENS – A REVIEW

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Abstract

Feed additives are an alternative to antibiotics to support sustainable poultry production, improving health and productivity, while meeting consumer demand for organic and environmentally-friendly products. Antimicrobial resistance has become a major global concern, which is encouraging the poultry industry to adopt nutritional immunomodulation as a strategy to produce healthy birds without antibiotics. This approach relies on nutritional science to replace antibiotics with immune-boosting feed additives, which is necessary because genetic selection of commercial poultry strains has improved productivity but reduced birds' ability to combat diverse disease challenges. A deeper understanding of immune system physiology can help optimize feed additives to enhance immune function without compromising performance. This review explores the poultry immune system's physiology and mechanisms, followed by an analysis of widely used synthetic feed additives: prebiotics, probiotics, enzymes, betaine, and L-carnitine. Pre- and probiotics modulate gut microbiota composition, favoring beneficial bacteria that produce short-chain fatty acids and competitively exclude pathogens, indirectly bolstering gut-associated lymphoid tissue development and function. Enzymes, either provided as a single enzyme or combination of enzymes, improve feed digestibility stabilize the intestinal microflora. Two further additives are valuable in boosting cellular immunity: betaine and L-carnitine. Betaine is mainly known as an osmotic regulator of cellular function, but

it has important immunocompetence strengthening effects. L-carnitine is best known for its effects on mitochondria, but it too can have substantial immunocompetence benefits. Furthermore, many immunomodulatory micronutrients and phytochemicals, including selenium, vitamin E, zinc, and various plant extracts rich in polyphenols and flavonoids, act as powerful antioxidants, mitigate oxidative stress, and directly influence the proliferation and differentiation of immune cells such as T and B lymphocytes, consequently strengthening both humoral and cell-mediated immunity. These alternatives aim to compensate for antibiotic removal while supporting immune modulation and maintaining productivity. A multifaceted approach integrating these additives is essential for poultry health, welfare, and performance.

Key words: antimicrobial resistance, chickens, feed additive, immune system, nutritional immunomodulation

The rapid growth of chicken meat production compared to other animal protein sources is a response to increasing demand and the opportunities for intensification of this industry (Hosseintabar-Ghasemabad et al., 2024). In 2023, 140 million tons of poultry meat were produced (Hosseintabar-Ghasemabad et al., 2024), 38% of the global meat market and 65% of the global demand for animal protein products (Dong et al., 2024). This growth will lead to increased stress and strain on the birds' immune system, which the industry has responded to by increasing biosecurity to minimize the exposure of birds to pathogens, thereby reducing the reliance of birds on a strong innate immune response (Korver, 2012). However, the growth and evolution of diseases and infectious agents is always faster than that of parameters related to the immune system (Seal et al., 2021). Therefore, nutritional solutions, including balancing nutrients and additives known to improve flock health, must be sought (Phillips et al., 2023; Seidavi et al., 2023 a; Madhulika et al., 2025).

Many strategies to manipulate chickens' immune function are utilized by the poultry industry, including vaccination, reduction or elimination of specific pathogens (e.g., biosecurity), use of growth-promoting antibiotics, and modification of nutrition (Korver, 2012). In recent years, there have been promising improvements in the development of feed

additives for both humans (Liu et al., 2023; Chuai et al., 2023; Zhen et al., 2024) and other animals (Movahhedkhah et al., 2019; Chand et al., 2021; Osita et al., 2022; Das et al., 2023). In the poultry industry, the use of additives is increasingly common in feeding programs in order to improve production efficiency and flock health, with significant economic benefits (Seidavi et al., 2023 a, b). In recent decades, overuse and misuse of antibiotics in clinical settings has led to an increase in antibiotic resistance (Ali et al., 2024). In chicken production, the widespread use of antibiotics as growth promoters in feed has led to the persistence of antibiotic residues in the products and subsequent increased concerns about antimicrobial resistance (AMR). Alternatives to antibiotic additives could prevent the growth in AMR. Antibiotic growth promoters were effective in the past at improving performance and regulating the immune system of the flock against pathogens, but the increase in bacterial resistance has jeopardized the health of poultry, the environment and human health, hence their use has been limited and banned in many countries (Baghban-Kanani et al., 2019; Phillips et al., 2023). Antibiotics have two modes of action for the host: bactericidal and bacteriostatic, the former destroying the microorganisms they are intended to combat and the latter preventing their reproduction and maintaining a constant population. Many antibiotics

bind to the active sites of enzymes, inactivating them. Thus, an antibiotic can either inhibit essential cellular functions or prevent the production of new proteins during cell growth by blocking enzymes (Ali et al., 2024). Restoring chickens' enzyme complement is one strategy to boost their immune system.

Intensive genetic selection for specific traits in poultry has depleted the immune system of modern chickens and increased the risk of contracting pathogenic organisms, making the birds more vulnerable (Kleyn and Ciacchiariello, 2025). Changes in regulations regarding the use of antibiotics in poultry feed, along with parallel efforts to meet consumer demand for "natural" products, and the approach of modulating the immune system through poultry nutrition with the help of feed additives is the most attractive solution for AMR in this industry (El-Sabrou et al., 2025).

Nutritional science embraces a preventive approach to pathogens in chickens, including practical solutions to regulate and strengthen their immune system (Seidavi et al., 2023 a). There is increasing awareness of consumers about the importance of healthy eating and food safety, and the expectations from the poultry industry are rising and putting pressure on production managers (Seidavi et al., 2020; Baghban-Kanani et al., 2023; Janmohammadi et al., 2023). Modulating nutritional immunity goes beyond functional disorders through deficiency or excess of various nutrients,

using specific nutrients to achieve functional goals, including changes in immune function and the balance between innate and acquired responses. This must be balanced with promoting and improving production traits, avoiding over-reliance on one or a few immune measures, which may predispose birds to other diseases and reduce other production traits (Korver, 2012).

The chicken's immune function is influenced by age, genetic potential, dietary composition, stress, environment, and other factors (Korver, 2012). The strategy of nutritional immune modulation, including targeted nutritional supplements to change some aspects of immune system function in order to achieve the desired goals through a series of additives, can be a low-risk and acceptable solution for the poultry industry (Korver, 2012). In this review, we initially review the chicken's immune system and then introduce and review a series of common additives available to the poultry industry whose effectiveness on the poultry immune system has been verified (Figure 1). It is likely that in the future, the feed additives reviewed in this report will be given more attention by researchers and poultry industry production managers to determine optimum doses and forms of consumption (microcapsules or nano), so that the improvement of the flock's immune system can be achieved in the simplest and most cost-effective way (Seidavi et al., 2023 a, b).

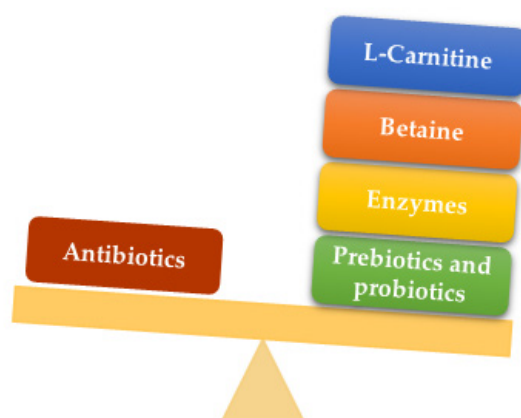


Figure 1. Schematic view of the trend in the use of feed additives in the 21st century.

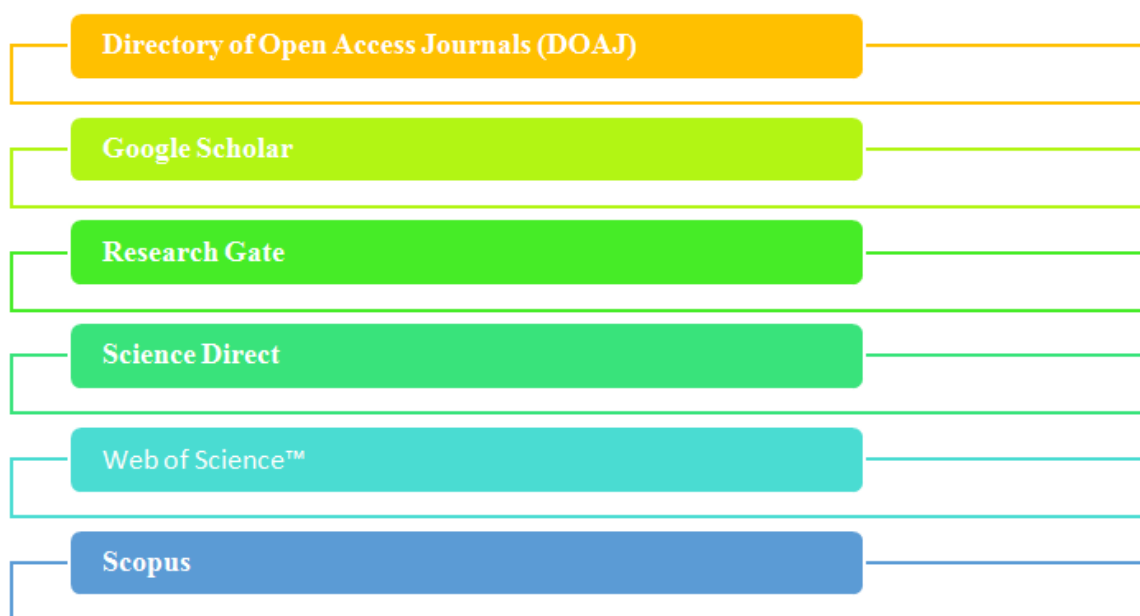


Figure 2. Electronic databases used.

Scientific review and search approach

The present review report was initiated using the electronic database (Figure 2). The search language was based on English and the keywords in the search were planned as shown in Figure 3. For the purpose of the search, the authors

selected and studied more than 90% of the reports of the last 5 years. A brief part of the scientific search period from 2002 to 2019 was selected and studied by selecting and studying prominent and important articles related to the report. The last search date in all databases was for September 2025.

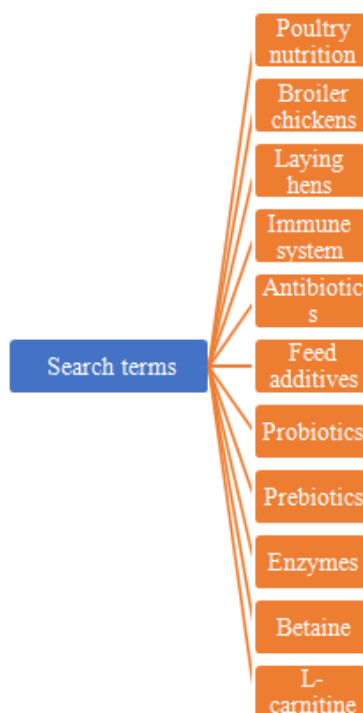


Figure 3. Keywords for search.

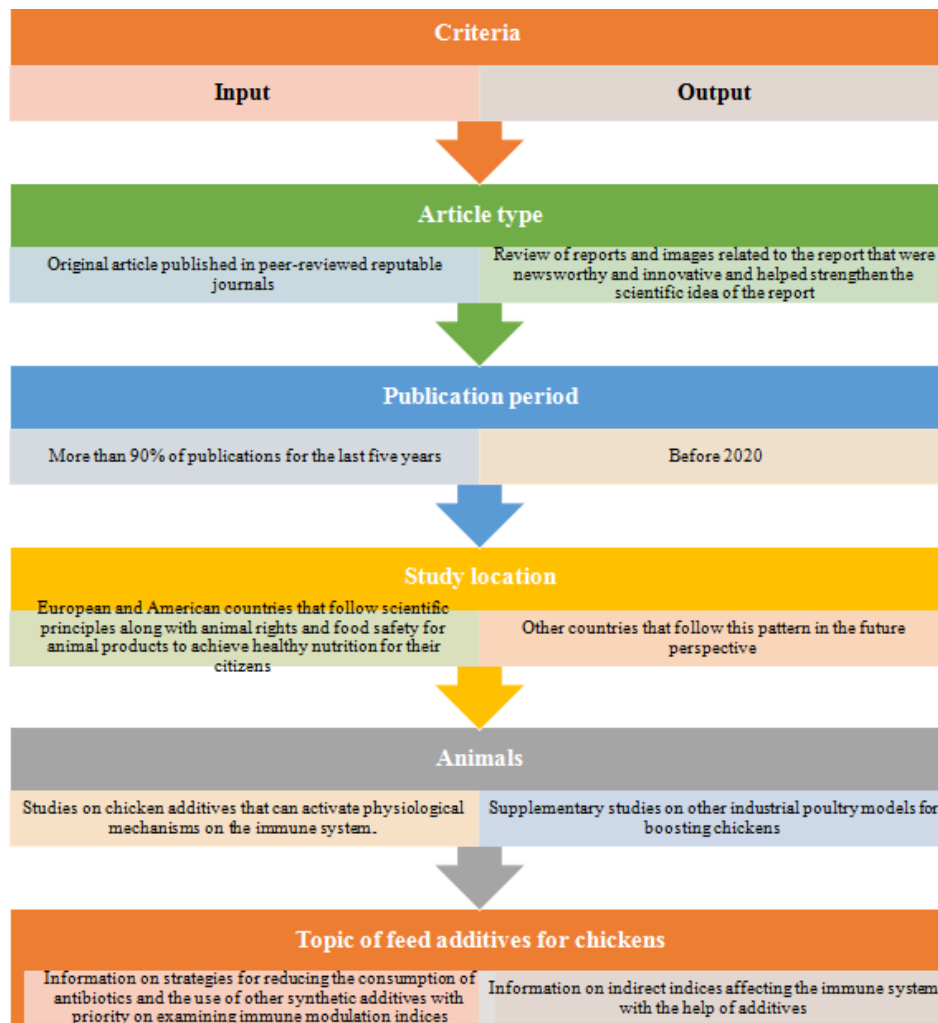


Figure 4. Criteria for reviewing the research under study.

In total, about three hundred specialized articles were selected with the help of the mentioned keywords and after studying their abstracts, about one hundred articles were selected and their detailed reports including abstract, introduction, materials and methods, results, discussion and conclusions were studied and their important points were interpreted for this review study. In order to keep the information up-to-date, new articles were used for citation and duplicates were removed. In all databases, the Endnote® scientific referencing management system was used for all articles. Of course, the manual management and control of the reference list was organized with the cooperation of all authors. It should be noted that the inclusion and ex-

clusion criteria were formulated according to Figure 4.

An introduction to the physiology and mechanisms of action of the immune system in chickens

The poultry immune system is composed of two parts: primary immune organs and secondary lymphoid organs. Primary immune organs include the thymus and bursa of Fabricius, and secondary lymphoid organs include the cecal tonsils, bone marrow, spleen, mucosa-associated lymphoid tissues (MALT), mucosal immune system (MIS), gut-associated lymphoid tissue (GALT), nasal-associated lymphoid tissue (NALT), bronchial-associated lymphoid tissue (BALT), and conjunctiva-associated

lymphoid tissue (CALT) (Sturkie, 2012; Wingfield, 2022; Khamas and Rutllant, 2024).

Many industries have shifted from improving production efficiency to increasing product quality, including public health impact. In the selection of animal feed, feed supplements and functional additives for the poultry industry, the improvement of animal health and immunity is gaining more support (Seidavi et al., 2023 a). Nutritional knowledge has advanced to the stage where we can formulate feeds that strengthen the immune system in a preventive approach. Nutritional immune modulation requires a deep understanding of the functioning of the avian immune system, and the balance between innate and acquired immunity is crucial (Korver, 2012). These two arms are complementary and work together in an integrated manner to protect the host, under the influence of the chickens' feed, and in particular feed additives. Genetic selection of poultry for increased adaptive immune activity (i.e., antibody response) usually decreases innate immunity, and vice versa. Increased cytokines that favor one type of response are countered by decreased production of other cytokines.

The innate immunity system's T helper (Th) cells enhance the bactericidal activity of heterophils and macrophages. The initial recognition of the pathogen by the innate immune system may destroy the pathogen, but the rapid response is metabolically and physiologically costly for the host (Korver, 2012). Although the metabolism of innate immune cells does not require many nutrients, an inflammatory response is nutritionally expensive. Following a proinflammatory challenge, acute phase proteins play a role in various aspects of host defense by reducing microbial growth and restoring homeostasis. Other innate immune cells protect the host by activating complement proteins to target pathogens for destruction by other cells. There is no immunological

memory for the innate immune response, i.e., a given pathogen elicits and defends a similar innate response each time it is encountered.

The adaptive immune system generates effector cells and adaptive immune molecules, which clear the infection and commence immunological memory. This includes B cells that produce antibodies to the antigen and cytotoxic T cells that destroy specific invading pathogens. Subsequent presentations of the antigens elicit a much more rapid response. The adaptive response is influenced by nutrient supply, but the nutrients required for antibody production and expansion of the B cell population are minimal (Korver, 2012). However, nutrients such as betaine and L-carnitine can enhance the adaptive response by specific effects on the immune system, including phagocytosis, that are detailed in a later section.

Phagocytic cells such as heterophils (the avian equivalent of mammalian neutrophils), dendritic cells, and macrophages destroy pathogens by releasing toxic chemicals, such as reactive oxygen species and nitric oxide, and by degranulation. Phagocytes also process and present antigens to cells of the adaptive immune system. To combat infectious and potentially harmful agents, submucosal and mucosal lymphoid tissue GALT develops in the intestines (Kiczorowska et al., 2017), comprising at least 75% of all lymphoid cells in the entire immune system. Approximately 80% of all immunoglobulins and 50% of lymphocytes are produced in the gut. One of the characteristics of the GALT system is the production of IgA antibodies, secreted on mucosal surfaces, whose main function is to receive antigens and prevent them from passing through the mucosa into the body. Antigens are produced by specialized antigen-providing cells (APCs), which lead to the secretion and absorption of effective cytokines, thereby helping to decrease inflammation (Kiczorowska et al., 2017).

Enterocytes are the cornerstone of the intestinal mucosa, which protects the host from the external environment. Joined together by tight junctions (TJs), enterocytes form a continuous band in the integral trans membranes (junctional adhesion molecules [JAMs], tricellulin, occludin, and claudins) and peripheral membranes, including the zonula occludens (ZO). TJ proteins are located between adjacent enterocytes, closing the paracellular space and regulating intestinal barrier permeability (Shehata et al., 2022), inhibiting the absorption of bacteria, toxins and other antigens from the intestine into the circulatory system. The formation and operation of strong junctions are regulated by the following signal transmission pathways in the cells: 1) protein kinase C, G and A signaling, 2) phosphatase Rho, myosin light chain (MLC) kinase (MLCK) and MAPK signaling, 3) the PI3K/Akt pathway environment (Seidavi et al., 2023 a).

In poultry, a number of acute phase proteins have been identified, including metallothionein, ovotransferrin, ceruloplasmin, and interleukins (Korver, 2012). The acute phase response is important for the survival of the bird when exposed to a pathogen, with a shift in metabolism from growth to pathogen elimination. During an inflammatory response, innate immune cells release signals that travel throughout the body to both recruit distant immune cells to the site of infection and alter the bird's metabolism, thereby creating an unfavorable environment for the invading pathogen by increasing metabolic rate (fever), decreasing feed intake, and initiating preferential breakdown of skeletal muscle to support gluconeogenesis and synthesis of acute phase proteins in the liver (Korver, 2012). Acute phase proteins respond actively to inflammatory cytokines and can be used as biomarkers of inflammation, infection and trauma. They appear in the blood much more rapidly than specific antibodies, and their levels return to normal when the inflammatory response resolves.

When designing a program to manipulate immune function in poultry, the interplay between innate and adaptive immunity must be considered. Immunomodulatory nutrients interact with other nutrients and produce multiple physiological effects in addition to altering immune function. Factors that increase the activity of one component often decrease the activity of the other. Therefore, caution should be exercised when attempting to use nutrition to modulate immunity.

The role of the gastrointestinal tract in the immune system

The gastrointestinal tract is not only specialized in the digestion and absorption of nutrients, it is a very important barrier to antigen entry. Immune cells are produced in special tissues such as the bursa of Fabricius, the cecal tonsils, Meckel's diverticulum, scattered lymphoid tissues, and the extensive mucosal layer in the gastrointestinal tract – the lamina propria (Perry, 2006; Smith, 2022).

The post-hatching period is key for the development of the gastrointestinal tract. With the introduction of feed and the colonization by microbes, the immune system must be able to distinguish between components of feed and harmful pathogens. It is not mature at this stage, and chickens rely on innate mechanisms and maternal antibodies, mainly Y immunoglobulin (IgY), transmitted to chicks through the yolk. This immunodeficiency makes the chicks vulnerable to pathogens until the acquired immune system is sufficiently developed and able to produce effective immune responses. This depends on the arrival and proliferation of specific white blood cells in key areas of the gastrointestinal tract, especially B and T lymphocytes (Perry, 2006; Smith, 2022). The clearance of the pathogen *Salmonella enterica* from the gastrointestinal tract depends on the ability to produce strong acquired immune responses. Digestive resistance to *Salmonella* begins

at 8–9 weeks of age regardless of the age of initial exposure, demonstrating the importance of a fully mature immune system in the gastrointestinal tract to control infection (Shields, 2000; Perry, 2006; Smith, 2022).

The gastrointestinal tract, a highly specialized organ for digestion, requires a large amount of mucus to maximize the absorption of nutrients, secreted by millions of villi lined with columnar epithelial cells that are constantly renewed. It is home to commensal organisms, some of which can break down cellulose and uric acid in the cecum and are involved in digestion. The defense mechanisms in the mucosal surfaces of the gastrointestinal tract are essential to protect the host against disease. These defenses include physical, chemical, and cellular barriers against the entry of pathogens, along with a highly specialized digestive immune system.

GALT consists of lymphoid cells in the lamina propria as well as specific lymphoid structures at specific sites throughout the gastrointestinal tract (Shields, 2000; Perry, 2006; Şık, 2020). GALT is the major component of the immune mechanism, with more lymphocytes than any other lymphocyte generating tissue (Shields, 2000; Perry, 2006; Smith, 2022).

Digestive immune responses include chemical and cellular responses, and the ability of different cell communities to interact is important, mediated by co-stimulatory molecules (direct cell-to-cell contact) and by the release of soluble mediators such as cytokines.

Development of the immune system of the gastrointestinal tract

After hatching, significant changes occur in the structure and organization of GALT. In the first 5 days, the epithelial cells in the crypts and villi multiply rapidly, and the intestinal epithelial cells also undergo rapid changes. Within 24 h of hatching, round and non-polar enterocytes become elongated,

this change in the structure of intestinal epithelial tissue having a great impact on the ability of newly-hatched chicks to resist intestinal pathogens. The increase in the number and variety of cells in the intestinal epithelium and lamina propria, and the development of lymphoid tissues, is due to the migration of thymus cells and bursal B cells from these organs. Thus, there are very few lymphoid cells in the intestine during hatching, but the number of epithelial lymphocytes increases with age in parallel with the migration of cells from the thymus, so that their maximum number is approximately 8 weeks after hatching. A significant increase in the number of B cells can be seen first in the small intestine on day 4 and then in the large intestine and cecal tonsils on day 6. However, B cell-specific mRNA expression is higher during hatching than in any other part of the gastrointestinal tract. GALT lymphoid cells are initially immature in terms of function, and the maturation of B and T cells in terms of function is in the first two weeks after hatching (Sturkie, 2012).

Feed additives to boost the immune response

The main purpose of immunomodulatory feed additives is to decrease local inflammation and strengthen the immune system, thus improving the animals' health status (Kiczorowska et al., 2017). This review covers the most important additives for immune responses, pro- and prebiotics, enzymes, betaine and L-carnitine. As well as these, there are many micronutrient and phytochemical additives that boost cellular and humoral immunity, such as selenium, vitamin E, zinc, and various plant extracts rich in polyphenols and flavonoids, which act as powerful antioxidants, mitigating oxidative stress, and boosting the production of T and B lymphocytes.

Probiotics and prebiotics

Poultry are usually raised in intensive production systems, where they are ex-

posed to a variety of infections that decrease production performance and immunity (Kiczorowska et al., 2017; Phillips et al., 2023). Probiotics and prebiotics focus on preventing pathogenic infections and thus improving animal welfare, which also modulates the immune response of the host via beneficial effects on the microbiome (Kiczorowska et al., 2017).

Probiotics are live micro-organisms that when administered in adequate amounts convey a health benefit on the host. They are non-pathogenic and stimulate the intestinal microbiota in their host (Ali et al., 2024), working by competitive exclusion and immune modulation (Halder et al., 2024). Potentially they could replace commercial antibiotics (Halder et al., 2024). A mutualistic interaction is established, a symbiosis of non-pathogenic bacteria with the host (Naeem and Bourassa, 2025), promoting microbial diversity in the intestine, increasing immunoglobulin levels and improving immunity (Akbarimehr et al., 2023).

The use of probiotics to modulate and enhance immunity is usually effective, but if the host's immune system is very weak, the use of probiotics may lead to infection and gastrointestinal disorders (Ali et al., 2024). Usually, they work with the primary immune responses, such as the secretion of interferon- γ and interleukin-2 against *Eimeria acervulina* to improve the local defense against coccidiosis. As immune modulators, probiotic additives reduce the expression of proinflammatory cytokines in the intestine, increase IgA secretion and intestinal epithelial barrier integrity and antibody levels (Seidavi et al., 2023 b). The increase in antibody production creates an immune response sufficient to increase resistance against pathogenic bacteria. Kabir et al. (2004) observed an increase in antibody production and resistance against microbial pathogens in broiler chickens by giving 2 mg of probiotic protoxin in 10 L water. Khaksefidi and Ghoorchi (2006) found that the consumption of 50 mg of probiotic/

kg feed increased antibody titers at days 7 and 14.

Some strains of probiotics such as *Lactobacillus*, *Lactobacillus acidophilus*, *Bifidobacterium lactis* stimulate macrophage and neutrophil production (O'Mahony et al., 2001). Dalloul et al. (2005) reported a positive effect of lactobacilli probiotics on intestinal immune responses during infection with *Eimeria acervulina*. There are more lymphoid follicles in the ileum when lactobacilli probiotics are included in the diet of laying hens, with increased IgA and antibodies (Nahashon, 1994). Lactic acid-producing bacteria activate a non-specific immune response and macrophages enhance the activity of multiple strains of live bacteria; subsequently lactic acid leads to the release of inflammatory cytokines, tumor necrosis factor- α and interleukin-6 (Seidavi et al., 2023 a). Probiotic bacterial strains of lactobacilli increase the concentration of lactic acid and volatile fatty acids (VFAs), preventing the spread of pathogens (Seidavi et al., 2023 a). Lactic acid increases acetic acid in the ileum and colon of poultry, preventing the proliferation of pathogenic bacteria by reducing intestinal pH (Wang et al., 2016).

Probiotics also stimulate the intestinal microbiota by binding to specific microbial receptors in the intestinal mucosa, competing with pathogens for nutrients, and producing antimicrobial agents to suppress their growth. They also regulate pathogenic microbial populations by pH reduction, organic acid production, and host immune modulation (Ali et al., 2024). They maintain the integrity of the intestinal barrier and immune tolerance, thereby reducing the transmission of pathogenic microbes in the intestinal defense. *Lactobacillus*, *Bifidobacterium*, and *Bacillus* strains all have a positive effect on the intestinal microbiota, improving nutrient absorption, immune function, and overall digestive health (Prakash et al., 2024). *Streptococcus*, enterococci and yeasts are also used (Prakash

et al., 2024). Different strains of *Bifidobacterium longum* can induce cytokine production by mononuclear cells in the blood (Prakash et al., 2024). Both *Bifidobacteria* and lactobacilli increase the ability to synthesize and secrete mucin in the intestine, which leads to enhanced ability of macrophages to stimulate cytokine production, as well as improvement of humoral immunity parameters (immunoglobulin) and innate (non-specific) immune factors (Spring et al., 2015; Seidavi et al., 2023 a).

In the context of competitive exclusion, inhibitory compounds such as antimicrobial peptides, bacteriocins, organic acids, and hydrogen peroxide in probiotics help reduce pathogen invasion (Halder et al., 2024). Their immunomodulatory effects include interaction with epithelial cells, dendritic cells, macrophages and lymphocytes (Halder et al., 2024). They stimulate mucus secretion and antimicrobial peptides, protecting intestinal epithelial cells against pathogenic bacteria (Halder et al., 2024). Intestinal epithelial cells and dendritic cells interact with intestinal microorganisms through pattern recognition receptors (PRRs), leading to the release of T and B cells (Halder et al., 2024). Probiotics of *Lactobacillus* origin reduce the expression of proinflammatory cytokines, namely IL-6, IL-10, IL-1 β , INF- γ , and TNF- α , and hence they diminish inflammation (Halder et al., 2024). In addition, probiotics increase production of T lymphocytes, CD⁺ cells and antibody-secreting cells (Seidavi et al., 2023 a).

Probiotics reduce ammonia production by increasing the activity of digestive enzymes and reducing the activity of some bacterial enzymes, thereby improving feed intake and digestion (Seidavi et al., 2023). Another mechanism of probiotics is enhancing the immune system by creating competition for adhesion to the intestinal epithelium, preventing the formation of colonies of pathogenic bacteria (Khan and Naz, 2013). Lymphoid tissue and lympho-

cyte production are enhanced (Idowu et al., 2025). Probiotics also reduce the activity of the bacterial enzymes beta-glucosidase and beta-glucuronidase in the intestine and feces, preventing the production of toxins in the bird's body, increasing nutrient absorption and enhancing the immune system (Khan and Naz, 2013; Seidavi et al., 2023 a, b; Jha et al., 2020). Probiotics also increase antioxidant capacity and resistance to heat stress when included in the diet of chickens (Hashemitabar and Hosseinian, 2024). The antioxidant status of poultry is also related to their immune responses through phytochemicals (Lee et al., 2018).

Prebiotics can play a very effective and helpful role in promoting the immune system and subsequently the health of birds through a wide range of processes, including effects on the microbiota of the digestive tract of birds, modulation of the immune system, morphological modification of the digestive tract and competitive elimination of pathogens (Ali et al., 2024). Prebiotics are non-digestible compounds that can selectively enhance the growth of one or more bacteria in the lower gastrointestinal tract (cecum and colon) and stimulate and modulate immunity (McFarlane et al., 2023; Seidavi et al., 2023 a). One advantage of prebiotics over probiotics is their ability to stimulate the growth of beneficial bacteria that are already present in the host organism and have adapted to the host environment. They act as substrates for microbes, for example, oligosaccharides can reduce the risk of disease by inhibiting the proliferation of pathogenic species (Ali et al., 2024). Prebiotics can play a pivotal role in improving host performance and immunity by feeding beneficial bacteria while simultaneously suppressing pathogenic bacteria. They improve the intestinal bacterial balance of broiler chickens by promoting the growth of non-pathogenic microorganisms and reducing the colonization of harmful bacteria, such as *Salmonella* and *E. coli*, and they improve poultry health

by both directly influencing immunological responses and indirectly altering fermentation patterns and composition of the gut microbiota. Shehata et al. (2022) hypothesized that prebiotic consumption leads to increased intestinal acidity and thereby helps reduce infection in the intestines of chickens. When prebiotics are fermented by gut bacteria, the short-chain fatty acids generated play a key role as a major inhibitory mechanism against pathogens (Liu et al., 2021; Abd El-Aziz et al., 2024). Other common benefits of both probiotics and prebiotics include thickening the intestinal wall, increasing resistance, increasing glucose absorption, increasing short-chain fatty acids and lactic acid, reducing the concentration of branched-chain fatty acids, encouraging the growth of gut-associated lymphoid tissue and increasing GALT colonization by T cells (Abd El-Aziz et al., 2024).

Prebiotics stimulate and activate the cellular components of the innate immune system (macrophages and heterophil antibodies) to defend the animal against digestive infections. Increases in both IgG and IgA have been observed in chickens fed with prebiotics (Savage et al., 1996). By stimulating bacteriocins (for example, lactic acid-producing bacteria), beneficial microflora are enhanced that can prevent the growth of potentially pathogenic bacteria and increase homeostasis in the digestive tract. The proper functioning of the mucosal immune system depends on the presence of intestinal bacteria. Intestinal bacteria synthesize antibacterial compounds, thereby strengthening the intestinal immune system (Kiczorowska et al., 2017). The intestinal microbiota is a hidden metabolic “organ” involved in the immunology of the body due to its control of metabolism, physiology, nutrition, and immune function of the host. The relationship between intestinal microbiota and their respective host animal is dynamic and generally beneficial to both parties (Shehata et al., 2022). This

complex interaction is a major determinant of whether a bird is healthy or diseased. Intestinal dysbiosis (intestinal microflora imbalance) is associated with several metabolic diseases (Shehata et al., 2022).

Prebiotic additives can enhance digestion and absorption of nutrients by limiting the growth of pathogenic bacteria and stimulating the growth of beneficial bacteria, improving intestinal health and integrity, improving intestinal morphological parameters and strengthening the intestinal immune system (Shehata et al., 2022). Intestinal permeability is controlled by intestinal microbiota, digestive secretions, physical barriers (mucin, intestinal epithelial cell lining, and tight attachments), and immune system messengers such as cytokines (Shehata et al., 2022). Proinflammatory cytokines modulate adaptive immunity and stabilize the intestinal microbiome.

Prebiotics used against pathogens in chickens include fructooligosaccharides (FOS), xylooligosaccharides (XOS), mannan-oligosaccharides (MOS), galactooligosaccharides (GOS), agaro-oligosaccharides (AOS), arabinoxylans, cyclodextrins, inulin, isomaltose, lactose (for poultry), lactulose, MOS oligofructose, raffinose, stachyose, xylooligosaccharides (XOS), and β -galactooligosaccharides (β -GOS) (Pirgozliev et al., 2019; Hasted et al., 2021; Seidavi et al., 2023 a). The type of carbohydrate in the prebiotic influences its effectiveness on immunity. For example, mannan-oligosaccharides have a positive effect on immunoglobulin status humoral immunity against certain pathogenic bacteria (Chacher et al., 2017). They stimulate the production of mannose-binding lectins and have a direct effect on intestinal immune cells by entering mast cells in the superficial glands of the intestine. Fructooligosaccharides facilitate the secretion of immunoglobulin A in the intestinal mucosa and subsequently render pathogens unstable to the phagocytic activity of intestinal lymphocytes. Another prebiotic that affects the

immunity of chickens is inulin. Huang et al. (2015) increased the intestinal immunity of young broiler chickens by feeding inulin at levels of 5–10 g/kg. Chitosan, a natural biopolymer that is a major component of fungal cell walls and arthropod exoskeletons, can also have a positive effect on immunity and can increase IgA levels, cementing its potential to be a prebiotic (Shehata et al., 2022; Seidavi et al., 2023 a, b).

In summary, regulating the intestinal microbiota can address diseases that are closely related to inflammatory and metabolic disorders, with benefits to animal health, function, and safety. Supplementing with prebiotics, probiotics and the combination of the two, synbiotics, has this effect (Shehata et al., 2022). Synbiotics exert their effects indirectly by manipulating the quality of the intestinal microbiota and improving the intestinal epithelial barrier, improving the integrity of strong bonds by controlling tightly bound proteins and inflammatory signaling pathways in host animal (Shehata et al., 2022; Seidavi et al., 2023 b).

Enzymes

Single exogenous enzyme additives

Exogenous enzymes are feed additives that affect the performance and health of poultry, and their internal environment, by improving feed digestibility and/or stabilization of intestinal microflora (Pirgozliev et al., 2019). Coccidiosis, a common disease in chicken flocks, could be prevented by using enzymes (Morgan et al., 1995). They are among the most widely used feed additives. Enzymes most improve the nutritional value of diets with low nutrient concentrations (Bedford and Apajalahti, 2022). A concern for all enzymes is the variability of responses, which is sometimes due to substrate variability. Methods have been developed to determine the optimal dose of enzyme in diets based on wheat and barley, and efforts are underway to determine their optimal dose in diets based on corn (Khattak et al., 2006;

Alagawany et al., 2018; Bedford and Apajalahti, 2022).

The nutritional benefits of using enzymes that may enhance the immunity of poultry include a decrease in the viscosity of digestive materials, increase in digestion and absorption of nutrients (especially fat and protein), improving the apparent digestibility of dietary energy, increasing feed intake, body weight and feed conversion efficiency, reducing feed adhesion, water consumption, ammonia production and reduced excretion of nitrogen and phosphorus in excreta, as well as moderating the digestive microorganism population (Wang et al., 2005).

Phytase (myo-inositol hexakisphosphate phosphohydrolase) is the most well-known enzyme for non-ruminant diets. It hydrolyzes the ester bonds between the phosphate groups and the inositol ring in phytates and increases the available phosphorus in the diet. Phytase is also used to increase access to plant phytate phosphate and decrease phosphorus contamination of the environment, as well as decreasing the amount of inorganic phosphate. The benefits of using dietary phytases are not limited to improving mineral retention, but may improve immune function, energy and amino acid availability (Selle et al., 2007; Pirgozliev et al., 2019; Attia et al., 2025). Phytase acts on the phytate complexes of plant compounds and splits the cell wall (Pirgozliev et al., 2019; Bedford and Apajalahti, 2022).

In chickens, phytase enzyme activity is mainly found in the upper part of the digestive system, including the proventriculus and gizzard. The efficiency of feed phytase in the digestive tract depends on the diet and the type of chicken (Singh et al., 2017). Although birds can produce some endogenous phytase, it is not enough to effectively hydrolyze dietary phytates. Harmful effects of phytates in chicken diets can therefore be reduced by adding microbial phytases (Pirgozliev et al., 2019; Bedford and Apajalahti, 2022).

Vertebrate animals cannot hydrolyze non-starch polysaccharide (NSP) in feed. Dietary NSPases break the bonds between NSP glucose units and decrease the viscosity of intestinal contents (Pirgozliev et al., 2019; Bedford and Apajalahti, 2022). Lower intestinal viscosity will lead to more complete digestion and absorption of nutrients, decreased microbial proliferation, and improved intestinal health (Perry, 2006). NSP-degrading enzymes can also produce certain types of oligosaccharides that may act as prebiotics in poultry intestines (Pirgozliev et al., 2019). Dietary NSPases can also improve the hepatic antioxidant capacity of broilers.

Protease supplementation improves the digestibility of proteins and amino acids, especially in very young animals that have relative activity of endogenous proteases (Pirgozliev et al., 2019). In addition, protease supplementation may improve feed quality by decreasing the negative effects of trypsin inhibitors or heat-stable lectins.

A primary phase lipase (LIP) and lecithin (LEC) dietary supplement has been developed with performance and immunity benefits in broilers (Shabani et al., 2021). Expression of LIP mRNA increases in older chickens (Sato and Akiba, 2002). LIP activity and fusion of fatty acids into micelles can be expected to improve with the addition of LIP enzymes or emulsifiers such as LEC.

Multi-enzyme additives

Combinations of enzymes have been developed to have the most benefit in poultry production. Supplementing sesame seed meal with a combination of phytase and lysine increased serum TSOD and T-AOC activity and improved the bioavailability of plant compounds for layers (Baghban-Kanani et al., 2019). Enzymatic antioxidants such as TSOD play an important role in converting reactive oxygen species to oxygen and water. Scavenger enzymes prevent oxidative stress in cells and improve the

immune system. A multi-enzyme additive containing alpha-galactosidase improved humoral immunity and increased the antibody titer by the 35th day on broilers (Llomas-Moya et al., 2020). Another multi-enzyme additive (Natuzyme P₅₀) reduced the cholesterol content of eggs, although it did not affect the antioxidant status or health of layers (Baghban-Kanani et al., 2018). A combination of a multi-enzyme additive (Combo), manganese and probiotic supplements for broilers increased antibodies against Newcastle disease virus, and also the concentration of IgG, IgM and total immunoglobulin produced against sheep red blood cells (Babaei et al., 2021). A Galzym-M multi-enzyme fed to broilers increased content of lymphocyte, hemoglobin and the weight of organs related to immunity, including thymus, spleen and bursa of Fabricius, indicating improvement of immune indicators after consuming the enzyme (Attia et al., 2020). However, supplementing rice gluten meal fed to broilers with a multi-enzyme containing protease and xylanase did not affect the immune response (Dinani et al., 2020).

Further research in this area will lead to more efficient use of common enzymes and effective development of heat-resistant enzymes in diets that require heat treatment (Khattak et al., 2006; Alagawany et al., 2018; Bedford and Apajalahti, 2022).

Betaine

Betaine is a derivative of the amino acid glycine with three methyl groups that is found in the tissues of plants and animals. It is abundant in marine invertebrates and sugar beet, as well as in wheat, wheat products and alfalfa powder. Sugar beet and its by-products, including molasses, are the most natural sources.

Betaine's benefits to humoral immune responses stem from its regulation of cytokines produced by the liver cell macrophages, inhibition of prostaglandin through its osmotic effects on Kupffer cells

(Zhang et al., 1996), and enhancement of nitric oxide (NO) release from heterophils and macrophages, which benefits intestinal integrity during a bacterial challenge (Klasing et al., 2002). There have been numerous studies demonstrating benefits to humoral immunity in chickens, usually by increasing antibody production, with only a few failing to demonstrate a response, for example when chicks were inoculated with betaine *in ovo* (Abd El-Ghany and Babazadeh, 2022).

Betaine plays an important role in regulating the osmotic pressure of plants, bacteria and marine organisms. Monocytes and macrophages use betaine to regulate cellular osmotic pressure, with consequent control of phagocytosis, NO and inflammatory cytokine release (Kettunen et al., 2001; Klasing et al., 2002). This will help to protect against coccidiosis, since both phagocytosis and NO release are effective defense against parasites, including coccidia. As a methyl group donor, it participates in transamination reactions and provides the methyl group required for the synthesis of active metabolic compounds such as creatine and carnitine. Therefore, betaine supplementation may decrease the need for other methyl group donors, such as methionine and choline (Eklund et al., 2005). The osmotic properties of betaine have beneficial effects on the cells and microbes of the chicken's gut. In addition, betaine increases the digestibility of fiber and minerals, as well as being involved in energy and protein metabolism at the metabolic level (Saeed et al., 2017; Ahmed et al., 2018). Betaine has an antioxidant function, scavenging free radicals to reduce tissue damage from lipid peroxidation.

Betaine is added to chicken diets as a purified additive in the form of anhydrous betaine, betaine monohydrate, and betaine hydrochloride to animal feed. Its role as a methyl donor helps to convert homocysteine into methionine, an essential amino acid that plays a role in several important

biological processes, such as protein storage and S-adenosylmethionine synthesis. S-adenosylmethionine is either used by transmethylation reactions or converted into spermidine and spermine. Therefore, the use of betaine leads to a saving of methionine that is needed for a series of biosynthesis processes (Saeed et al., 2017; Ahmed et al., 2018). After converting into homocysteine and reacting it with serine, methionine produces cystathionine and, as a result, provides the necessary sulfur for cysteine production. Methionine plays an important role in cellular and humoral immunity; indeed, it is required for immune responses more than for growth (Saeed et al., 2017; Ahmed et al., 2018). Both protein synthesis reactions and S-adenosylmethionine production compete for access to methionine. Therefore, methylating compounds may replace methionine to allow methyl, or provide the methyl group needed to convert homocysteine to methionine. The ability of dietary betaine to replace part of the role of methionine is highly variable (Zhan et al., 2006).

Betaine is the most effective osmolyte of organic osmolytes, such as proline, glycine and glutamine due to its dipolar zwitterion and high water solubility. Betaine can reduce vascular perfusion and prevent water loss from the extracellular fluid (ECF) during hyperthermia by binding and retaining water molecules and improving renal electrolyte balance. In addition, betaine has anti-apoptotic effects and increases cell proliferation in hyperosmotic environments. Furthermore, betaine can increase intestinal cell proliferation and nutrient absorption levels, and subsequently improve performance, immunity, and bird health. Betaine is largely absorbed in the duodenum and jejunum (Klasing et al., 2002). Also, controlling diarrhea, reducing susceptibility to various infections, and reducing atmospheric ammonia levels are secondary benefits of using this supplement in poultry. Overall,

betaine supplementation in chicken diets acts as a strong antioxidant with ROS reduction power, which can play an important role in improving poultry immunity and health (Patel et al., 2024).

Betaine has a chemical structure that protects beneficial intestinal cells and microbes, and subsequently neutralizes any decrease in performance caused by heat stress and coccidiosis. In addition, both microbes and intestinal cells are subjected to various osmotic stresses. Osmotic properties of betaine may also be essential for intestinal cell support, function, and proliferation (Saeed et al., 2017; Ahmed et al., 2018). Betaine seems to have a positive effect in the fight against coccidiosis. In Sweden, Waldenstedt et al. (1999) showed that the addition of betaine to the diet of chickens decreased weight loss during coccidia infection by various *Eimeria* species. There are also protective effects of betaine on intestinal cells against parasitic infection.

Stress is one of the factors that reduces the effectiveness of the poultry immune system. Betaine can have a role in improving the health and safety of heat stressed poultry. As a methyl donor, and a potent osmolyte and antioxidant, it plays an important role in reducing heat stress (Patel et al., 2024). High ambient temperature is harmful for the productive performance of broilers and laying hens and leads to significant economic losses. Heat stress leads to a decrease in digestibility of nutrients by decreasing feed intake and negatively affecting intestinal health. In laying hens, reduced feed intake decreases feed efficiency, egg production and egg quality. In addition, a decrease of immune system function, such as thyroid activity and antibody production, is noticeable in poultry exposed to heat stress. Heat stress increases the production of oxidants and causes oxidative stress and lipid peroxidation of the cell membrane. Betaine has osmotic protective properties that help to protect cellular proteins and intestinal enzymes against

environmental stress, restoring performance and immunity (Ratriyanto and Mo-senthin, 2018).

Broilers do not have a fully developed immune system at the time of hatching, both the cellular immune system and the humoral immune system develop in the first few days after hatching (Santos et al., 2020). Accelerating the maturation of the digestive system is important for the development of the immune system in broiler chickens in the early days after hatching. The development of the immune system depends on the availability and bioavailability of nutrients and energy through the digestive tract, and the gut as an important organ plays a key role in the development and evolution of the immune system. Adding betaine to the diet of chicks from hatching to 9 days of age decreased gene expression for the tight junction regulators occludin, claudin 1, claudin 5 between days 4 and 9, suggesting downregulation of genes involved in pathogen absorption (Santos et al., 2020). Maintaining the status of immune balance in the digestive tract is metabolically expensive for the animal. At the time of birth of chicks, the enterocytes of the digestive tract are not connected to each other, and large molecules pass through paracellular pathways directly into the interstitial spaces. These paracellular passages will be convenient for the entry of pathogenic bacteria and viruses at an early age. During the growth of the bird, the lymphatic tissues of the intestine, which are the place of accumulation of immune cells, protect the host body against the passage of microbiota from the digestive tract to other organs. Because the gut microbiota changes as soon as the animal eats, the composition of the feed affects the immune status of the animal and the bacterial species that are effective in the digestive system can affect the osmolarity of the intestines (Santos et al., 2020). The use of additives that affect the osmolarity and gut microbiota can improve the immune system.

As described above, betaine has two main physiological roles in the body, methyl donor for the synthesis of the amino acid methionine and osmolyte to facilitate water retention in cells to protect cells from osmotic stress. The methionine-sparing effect, an osmotic protector and cell protection are the central roles of this additive in modulating the immune system. Betaine also lengthens the intestinal villi (Santos et al., 2020) and strengthens humoral immunity, particularly in birds on high-protein diets (Ghasemi and Nari, 2020). Added to the diet of broilers, betaine improves bird health by increasing the antibody titer against infected bursa and decreasing heterophil to lymphocyte ratio, alanine aminotransferase and aspartate aminotransferase enzymes (Gudev et al., 2011; Awad et al., 2014; Nofal et al., 2015; Maddahian et al., 2021). Chand et al. (2017) observed that betaine added to the diet of broiler chickens under heat stress in the summer season increased the number of both heterophils and lymphocytes in blood serum, decreasing the heterophil to lymphocyte ratio, and also the antibody titer. Thus, there is substantial evidence that, particularly under conditions of heat stress, supplementing the diet of broilers with betaine leads to improvement of the immune system (Farooqi et al., 2005).

L-carnitine

L-carnitine is produced in the liver, and two essential amino acids, lysine and methionine, are the main substrates of L-carnitine biosynthesis, with pyridoxine, nicotinic acid, vitamin C and folate as cofactors in its production. It plays a role in immunity by boosting antibody production, enhancing the acute phase protein responses, and a glucocorticoid-like effect, but its precise mode of action is unclear (Adabi et al., 2011). It increases growth of the spleen and thymus weights, and at high concentrations growth of the bursa of Fabricius, which plays a key role in the development

of B lymphocytes and production of antibodies (Adabi et al., 2006). It inhibits apoptosis of lymphocytes and enhances their response to mitogens, as well as stimulating IgG production (Adabi et al., 2011).

Its key cellular role is in energy metabolism, being responsible for transfer of a fatty acyl group from cytosol into mitochondria. It is also involved in glucose and fat metabolism by regulating the concentration of coenzyme A in the cytosol and mitochondria. Endogenous production of L-carnitine normally suffices for the natural requirements of poultry for this compound, however, during rapid metabolism and periods of high energy needs (such as during rapid growth, egg laying, thermal stress and when diets are rich in fat), L-carnitine supplementation or its precursors and cofactors may be needed. Many factors (species, breed, lipid stores, and the rate of decomposition in the intestine) affect the use of L-carnitine. L-carnitine supplementation is particularly required when there is limited absorptive capacity of the intestine and significant microbial decomposition there (Adabi et al., 2011).

As a supplement, it can improve cecum microflora and birds' health, stimulating antibody production against Newcastle and Gumboro diseases in broilers (Hosseintabar et al., 2013, 2015; Ghoreyshi et al., 2019; Tufarelli et al., 2020). It also increases lipid catabolism, acts a strong antioxidant by neutralizing free radicals, strengthening the immune system by increasing IgA, IgG, and IgM and reducing apoptosis of B and T lymphocytes (Geng et al., 2007; Fortin 2011; Azizi-Chekosari et al., 2021; Sözcü et al., 2023). It may also increase the release of hormones such as insulin and growth hormone (IGF-1), the transfer of mitochondrial fatty acids and synthesis of acetylcholine (Thangasamy et al., 2008; Vaziri-Harami and Delkash, 2022). Particularly in older birds when a decrease in the activity of glucose-6-phosphate dehydrogenase reduces macrophage production

(Shehata et al., 2022). Similar improvements have been observed in roosters (Ennab et al., 2023).

L-carnitine metabolite concentrations are reduced by betaine supplements, and the greatest benefits for immunity are obtained when both supplements are provided simultaneously (Jewell et al., 2024). This increases the concentration of asparagine, serine and choline, the production of immune cells, including lymphocytes and macrophages, and the activity of ornithine decarboxylase enzyme, which increases polyamine synthesis.

Summary of main modes of action of additives

We summarize the modes of action of the additives in Table 1.

Conclusions

Supplementing poultry diets with popular additives, as an alternative to antibiotics,

creates the opportunity to balance acquired and innate immunity of chickens more effectively. Feed programs can be planned to modulate and strengthen the immune system. The routine use of probiotics, prebiotics, enzymes, betaine, and L-carnitine as dietary supplements can, through a series of specific metabolic responses, improve the poultry immune system to strengthen their health and make antibiotic additives redundant.

Author contributions

Conceptualization, B.H.G. and A.S.; writing – original draft preparation, B.H.G., C.J.C.P., M.R.V., F.Z., O.V.K., E.V.S., A.V.B., S.S.E., F.A., M.G.Z., and A.S.; writing – review and editing, C.J.C.P., M.R.V., F.Z., O.V.K., M.G.Z., and A.S.; supervision, M.R.V., F.Z., O.V.K., E.V.S., and A.V.B.; funding acquisition, M.R.V., F.Z., O.V.K., E.V.S., and A.V.B. All authors have read and agreed to the published version of the manuscript.

Table 1. Summary of main modes of action of the major additives used in chicken diets

	Probiotics	Prebiotics	Enzymes	Betaine	L-carnitine
Expression of proinflammatory cytokines in intestine	↓				
IgA	↑	↑			↑
Intestinal epithelial barrier integrity	↑	↑		↑	
Antibodies	↑			↑	↑
Gut microbiota modulation	↑	↑		↑	↑
Antioxidant activity	↑			↑	↑
Resistance to heat stress	↑			↑	↑
Lymphocyte proliferation	↑	↑			↑
Reduction of ammonia production	↑	↑	↑	↑	
Enhancement of nutrient absorption	↑	↑	↑	↑	

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Conflicts of Interest

The authors declare no conflicts of interest.

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