

# Feed Additives for Layer Chicken Health and Production: A Review

## Abstract

The poultry industry continuously seeks to improve the health and productivity of layer chickens to meet rising demands for eggs. Feed additives, including probiotics, prebiotics, enzymes, organic acids, and herbal extracts, have gained attention for their potential to improve health and production without relying on antibiotics. This review evaluates recent research on feed additives, focusing on their effects on egg production, quality, and immunity in layer chickens. New studies indicate that probiotics, such as *Lactobacillus* strains, and prebiotics, like mannan oligosaccharides (MOS), can improve nutrient absorption, enhance immunity, and reduce pathogenic bacteria. Enzyme supplements, especially those targeting non-starch polysaccharides, have shown promise in increasing digestive efficiency and nutrient bioavailability, ultimately supporting greater egg production. Organic acids, such as citric and lactic acid, were found to lower gut pH, suppress harmful bacteria, and improve feed conversion ratio. Additionally, herbal extracts like turmeric and oregano exhibited antioxidant properties, contributing to improved egg quality and bird health. The review highlights the importance of selecting the right additive combinations for optimal effects. This comprehensive review suggests that feed additives offer sustainable and antibiotic-free options to improve layer chicken health and productivity, aligning with current consumer and regulatory demands.

**Keywords: Feed additives, Probiotics, Prebiotics, Gut health, Layer productivity**

## Introduction

The poultry industry plays a critical role in global food production, with layer chickens being fundamental to the supply of eggs, a staple source of affordable protein worldwide. As consumer demand for high-quality, nutritious eggs rises, producers face pressures to maintain productivity and flock health while minimizing environmental impact and adhering to consumer preferences for antibiotic-free products (**Haque *et al.*, 2020**). Traditionally, antibiotics have been used to support growth and health in poultry; however, growing concerns about antibiotic resistance and stricter regulations have led to a search for sustainable alternatives. In this context, feed additives have emerged as a promising solution to support the health and productivity of layer chickens without the need for antibiotics (**Bist *et al.*, 2024**).

Recent research has identified various categories of feed additives such as probiotics, prebiotics, enzymes, organic acids, and herbal extracts that positively impact layer chicken performance by enhancing gut health, immune function, and nutrient absorption. Probiotics, for instance, have been shown to improve gut microbiota composition, which in turn boosts nutrient absorption and immune responses (**Rastogi and Singh, 2022**). A recent study using *Lactobacillus reuteri* in layer feed demonstrated improved intestinal health and a 5-10% increase in egg production rates, highlighting the benefits of probiotics as a viable alternative to antibiotics. Similarly, prebiotics such as mannan oligosaccharides have been found to selectively stimulate beneficial gut bacteria, reducing pathogen loads and enhancing nutrient uptake. This combination of improved digestion and immune support contributes to better feed efficiency and egg quality, addressing both health and productivity goals (**Waqas et al., 2024**). In addition to probiotics and prebiotics, enzyme additives, especially those targeting non-starch polysaccharides, have gained attention for their ability to improve nutrient bioavailability by breaking down otherwise indigestible components in feed (**Ibrahim et al., 2023**). Recent studies indicate that enzyme supplementation can lead to a 3-8% increase in feed efficiency and a corresponding improvement in egg production, as more nutrients become available for absorption. Organic acids, another additive category, help regulate gut pH, creating an environment less favorable to pathogens while supporting beneficial bacteria. Studies show that adding citric acid and lactic acid to layer diets can enhance gut health, boost feed conversion, and increase overall production performance (**Bedford and Apajalahti, 2022**).

Herbal extracts and phytogenics, such as oregano and turmeric, are also being explored for their antioxidant and antimicrobial properties. New findings suggest that these natural additives not only support immune function but also improve egg quality, with turmeric-supplemented diets leading to enhanced yolk color and egg shell strength in some trials. These additives align well with consumer demands for natural products and offer a promising route to enhancing bird health and product quality (**Jasim et al., 2024**). As more research emerges on the specific mechanisms and optimal combinations of feed additives, the potential for achieving healthier, more productive flocks through natural means continues to grow, offering a path forward for antibiotic-free, sustainable poultry production (**Placha et al., 2022**).

## **Probiotics**

Probiotics are gaining a lot of interest as feed additives to improve layer chicken production and health. These beneficial microorganisms, commonly including *Lactobacillus*, *Bifidobacterium*, and *Bacillus* strains, positively influence the gut microbiota, improving nutrient absorption, immune function, and overall digestive health (Jha *et al.*, 2020). Recent research highlights that probiotics not only support the balance of gut bacteria but also reduce pathogenic bacterial colonization, leading to improved feed conversion ratios, egg production, and egg quality (Krysiak *et al.*, 2021). Studies have demonstrated that hens fed with probiotic-enriched diets show increased egg weight and shell strength, likely due to enhanced nutrient uptake and better gastrointestinal health (Nour *et al.*, 2021). According to Krawczyk *et al.* (2021) the most popular strains are represented by the following genera: *Lactobacillus*, *Streptococcus*, and *Bifidobacterium*, but other organisms including enterococci and yeasts have also been used as probiotics. Medina *et al.* (2008) evaluated the ability of different strains of *Bifidobacterium longum* to induce cytokine production by peripheral blood mononuclear cells. *B. longum* live cells of all strains induced specific cytokine patterns, suggesting that they could drive immune responses in different directions. *L. casei ssp. rhamnosus* has shown to be a promising probiotic in preventing the colonization of the gastrointestinal tract by pathogenic bacteria such as enteropathogenic *E. coli*, enterotoxigenic *E. coli*, and *Klebsiella pneumoniae* using in vitro model with Caco-2 cell line (Qin *et al.*, 2022). Probiotics also have been associated with decreased mortality rates, suggesting a role in boosting immune resilience (Garcias *et al.*, 2024). Overall, by improving layer chicken performance and health outcomes, the addition of probiotics to diets for poultry presents a viable strategy for sustainable production.

### **Prebiotics**

Prebiotics, such as fructo-oligosaccharides, inulin, and mannan-oligosaccharides, are increasingly used as feed additives in layer chicken diets to improve gut health and production outcomes. A polymer of fructose molecules joined by beta (2–1) glycosidic bonds is called inulin. It is categorized as a fructans, meaning that fructose units with a terminal glucose molecule make up its structure. Inulin chains come in different lengths; shorter chains are called oligofructans, and longer chains are called polysaccharides. It evades digestion in the upper gastrointestinal tract and enters the colon undigested, inulin is regarded as a prebiotic. There, it acts as a substrate for fermentation by beneficial bacteria (Wan *et al.*, 2020). Fructooligosaccharides (FOS) is made up of short chains of fructose molecules with a glucose

molecule at one end. They are present in some fruits, vegetables, and grains in their natural form. FOS has been demonstrated to have a bifidogenic effect, which means that they specifically encourage the growth of gut-dwelling bifidobacteria. By increasing stool frequency and improving stool consistency, this can help control bowel movements and relieve constipation symptoms (**Costa et al., 2022**). These non-digestible dietary fibers stimulate the growth of beneficial gut bacteria, thereby enhancing intestinal health, nutrient absorption, and immune response (**Jahan et al., 2022**). Recent studies suggest that the inclusion of prebiotics in poultry feed can lead to better egg production, increased egg weight, and enhanced shell quality due to optimized digestive function and improved gut morphology (**Xu et al., 2023**). Prebiotics are described as diverse fermentation component that alter the activity or composition of the gut microbiota to the host's advantage (**Rossen et al., 2015**). By supporting a balanced gut microbiota, prebiotics reduce pathogen load and decrease the need for antibiotic use, promoting a more sustainable and health-conscious approach to poultry management. Prebiotics also associated with a lower mortality rate and enhanced resistance to stress, making them a valuable component in the nutrition of layer chickens aiming for enhanced productivity and health (**Alagawany et al., 2021**).

### **Organic Acids**

Organic acids, such as formic, acetic, lactic, and citric acids, are valuable feed additives in layer chicken diets, known for their ability to improve gut health, feed efficiency, and egg production (**Abbas et al., 2022**). Organic acid addition composed of individual organic acids and blends of several acids have been found to execute antimicrobial actions similar to those of antibiotics (**Khan et al., 2022**). The European Union (EU) allowed the usage of organic acids and their salts in poultry because these are generally considered harmless (**Ben et al., 2021**). Commercial feeds have been using organic acids for decades, mostly for feed preservation, where propionic and formic acids work especially effective (**Tugnoli et al., 2021**). Acidification of drinking water is now another technique employed in the poultry business to enhance performance. According to previous research, adding organic acid to drinking water can help lower the amount of pathogens in the water that control gut microbiota, enhance feed digestion, and boost growth performance (**Pearlin et al., 2020**). According to **Abdel and Emam, (2020)**, adding 0.5% organic acids (lactic, acetic, or formic acid) to drinking water during pre-transport feed withdrawal might lessen the amount of Salmonella and Campylobacter that contaminate

carcasses during processing. They proposed that the lactic acid in drinking water lowers pH and might serve as a short-term carbon source for good bacteria. Recent studies indicate that organic acids enhance nutrient digestibility and absorption, leading to improved feed conversion ratios and higher egg production rates in layers (**Kim *et al.*, 2021**). They have also been shown to strengthen egg quality, increasing shell thickness and strength, likely due to enhanced mineral absorption. By supporting intestinal health and reducing pathogen loads, organic acids reduce the need for antibiotics, promoting a more sustainable poultry production model (**Choi *et al.*, 2023**).

### **Enzymes**

Enzymes are widely used as feed additives in layer chicken diets to improve nutrient bioavailability and feed efficiency (**Zampiga *et al.*, 2021**). Common important enzymes like phytase, xylanase, and protease help break down complex feed ingredients, enhancing the digestibility of proteins, starches, and minerals (**Alagawany *et al.*, 2018**). Recent research highlights that adding enzymes to poultry feed improves nutrient absorption, leading to higher egg production and better feed conversion ratios (**Singh *et al.*, 2024**). Phytase, for example, breaks down phytate-bound phosphorus, making it accessible for absorption and reducing phosphorus excretion into the environment (**Hussain *et al.*, 2022**). Xylanase and protease assist in breaking down non-starch polysaccharides and proteins, respectively, reducing digestive stress and energy loss (**Lewko *et al.*, 2022**). Studies also show that enzymes contribute to better eggshell quality by increasing mineral bioavailability (**Elnesr *et al.*, 2024**). Overall, enzyme supplementation promotes sustainable and efficient egg production by maximizing nutrient use, lowering feed costs, and reducing waste, benefiting both poultry health and environmental sustainability.

### **Phytogenics (Plant Extracts)**

Phytogenics, including essential oils, herbs, and plant extracts such as oregano, thyme, and garlic, are increasingly used as natural feed additives to enhance layer chicken health and productivity (**Verma *et al.*, 2020**; **Vlaicu *et al.*, 2023**). Known for their antimicrobial, antioxidant, and anti-inflammatory properties, phytogenics help support a balanced gut microbiota and reduce the colonization of harmful pathogens (**Saleh *et al.*, 2018**). **Fascina *et al.* (2012)** report that by using phytogenic additives in form of turmeric extract, citrus extract, and grape seed extract with Chinese cinnamon essential oil, Chile Boldo leaves, and fenugreek seed phytogenic additives probably due to the effects of cinnamaldehyde and turmeric, the main active

ingredients in cinnamon and curcumin, pancreatic and intestinal enzyme secretion is stimulated and concurrently, production of bile, bile salts, pancreatic and intestinal lipase is increased, leading to more effective nutrient absorption. In a study, **Hong et al. (2012)** used the addition of 125 ppm of essential oil from oregano, anise, and citrus peel powder. The authors reported that essential oil supplementation improved the survival rate by approximately 10%; serum cholesterol levels were reduced, very low density lipoprotein levels decreased, total polyphenolic compounds and total flavonoids increased; breast muscles were more tenderer and thigh muscles were juicier for birds in the essential oil group as compared to the control group. Recent researches also shows that adding phytogetic compounds to layer diets improves feed intake, nutrient absorption, and overall egg production (**Rawat et al., 2020; Khukhodziinai et al., 2023**).

### **Mineral Supplements**

Mineral supplements, including essential elements like calcium, phosphorus, selenium, zinc, and magnesium, are crucial feed additives in layer chicken diets to support bone health, eggshell quality, and immune function (**Elnesr et al., 2024**). Calcium and phosphorus are especially important for eggshell formation and skeletal strength, while trace minerals like selenium and zinc play key roles in antioxidant defense and metabolic processes (**Medeiros et al., 2023**). Recent studies demonstrate that optimized mineral supplementation improves eggshell thickness, reduces breakage, and enhances egg production efficiency (**Liu et al., 2023**). Adequate mineral intake has also been linked to improved immune response and resistance to stressors, contributing to lower mortality rates and better overall health. **Byrne et al. (2023)** using Cu, Fe, Mn, and Zn in study and found hen-day production was greater by +2.07% and FCR were lowered by 51.28 g feed/kg egg and 22.82 g feed/dozen eggs, respectively. Positive impacts were also observed on egg quality traits, with egg mass greater by 0.50 g/hen/day and egg weight higher by 0.48 g per egg, on average. According to study of **Ghasemi et al. (2022)** advanced mineral formulations, such as organic chelates, are shown to improve bioavailability, further supporting layer productivity and health. By addressing mineral deficiencies, these supplements not only increase production performance but also promote welfare, making them essential for sustainable poultry management practices (**Biabani et al., 2024**).

**Table 1: The roles of essential minerals as feed additives in layer diets**

S. No.	Minerals	Health importance	Reference
1.	Calcium (Ca)	Vital for eggshell formation and skeletal strength; deficiencies lead to weak bones and poor shell quality	<b>Singh <i>et al.</i>, 2021</b>
2.	Phosphorus (P)	Works with calcium for bone and eggshell development; essential for energy metabolism	<b>Li <i>et al.</i>, 2017</b>
3.	Magnesium (Mg)	Supports enzyme function, bone health, and energy metabolism	<b>Belkameh <i>et al.</i>, 2021</b>
4.	Sodium (Na)	Essential for fluid balance, nerve function, and nutrient transport	<b>Bernal <i>et al.</i>, 2023</b>
5.	Potassium (K)	Helps maintain electrolyte balance, nerve function, and muscle contraction	<b>Tomaszewska <i>et al.</i>, 2020</b>
6.	Chlorine (Cl)	Works with sodium and potassium to maintain osmotic balance and pH	<b>Martínez <i>et al.</i>, 2021</b>
7.	Zinc (Zn)	Important for immune function, feathering, skin health, and reproduction; supports enzyme activity	<b>Huang <i>et al.</i>, 2019</b>
8.	Iron (Fe)	Necessary for hemoglobin formation and oxygen transport in blood	<b>Tan <i>et al.</i>, 2021</b>
9.	Copper (Cu)	Supports immune function, iron metabolism, and bone health; also aids in pigmentation of feathers	<b>Sharif <i>et al.</i>, 2021</b>
10.	Manganese (Mn)	Crucial for bone formation, eggshell quality, and enzyme activation	<b>Zhang <i>et al.</i>, 2022</b>
11.	Selenium (Se)	Acts as an antioxidant, protecting cells from oxidative damage; supports immune function	<b>Zheng <i>et al.</i>, 2022</b>
12.	Iodine (I)	Required for thyroid hormone production, supporting metabolism and growth	<b>Soliman <i>et al.</i>, 2018</b>

## Vitamins

Vitamins play a crucial role as feed additives in enhancing the health and productivity of layer chickens. Key vitamins like A, D, E, and B-complex supports various metabolic functions, boost immunity, and improve egg production and quality (**Das *et al.*, 2021**). For instance, vitamin D is essential for calcium absorption, ensuring strong eggshells and bone health, while vitamins E and C reduce stress and oxidative damage, particularly under high-production or stressful conditions (**Hafez and Attia, 2020**). Recent Studies suggest that optimal vitamin supplementation tailored to a layer's life stage and environmental conditions improves not only egg yield but also supports longevity and resilience against disease (**El-Sabroun *et al.*, 2022**). Additionally, enhanced levels of vitamin B complex facilitate efficient nutrient metabolism, which contributes to energy balance and sustained productivity in high-yield breeds (**Gaikwad *et***

*al., 2020*). This balanced vitamin approach has become essential for modern poultry operations focused on optimizing performance and economic efficiency (**Bist *et al.*, 2024**).

**Table 2: The roles of essential vitamins as feed additives in layer diets**

S. No.	Vitamins	Health importance	Reference
1.	Vitamin A	Supports vision, skin health, and immune function; crucial for reproductive health and egg quality	<b>Beer <i>et al.</i>, 2024</b>
2.	Vitamin D	Facilitates calcium and phosphorus absorption, essential for strong eggshells and bone health	<b>Liu <i>et al.</i>, 2023</b>
3.	Vitamin E	Acts as an antioxidant, protecting cells from oxidative damage; enhances immunity and stress resistance	<b>Meydani <i>et al.</i>, 2020</b>
4.	Vitamin K	Important for blood clotting and bone health, helps reduce bleeding risks	<b>Halder <i>et al.</i>, 2019</b>
5.	Vitamin B1	Supports nerve function and energy metabolism, ensuring efficient nutrient use	<b>Tardy <i>et al.</i>, 2023</b>
6.	Vitamin B2	Vital for growth and egg production, assists in metabolizing fats, proteins, and carbohydrates	<b>Pathan <i>et al.</i>, 2023</b>
7.	Vitamin B6	Plays a role in protein metabolism and aids in immune function	<b>Aslam <i>et al.</i>, 2017</b>
8.	Vitamin B12	Supports red blood cell formation, energy metabolism, and egg production	<b>Ahmad <i>et al.</i>, 2019</b>
9.	Vitamin B3	Essential for energy production and metabolic health; prevents skin lesions and improves feather condition	<b>Wahab <i>et al.</i>, 2024</b>
10.	Pantothenic Acid	Important for energy metabolism and stress management	<b>Wang <i>et al.</i>, 2024</b>
11.	Vitamin C	Enhances immunity, acts as an antioxidant, and helps manage stress	<b>Shakeri <i>et al.</i>, 2020</b>

### **Amino Acids**

Amino acids serve critical roles as feed additives in layer poultry nutrition, enhancing production and health. Methionine, for instance, is vital for optimal egg production, feather development, and overall growth, particularly under stressful conditions like high temperatures (**Reda *et al.*, 2020**). Supplementing methionine has shown improvements in egg mass, egg quality, and feed efficiency, while reducing oxidative stress and enhancing immunity (**Vandana *et al.*, 2021**). Lysine, another essential amino acid, supports protein synthesis and boosts egg mass and egg weight, contributing to efficient feed conversion rates (**Macelline *et al.*, 2021**). Threonine aids in gut health by supporting mucosal protein synthesis, which fortifies the gut against pathogens (**Tang *et al.*, 2021**). Arginine and tryptophan are also crucial, with roles in

immune response, reproductive health, and stress reduction, improving egg quality under environmental stressors (Lee *et al.*, 2023). Modern amino acid recommendations are based on ideal protein profiles to ensure balanced nutrition that optimizes production outcomes in laying hens.

**Table 3: The roles of essential amino acids as feed additives in layer diets**

S. No.	Amino Acids	Health importance	Reference
1.	Methionine	Aids in feather development, egg production, and protein synthesis; acts as an antioxidant and supports liver health	Reda <i>et al.</i> , 2020
2.	Lysine	Critical for muscle growth, egg size, and overall protein synthesis; helps with calcium absorption for strong bones and eggshells	Ma <i>et al.</i> , 2021
3.	Threonine	Supports immune function, digestive health, and overall protein balance; important for mucus production in the gut	Wu <i>et al.</i> , 2021
4.	Tryptophan	Helps in serotonin production, which supports stress reduction and improved feed intake; essential for growth and immunity	Lu <i>et al.</i> , 2024
5.	Arginine	Key for growth, immune function, and reproductive health; essential for nitric oxide production, which aids blood flow	Kulshreshtha <i>et al.</i> , 2020
6.	Valine	Important for muscle protein synthesis, feather development, and overall growth	Liu <i>et al.</i> , 2024
7.	Isoleucine	Supports muscle development, feather formation, and immune health	Uyanga <i>et al.</i> , 2022
8.	Leucine	Critical for protein synthesis, muscle growth, and repair; helps regulate blood glucose levels	Niu <i>et al.</i> , 2021
9.	Histidine	Essential for growth and tissue repair; involved in hemoglobin formation and immune responses	Moro <i>et al.</i> , 2020
10.	Phenylalanine	Supports protein synthesis, feather pigmentation, and is a precursor for the hormone dopamine, influencing mood and stress resilience	Fuchs <i>et al.</i> , 2018

## Conclusion

In conclusion, feed additives are essential for maximising the welfare, productivity, and health of layer hens. Minerals, vitamins, organic acids, enzymes, phytogenics, probiotics, prebiotics, and amino acids are a few examples of additives that greatly enhance gut health, immunological response, and nutritional utilisation. Recent studies has shown that strategic supplementation enhances egg quality, boosts resistance to disease, and supports layers in stressful conditions, aligning well with the industry's shift toward sustainable, antibiotic-free production. Use of feed

additive, creating more targeted and efficient solutions for the evolving demands of poultry health and productivity.

## Reference

1. Abbas, G., Arshad, M., Saeed, M., Imran, S., Kamboh, A.A., Al-Taey, D.K., Aslam, M.A., Imran, M.S., Ashraf, M., Asif, M. and Tanveer, A.J., 2022. An update on the promising role of organic acids in broiler and layer production. *Journal of Animal Health and Production 2022a*, 10(3), pp.273-286.
2. Abdel-Kader, I.A. and Emam, A., 2020. Testing two levels of lactic and citric acids as growth promoters in rabbits diets. *Egyptian Journal of Nutrition and Feeds*, 23(3), pp.435-444.
3. Ahmad, Z., Xie, M., Wu, Y. and Hou, S., 2019. Effect of supplemental cyanocobalamin on the growth performance and hematological indicators of the white Pekin ducks from hatch to day 21. *Animals*, 9(9), p.633.
4. Alagawany, M., Elnesr, S.S. and Farag, M.R., 2018. The role of exogenous enzymes in promoting growth and improving nutrient digestibility in poultry. *Iranian journal of veterinary research*, 19(3), p.157.
5. Alagawany, M., Elnesr, S.S., Farag, M.R., Abd El-Hack, M.E., Barkat, R.A., Gabr, A.A., Foda, M.A., Noreldin, A.E., Khafaga, A.F., El-Sabrou, K. and Elwan, H.A., 2021. Potential role of important nutraceuticals in poultry performance and health-A comprehensive review. *Research in veterinary science*, 137, pp.9-29.
6. Aslam, F., Muhammad, S.M., Aslam, S. and Irfan, J.A., 2017. Vitamins: key role players in boosting up immune response-a mini review. *Vitamins & Minerals*, 6(01).
7. Bedford, M.R. and Apajalahti, J.H., 2022. The role of feed enzymes in maintaining poultry intestinal health. *Journal of the Science of Food and Agriculture*, 102(5), pp.1759-1770.
8. Beer, K., Singh, A., Ravi, S.C., Gupta, A.K., Kumar, A. and Sharma, M.M., 2024. A comprehensive review on the role of Vitamin A on human health and nutrition. *nutrition*, 45, pp.645-653.
9. Belkameh, M.M., Sedghi, M. and Azarfar, A., 2021. The effect of different levels of dietary magnesium on eggshell quality and laying hen's performance. *Biological Trace Element Research*, 199(4), pp.1566-1573.
10. Ben Braïek., Olfa and Smaoui, S., 2021. Chemistry, safety, and challenges of the use of organic acids and their derivative salts in meat preservation. *Journal of Food Quality*, 2021(1), p.6653190.
11. Bernal, A., Zafra, M.A., Simón, M.J. and Mahía, J., 2023. *Sodium Homeostasis, a Balance Necessary for Life. Nutrients*. 2023; 15: 395
12. Biabani, N., Taherpour, K., Ghasemi, H.A., Gharaei, M.A., Hafizi, M. and Nazaran, M.H., 2024. Dietary advanced chelate technology-based 7-mineral supplement improves growth performance and intestinal health indicators during a mixed Eimeria challenge in broiler chickens. *Veterinary Parasitology*, 331, p.110277.

13. Bist, R.B., Bist, K., Poudel, S., Subedi, D., Yang, X., Paneru, B., Mani, S., Wang, D. and Chai, L., 2024. Sustainable poultry farming practices: A critical review of current strategies and future prospects. *Poultry Science*, p.104295.
14. Byrne, L., Ross, S., Taylor-Pickard, J. and Murphy, R., 2023. The Effect of Organic Trace Mineral Supplementation in the Form of Proteinates on Performance and Sustainability Parameters in Laying Hens: A Meta-Analysis. *Animals*, 13(19), p.3132.
15. Choi, J., Kong, B., Bowker, B.C., Zhuang, H. and Kim, W.K., 2023. Nutritional strategies to improve meat quality and composition in the challenging conditions of broiler production: a review. *Animals*, 13(8), p.1386.
16. Costa, G. T., Vasconcelos, Q. D. J. S., & Aragão, G. F. (2022). Fructooligosaccharides on inflammation, immunomodulation, oxidative stress, and gut immune response: A systematic review. *Nutrition Reviews*, 80(4), 709–722.
17. Das, R., Mishra, P. and Jha, R., 2021. In ovo feeding as a tool for improving performance and gut health of poultry: a review. *Frontiers in Veterinary Science*, 8, p.754246.
18. Elnesr, S.S., Mahmoud, B.Y., da Silva Pires, P.G., Moraes, P., Elwan, H.A., El-Shall, N.A., El-Kholy, M.S. and Alagawany, M., 2024. Trace minerals in laying hen diets and their effects on egg quality. *Biological Trace Element Research*, pp.1-16.
19. El-Sabrou, K., Aggag, S. and Mishra, B., 2022. Advanced practical strategies to enhance table egg production. *Scientifica*, 2022(1), p.1393392.
20. Fascina V.B., Sartori J.R., Gonzales E., de Carvalho F.B., de Souza I.M.G.P., Polycarpo G.D., Stradiotti A.C., Pelicia V.C. 2012. Phytogenic additives and organic acids in broiler chicken diets. *Revista Brasileira de Zootecnia*, 41, 2189–2197.
21. Fuchs, D., Gostner, J.M., Griesmacher, A., Melichar, B., Reibnegger, G., Weiss, G. and Werner, E.R., 2018. 37th International Winter-Workshop Clinical, Chemical and Biochemical Aspects of Pteridines and Related Topics. *Pteridines*, 29(1), pp.42-69.
22. Gaikwad, K.B., Rani, S., Kumar, M., Gupta, V., Babu, P.H., Bainsla, N.K. and Yadav, R., 2020. Enhancing the nutritional quality of major food crops through conventional and genomics-assisted breeding. *Frontiers in Nutrition*, 7, p.533453.
23. Garcias-Bonet, N., Roik, A., Tierney, B., García, F.C., Villela, H.D., Dungan, A.M., Quigley, K.M., Sweet, M., Berg, G., Gram, L. and Bourne, D.G., 2024. Horizon scanning the application of probiotics for wildlife. *Trends in Microbiology*, 32(3), pp.252-269.
24. Ghasemi, H.A., Hajkhodadadi, I., Hafizi, M., Fakharzadeh, S., Abbasi, M., Kalanaky, S. and Nazaran, M.H., 2022. Effect of advanced chelate compounds-based mineral supplement in laying hen diet on the performance, egg quality, yolk mineral content, fatty acid composition, and oxidative status. *Food Chemistry*, 366, p.130636.
25. Hafez, H.M. and Attia, Y.A., 2020. Challenges to the poultry industry: current perspectives and strategic future after the COVID-19 outbreak. *Frontiers in veterinary science*, 7, p.516.
26. Halder, M., Petsophonsakul, P., Akbulut, A.C., Pavlic, A., Bohan, F., Anderson, E., Maresz, K., Kramann, R. and Schurgers, L., 2019. Vitamin K: double bonds beyond

coagulation insights into differences between vitamin K1 and K2 in health and disease. *International journal of molecular sciences*, 20(4), p.896.

27. Haque, Md Hakimul, Subir Sarker, Md Shariful Islam, Md Aminul Islam, Md Rezaul Karim, Mohammad Enamul Hoque Kayesh, Muhammad JA Shiddiky, and M. Sawkat Anwer. "Sustainable antibiotic-free broiler meat production: Current trends, challenges, and possibilities in a developing country perspective." *Biology* 9, no. 11 (2020): 411.
28. Hong J.C., Steiner T., Aufy A., Lien T.F. 2012. Effects of supplemental essential oil on growth performance, lipid metabolites and immunity, intestinal characteristics, microbiota and carcass traits in broilers. *Livestock Science*, 144, 253–262.
29. Huang, L., Li, X., Wang, W., Yang, L. and Zhu, Y., 2019. The role of zinc in poultry breeder and hen nutrition: an update. *Biological Trace Element Research*, 192, pp.308-318.
30. Hussain, S.M., Hanif, S., Sharif, A., Bashir, F. and Iqbal, H.M., 2022. Unrevealing the sources and catalytic functions of phytase with multipurpose characteristics. *Catalysis Letters*, pp.1-14.
31. Ibrahim, D., El-Sayed, H.I., Mahmoud, E.R., El-Rahman, G.I.A., Bazeed, S.M., Abdelwarith, A.A., Elgamal, A., Khalil, S.S., Younis, E.M., Kishawy, A.T. and Davies, S.J., 2023. Impacts of solid-state fermented barley with fibrolytic exogenous enzymes on feed utilization, and antioxidant status of broiler chickens. *Veterinary Sciences*, 10(10), p.594.
32. Jahan, A. A., González Ortiz, G., Moss, A. F., Bhuiyan, M. M., and Morgan, N. K. (2022). Role of supplemental oligosaccharides in poultry diets. *World's Poultry Science Journal*, 78(3), 615-639.
33. Jasim, S.A., Al-Dhalimy, A.M.B., Zokaei, M., Salimi, S., Alnajjar, M.J., Kumar, A., Alwaily, E.R., Zwamel, A.H., Hussein, S.A. and Gholami-Ahangaran, M., 2024. The Beneficial Application of Turmeric (*Curcuma longa* L.) on Health and Egg Production, in Layers: A Review. *Veterinary Medicine and Science*, 10(6), p.e70115.
34. Jha, R., Das, R., Oak, S., and Mishra, P. (2020). Probiotics (direct-fed microbials) in poultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: A systematic review. *Animals*, 10(10), 1863.
35. Khan, R.U., Naz, S., Raziq, F., Qudratullah, Q., Khan, N.A., Laudadio, V., Tufarelli, V. and Ragni, M., 2022. Prospects of organic acids as safe alternative to antibiotics in broiler chickens diet. *Environmental Science and Pollution Research*, 29(22), pp.32594-32604.
36. Khukhodziinai, J.S., Das, P.K., Mukherjee, J., Banerjee, D., Ghosh, P.R., Das, A.K., Samanta, I., Jas, R., Mondal, S. and Patra, A.K., 2024. Effect of Dietary Benzoic Acid and Oregano Essential Oil as a Substitute for an Anti-Coccidial Agent on Growth Performance and Physiological and Immunological Responses in Broiler Chickens Challenged with *Eimeria* Species. *Animals*, 14(20), p.3008.

37. Kim, Y.B., Lee, S.H., Kim, D.H., Lee, H.G., Choi, Y., Lee, S.D. and Lee, K.W., 2021. Effects of dietary organic and inorganic sulfur on laying performance, egg quality, ileal morphology, and antioxidant capacity in laying hens. *Animals*, 12(1), p.87.
38. Krawczyk, B., Wityk, P., Gałęcka, M., and Michalik, M. (2021). The many faces of *Enterococcus* spp.—commensal, probiotic and opportunistic pathogen. *Microorganisms*, 9(9), 1900.
39. Krysiak, K., Konkol, D., and Korczyński, M. (2021). Overview of the use of probiotics in poultry production. *Animals*, 11(6), 1620.
40. Kulshreshtha, G., D'alba, L., Dunn, I.C., Rehault-Godbert, S., Rodriguez-Navarro, A.B. and Hincke, M.T., 2022. Properties, genetics and innate immune function of the cuticle in egg-laying species. *Frontiers in Immunology*, 13, p.838525.
41. Lee, J.T., Rochell, S.J., Kriseldi, R., Kim, W.K. and Mitchell, R.D., 2023. Functional properties of amino acids: Improve health status and sustainability. *Poultry Science*, 102(1), p.102288.
42. Lewko, P., Wójtowicz, A. and Róžańska-Boczula, M., 2024. Effect of Extruder Configuration and Extrusion Cooking Processing Parameters on Selected Characteristics of Non-Starch Polysaccharide-Rich Wheat Flour as Hybrid Treatment with Xylanase Addition. *Processes*, 12(6), p.1159.
43. Li, X., Zhang, D. and Bryden, W.L., 2017. Calcium and phosphorus metabolism and nutrition of poultry: are current diets formulated in excess?. *Animal Production Science*, 57(11), pp.2304-2310.
44. Liu, L., Zhang, G., Qu, G., Liu, B., Zhang, X., Li, G., Jin, N., Li, C., Bai, J. and Zhao, C., 2023. Effects of dietary *Lactobacillus rhamnosus* GG supplementation on the production performance, egg quality, eggshell ultrastructure, and lipid metabolism of late-phase laying hens. *BMC Veterinary Research*, 19(1), p.150.
45. Liu, M., Xia, Z.Y., Li, H.L., Huang, Y.X., Refaie, A., Deng, Z.C. and Sun, L.H., 2024. Estimation of Protein and Amino Acid Requirements in Layer Chicks Depending on Dynamic Model. *Animals*, 14(5), p.764.
46. Liu, W., Tang, C., Cai, Z., Jin, Y., Ahn, D.U. and Huang, X., 2023. The effectiveness of polypeptides from phosvitin and eggshell membrane in enhancing the bioavailability of eggshell powder calcium and its accumulation in bones. *Food Bioscience*, 51, p.102257.
47. Lu, C., Deng, Y., Ma, W., Wang, W., Li, P., Shi, P., Yan, T., Yin, Y. and Huang, P., 2024. Tryptophan Nutrition in Poultry and Ruminant Animals. *Tryptophan in Animal Nutrition and Human Health*, pp.127-157.
48. Ma, M., Geng, S., Liu, M., Zhao, L., Zhang, J., Huang, S. and Ma, Q., 2021. Effects of different methionine levels in low protein diets on production performance, reproductive system, metabolism, and gut microbiota in laying hens. *Frontiers in Nutrition*, 8, p.739676.

49. Macelline, S.P., Toghyani, M., Chrystal, P.V., Selle, P.H. and Liu, S.Y., 2021. Amino acid requirements for laying hens: a comprehensive review. *Poultry Science*, 100(5), p.101036.
50. Martínez, Y., Almendares, C.I., Hernández, C.J., Avellaneda, M.C., Urquía, A.M. and Valdivié, M., 2021. Effect of acetic acid and sodium bicarbonate supplemented to drinking water on water quality, growth performance, organ weights, cecal traits and hematological parameters of young broilers. *Animals*, 11(7), p.1865.
51. Medeiros-Ventura, W.R., Rabello, C.B., Santos, M.J., Barros, M.R., Silva Junior, R.V., Oliveira, H.B., Costa, F.S., Faria, A.G. and Fireman, A.K., 2023. The impact of phytase and different levels of supplemental amino acid complexed minerals in diets of older laying hens. *Animals*, 13(23), p.3709.
52. Medina, M., De Palma, G., Ribes-Koninckx, C., Calabuig, M., & Sanz, Y. (2008). Bifidobacterium strains suppress in vitro the pro-inflammatory milieu triggered by the large intestinal microbiota of coeliac patients. *Journal of Inflammation*, 5, 1-13.
53. Meydani, S.N. and Blumberg, J.B., 2020. Vitamin E and the immune response. In *Nutrient modulation of the immune response* (pp. 223-238). CRC Press.
54. Moro, J., Tomé, D., Schmidely, P., Demersay, T.C. and Azzout-Marniche, D., 2020. Histidine: A systematic review on metabolism and physiological effects in human and different animal species. *Nutrients*, 12(5), p.1414.
55. Niu, X., Qian, X., Feng, H., Yi, K., Li, D., Chen, W. and Ye, J., 2021. Growth and metabolic responses of grouper juveniles (*Epinephelus coioides*) fed diets containing varying levels of leucine. *Aquaculture*, 534, p.736281.
56. Nour, M.A., El-Hindawy, M.M., Abou-Kassem, D.E., Ashour, E.A., Abd El-Hack, M.E., Mahgoub, S., Aboelenin, S.M., Soliman, M.M., El-Tarabily, K.A. and Abdel-Moneim, A.M.E., 2021. Productive performance, fertility and hatchability, blood indices and gut microbial load in laying quails as affected by two types of probiotic bacteria. *Saudi journal of biological sciences*, 28(11), pp.6544-6555.
57. Pathan, A.S., Jain, P.G., Mahajan, A.B., Kumawat, V.S., Ahire, E.D., Surana, K.R., Rajora, A.K. and Rajora, M.A.K., 2023. Beneficial Effects of Water-Soluble Vitamins in Nutrition and Health Promotion. *Vitamins as Nutraceuticals: Recent Advances and Applications*, pp.235-251.
58. Pearlin, B.V., Muthuvel, S., Govidasamy, P., Villavan, M., Alagawany, M., Ragab Farag, M., Dhama, K. and Gopi, M., 2020. Role of acidifiers in livestock nutrition and health: A review. *Journal of animal physiology and animal nutrition*, 104(2), pp.558-569.
59. Placha, I., Gai, F. and Pogány Simonová, M., 2022. Natural feed additives in animal nutrition—Their potential as functional feed. *Frontiers in Veterinary Science*, 9, p.1062724.
60. Qin, D., Ma, Y., Wang, Y., Hou, X., and Yu, L. (2022). Contribution of Lactobacilli on intestinal mucosal barrier and diseases: perspectives and challenges of *Lactobacillus casei*. *Life*, 12(11), 1910.

61. Rastogi, S., & Singh, A. (2022). Gut microbiome and human health: Exploring how the probiotic genus *Lactobacillus* modulate immune responses. *Frontiers in Pharmacology*, *13*, 1042189.
62. Rawat, P., Ahmad, A. H., Pant, D., Verma, M. K., & Bisht, P. (2020). Evaluation of anti-osteoporotic potential of *Moringa olifera* Leaves in rats. *Journal Of Veterinary Pharmacology And Toxicology*, *19*(2), 25-28.
63. Reda, F.M., Swelum, A.A., Hussein, E.O., Elnesr, S.S., Alhimaidi, A.R. and Alagawany, M., 2020. Effects of varying dietary DL-methionine levels on productive and reproductive performance, egg quality, and blood biochemical parameters of quail breeders. *Animals*, *10*(10), p.1839.
64. Saleh, A.A., Ebeid, T.A. and Abudabos, A.M., 2018. Effect of dietary phytochemicals (herbal mixture) supplementation on growth performance, nutrient utilization, antioxidative properties, and immune response in broilers. *Environmental Science and Pollution Research*, *25*, pp.14606-14613.
65. Shakeri, M., Oskoueian, E., Le, H.H. and Shakeri, M., 2020. Strategies to combat heat stress in broiler chickens: Unveiling the roles of selenium, vitamin E and vitamin C. *Veterinary sciences*, *7*(2), p.71.
66. Sharif, M., Rahman, M.A.U., Ahmed, B., Abbas, R.Z. and Hassan, F.U., 2021. Copper nanoparticles as growth promoter, antioxidant and anti-bacterial agents in poultry nutrition: prospects and future implications. *Biological trace element research*, *199*(10), pp.3825-3836.
67. Singh, A., Kelkar, N., Natarajan, K. and Selvaraj, S., 2021. Review on the extraction of calcium supplements from eggshells to combat waste generation and chronic calcium deficiency. *Environmental Science and Pollution Research*, *28*, pp.46985-46998.
68. Singh, B., Pragya, Tiwari, S.K., Singh, D., Kumar, S. and Malik, V., 2024. Production of fungal phytases in solid state fermentation and potential biotechnological applications. *World Journal of Microbiology and Biotechnology*, *40*(1), p.22.
69. Soliman, M.M., Hassaan, S.F., El-Halim, A. and Osman, S., 2018. Role of dietary iodine on modulating productive, reproductive, physiological and immunological performance for local chickens 2-during laying period. *Egyptian Poultry Science Journal*, *38*(1), pp.91-101.
70. Tan, Z., Lu, P., Adewole, D., Diarra, M.S., Gong, J. and Yang, C., 2021. Iron requirement in the infection of *Salmonella* and its relevance to poultry health. *Journal of Applied Poultry Research*, *30*(1), p.100101.
71. Tang, Q., Tan, P., Ma, N. and Ma, X., 2021. Physiological functions of threonine in animals: beyond nutrition metabolism. *Nutrients*, *13*(8), p.2592.
72. Tardy, A.L., Pouteau, E., Marquez, D., Yilmaz, C. and Scholey, A., 2020. Vitamins and minerals for energy, fatigue and cognition: a narrative review of the biochemical and clinical evidence. *Nutrients*, *12*(1), p.228.

73. Tomaszewska, E., Świątkiewicz, S., Arczewska-Włosek, A., Wojtysiak, D., Dobrowolski, P., Domaradzki, P., Świetlicka, I., Donaldson, J., Hułas-Stasiak, M. and Muszyński, S., 2020. Alpha-ketoglutarate: an effective feed supplement in improving bone metabolism and muscle quality of laying hens: a preliminary study. *Animals*, 10(12), p.2420.
74. Tugnoli, B., Giovagnoni, G., Piva, A. and Grilli, E., 2020. From acidifiers to intestinal health enhancers: how organic acids can improve growth efficiency of pigs. *Animals*, 10(1), p.134.
75. Uyanga, V.A., Oke, E.O., Amevor, F.K., Zhao, J., Wang, X., Jiao, H., Onagbesan, O.M. and Lin, H., 2022. Functional roles of taurine, L-theanine, L-citrulline, and betaine during heat stress in poultry. *Journal of animal science and biotechnology*, 13(1), p.23.
76. Vandana, G.D., Sejian, V., Lees, A.M., Pragna, P., Silpa, M.V. and Maloney, S.K., 2021. Heat stress and poultry production: impact and amelioration. *International Journal of Biometeorology*, 65, pp.163-179.
77. Verma, M. K., Sharma, S., & Kumar, S. (2020). A review on pharmacological properties of *Artemisia annua*. *Journal of Pharmacognosy and Phytochemistry*, 9(6), 2179-2183.
78. Vlaicu, P.A., Untea, A.E., Panaite, T.D., Saracila, M., Turcu, R.P. and Dumitru, M., 2023. Effect of basil, thyme and sage essential oils as phytogetic feed additives on production performances, meat quality and intestinal microbiota in broiler chickens. *Agriculture*, 13(4), p.874.
79. Wahab, A.A., Chuppava, B., Shehata, A.A., Basiouni, S., Eisenreich, W. and Hafez, H.M., 2024. Nutritional Disorders in Fattening Turkeys. In *Turkey Diseases and Disorders Volume 2: Infectious and Nutritional Diseases, Diagnostics and Control Strategies* (pp. 215-256). Cham: Springer Nature Switzerland.
80. Wan, X., Guo, H., Liang, Y., Zhou, C., Liu, Z., Li, K., Niu, F., Zhai, X., & Wang, L. (2020). The physiological functions and pharmaceutical applications of inulin: A review. *Carbohydrate Polymers*, 246, Article 116589.
81. Wang, C., Liu, X., Sun, X., Li, Y., Yang, X. and Liu, Y., 2024. Dietary betaine supplementation improved egg quality and gut microbes of laying hens under dexamethasone-induced oxidative stress. *Poultry Science*, 103(11), p.104178.
82. Waqas, M., Nastoh, N.A., Çinar, A.A., Farooq, M.Z. and Salman, M., 2024. Advantages of the Use of Postbiotics in Poultry Production: A New Concept. *Brazilian Journal of Poultry Science*, 26(3), pp.eRBCA-2024.
83. Wu, L., Tang, Z., Chen, H., Ren, Z., Ding, Q., Liang, K. and Sun, Z., 2021. Mutual interaction between gut microbiota and protein/amino acid metabolism for host mucosal immunity and health. *Animal nutrition*, 7(1), pp.11-16.
84. Xu, H., Lu, Y., Li, D., Yan, C., Jiang, Y., Hu, Z., Zhang, Z., Du, R., Zhao, X., Zhang, Y. and Tian, Y., 2023. Probiotic mediated intestinal microbiota and improved performance, egg quality and ovarian immune function of laying hens at different laying stage. *Frontiers in Microbiology*, 14, p.1041072.

85. Zampiga, M., Calini, F. and Sirri, F., 2021. Importance of feed efficiency for sustainable intensification of chicken meat production: implications and role for amino acids, feed enzymes and organic trace minerals. *World's Poultry Science Journal*, 77(3), pp.639-659.
86. Zhang, Y.N., Wang, S., Huang, X.B., Li, K.C., Ruan, D., Xia, W.G., Wang, S.L., Chen, W. and Zheng, C.T., 2022. Comparative effects of inorganic and organic manganese supplementation on productive performance, egg quality, tibial characteristics, serum biochemical indices, and fecal Mn excretion of laying ducks. *Animal Feed Science and Technology*, 283, p.115159.
87. Zheng, Y., Xie, T., Li, S., Wang, W., Wang, Y., Cao, Z. and Yang, H., 2022. Effects of selenium as a dietary source on performance, inflammation, cell damage, and reproduction of livestock induced by heat stress: A review. *Frontiers in Immunology*, 12, p.820853.

UNDER PEER REVIEW