Rovedar

JWPS

Journal of World's Poultry Science. 2026; 5(1): 9-16.

DOI: 10.58803/jwps.v5i1.116

http://jwps.rovedar.com/

Review Article

# Nutritional Intervention: Impacts on Gut Microbiome, Health, and Productivity of Laying Hens

Emeka Austin Anyigor[1*], Eleleh Oluchi Jecinta[2], Ifeanyi Princewell Ogbuewu[2], and Edeheudim Bassey Etuk[2]

[1] Department of Animal Production Technology, Federal College of Land Resources Technology, Owerri, Imo State, Nigeria
[2] Department of Animal Science and Technology, Federal University of Technology, Owerri, Imo State, Nigeria

* ***Corresponding author:*** Emeka Austin Anyigor, Department of Animal Production Technology, Federal College of Land Resources Technology, Owerri, Imo State, Nigeria. Email: anyigoremeka@gmail.com

ARTICLE INFO

***Article History:***
Received: 03/02/2026
Revised: 29/02/2026
Accepted: 15/03/2026
Published: 31/03/2026

check for updates

***Keywords:***
Egg production
Gut microbiome
Laying hen
Nutritional intervention
Welfare

ABSTRACT

Modulating the gut microbiome is critical for maintaining the health and productivity of laying hens. Nutritional interventions are essential for preserving gut integrity under the pressures of high egg production and alternative housing systems. The present study aimed to provide a comprehensive synthesis of current knowledge on the relationship between nutritional strategies and gut microbiome modulation and productivity in laying hens. A systematic literature search of PubMed, Scopus, and ScienceDirect (2015-2026) retrieved 108 studies that met the eligibility criteria for qualitative analysis. The inclusion of probiotics, prebiotics, organic acids, exogenous enzymes, vitamins, minerals, and amino acids in laying hen diets was associated with improved microbial balance, intestinal function, immune status, and nutrient utilization, resulting in greater egg production and egg quality. These nutritional approaches provided sustainable alternatives to antibiotic growth promoters, supporting resilient egg production systems by improving gut health and maintaining productive performance in laying hens. Productive performance, short-chain fatty acid production, and reduced pathogenic colonization in laying hens were all positively influenced by nutritional interventions that effectively modulate the gut microbiome.

## 1. Introduction

Poultry eggs constitute a vital source of high-quality animal protein globally, playing an important role in human nutrition and food security[1-3]. Increased global demand, fueled by population growth and changing dietary habits, has led to intensified egg production through advancements in genetics, nutrition, and health management[2,4,5]. Modern commercial layer strains, such as ISA brown and Lohmann brown, have been genetically selected for high laying performance, with industry data indicating production of over 300 eggs per hen annually under commercial management conditions[6-8].

Despite progress in genetics, nutrition, and health management, intensified egg production poses significant challenges. These challenges include increased vulnerability to infectious diseases, diminished egg quality, and welfare concerns[9]. Prolonged high-intensity laying places considerable physiological stress on hens, which has been associated with impaired gastrointestinal function, altered metabolic status, and increased susceptibility to disease, particularly during the late laying phase[10,11]. These challenges may be exacerbated in resource-limited production systems, where optimal health management is more difficult to maintain[12]. Concurrently, rising consumer awareness of animal welfare has prompted a shift from conventional cage systems to alternative housing options such as free-range and cage-free production[13]. While these systems may enhance behavioral welfare, they also expose hens to greater environmental stressors, pathogens, and parasites[14-16]. Such exposures can increase risks to gut health, productivity, and food safety[17]. Disruptions to the gut microbiome in free-range and cage-free production environments have been linked to higher shedding rates of foodborne pathogens and reduced laying performance[18].

The gut microbiome of laying hens is a complex and dynamic ecosystem essential for nutrient metabolism, immune regulation, intestinal integrity, and overall productivity[19]. The gut microbiota contributes to host health through the production of short-chain fatty acids (SCFAs), competitive exclusion of pathogens, and regulation of intestinal barrier integrity[20-22]. However, different stressors, including dietary imbalances, environmental changes, heat stress, and pathogen exposure, can disrupt microbial

*Cite this paper as*: Anyigor EA, Jecinta EO, Ogbuewu IP, and Etuk EB. Nutritional Intervention: Impacts on Gut Microbiome, Health, and Productivity of Laying Hens. Journal of World's Poultry Science. 2026; 5(1): 9-16. DOI: 10.58803/jwps.v5i1.116

homeostasis, leading to dysbiosis and compromised performance[22,23].

Several nutritional interventions have emerged as effective and sustainable strategies for modulating the gut microbiome and enhancing the health, welfare, and productivity of laying hens[24,25]. Dietary supplementation with probiotics[24-28], prebiotics[26], organic acids, minerals, amino acids, exogenous enzymes, and vitamins, has been shown to improve microbial balance, enhance intestinal morphology, strengthen immune function, and optimize nutrient utilization. These effects can contribute to increased egg production, improved egg quality, and may support overall physiological resilience and health status. Importantly, these dietary strategies may serve as viable alternatives to antibiotic growth promoters, aligning poultry production with antimicrobial stewardship and welfare-oriented goals[2]. The present study aimed to synthesize current evidence on the effects of nutritional interventions on the gut microbiome, health, welfare, and productivity of laying hens.

## 2. Materials and methods

A systematic literature search was conducted using PubMed, Scopus, and ScienceDirect databases for articles published between January 2015 and January 2026. The search strategy employed combinations of keywords, including laying hens, gut microbiome, probiotics, prebiotics, organic acids, enzymes, vitamins, minerals, amino acids, egg production, and egg quality. A total of 312 records were obtained from PubMed (n = 112), Scopus (n = 112), and ScienceDirect (n = 98). After removal of 50 duplicates, 262 records were screened based on title and abstract, resulting in 86 exclusions. Full-text assessment was conducted for 176 articles, of which 68 were excluded for reasons including studies on broilers or other poultry (n = 35), absence of gut microbiome outcomes (n = 8), non-nutritional interventions (n = 13), and conference abstracts or unavailable full texts (n = 12). Inclusion criteria included studies that provided information on nutritional interventions in laying hens and included outcomes related to gut microbiome, performance, welfare, health, or egg quality. Eligible studies were conducted on laying hens at any stage of lay, contained sufficient methodological detail to enable evaluation, and presented unique quantitative or qualitative data relevant to the review topic. Only articles published in peer-reviewed journals between 2015 and 2026 were included. Ultimately, 108 studies met the inclusion criteria and were included in the qualitative synthesis, as illustrated by the PRISMA flow diagram (Figure 1). Data extraction focused on intervention type, microbiome outcomes, health and productivity measures, and study characteristics. Due to heterogeneity in study designs, interventions, and outcome measures, a meta-analysis was not feasible.

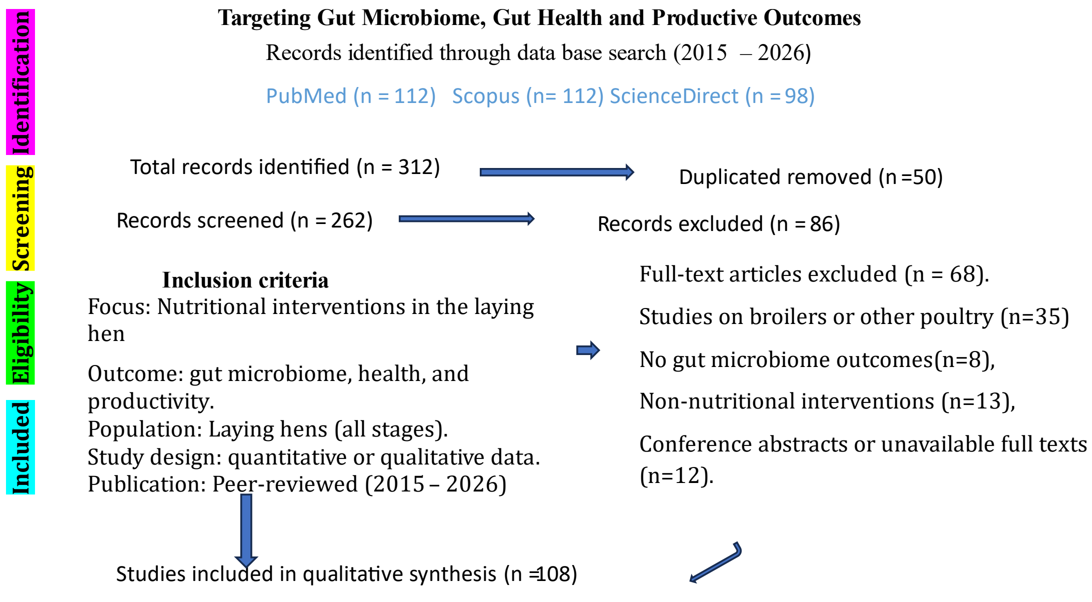


**Figure 1.** PRISMA flow diagram for systematic review of nutritional interventions in laying hens from January 2015 and January 2026

## 3. Gut microbiome in laying hens

The gut microbiome of laying hens is characterized by a delicate balance among commensal, beneficial, and potentially pathogenic microbes[2,10,12]. Key bacterial phyla in this ecosystem include *Firmicutes*, which are involved in energy harvesting and nutrient metabolism, *Bacteroidetes*, which contribute significantly to carbohydrate digestion and immune modulation, and *Proteobacteria*, which often include opportunistic pathogens[12,14]. Elevated proportions of *Proteobacteria* may indicate microbial imbalance or dysbiosis[13]. These microbial communities are constantly changing, influenced by age, diet, environmental conditions, and modifications in production systems. Early microbial colonization during the initial stages of life is crucial, as it helps establish a strong balanced gut microbiota. This foundation supports lifelong health, optimal egg production, and may improve the overall hen welfare[2,12]. Nutritional interventions play a pivotal role in shaping this microbial ecosystem. By modulating the abundance and activity of beneficial microbes and suppressing harmful bacteria, these dietary interventions influence gut health, nutrient utilization, and immune competence[1,12,13].

## 4. Effects of nutritional intervention on the gut microbiome

Nutritional interventions, including probiotics and prebiotics (Table 1), organic acids, exogenous enzymes, vitamins (Table 2), minerals (Table 3), and amino acids, are essential for maintaining a balanced and resilient gut microbiome[14]. These additives can enhance gut health and immune function, improve nutrient utilization and absorption, strengthen disease resistance, and reduce inflammation and stress. Ultimately, feed additives contribute to increased productivity and welfare in laying hens[15,16].

### *4.1. Probiotics*

Live beneficial bacteria, known as probiotics, form symbiotic interactions within the host's gastrointestinal tract and have been shown to enhance immunity, gut integrity, and performance[24-28]. Probiotics modify the gut microbiome by increasing beneficial microorganisms, improving gut integrity and nutrient absorption, stimulating mucin production, strengthening the mucosal barrier against pathogens and toxins, and producing SCFAs[24-27]. Additionally, certain probiotic strains have been associated with improved growth performance, egg production, egg quality, and overall physiological status in laying hens[28-31]. Several probiotic species, including *Lactobacillus*, *Bacillus*, *Faecalibacterium*, *Bifidobacterium*, and *Saccharomyces* (yeast), have been extensively studied for their roles in poultry nutrition. These microbes promote gut health by modulating the intestinal microbiota, enhancing food intake, and regulating immune responses[12,24,25].

*Lactobacillus* species are among the most extensively studied probiotics due to their ability to regulate intestinal microbial ecosystems and improve host function. Supplementation of late-phase laying hens with *Lactobacillus rhamnosus* GG has been shown to improve eggshell ultrastructure, lipid metabolism, and production performance[28,29].

Another important category of probiotics for laying hen nutrition includes *Bacillus* species. *Bacillus aryabhattai* CKNJh11 has been demonstrated to significantly improve the growth performance and egg quality of laying hens[29]. *Bacillus* species remain highly stable during feed preparation and storage due to their ability to form spores[30-32]. These species synthesize digestive enzymes and antimicrobial peptides, which can enhance feed digestibility and suppress pathogenic bacteria[30-32]. These effects contribute to improved intestinal health and microbial balance[30-32]. Supplementing laying hen diets with *Bacillus* has been associated with improvements in immunological homeostasis and stress resistance through modulation of the gut-brain axis, in addition to intestinal health[30-32]. Yeasts, particularly *Saccharomyces cerevisiae*, provide additional functional benefits in poultry diets[32-34]. These microorganisms offer bioactive metabolites, improve feed efficiency and stress tolerance, stabilize gut microbiota, and boost immunological responses in laying hens[24,27,34].

### *4.2. Prebiotics*

Prebiotics are non-digestible dietary fibers and oligosaccharides that selectively stimulate the growth and activity of beneficial intestinal microorganisms, such as *Lactobacillus* and *Bifidobacterium* species[35]. In poultry nutrition, common prebiotics include fructo-oligosaccharides (FOS), mannan-oligosaccharides (MOS), galacto-oligosaccharides (GOS), and another complex glycans[35-38]. These compounds resist digestion in the upper gastrointestinal tract and are fermented by gut microbiota in the lower gut[19,37]. Studies in laying hens have demonstrated that dietary supplementation with MOS and FOS enhances cecal fermentation, reduces populations of potentially pathogenic bacteria, and promotes beneficial microbial taxa[10,38]. These effects contribute to improved gut microbial balance and enhanced overall stability of the intestinal ecosystem[24,39]. Prebiotics act as fermentable substrates that selectively enhance populations of beneficial microbes and modulate gut microbiome composition[40-43]. Mechanistically, prebiotics increase production of key SCFAs such as acetate, propionate, and butyrate, which in turn improve nutrient solubility, support energy supply for epithelial cells, and inhibit pathogen proliferation[22,43]. Prebiotic supplementation has been associated with increased villus height and deeper crypts, which expand the absorptive surface area of the small intestine and contribute to improved nutrient uptake and digestive efficiency[37,42]. Morphological improvements correlate with a more diverse and balanced microbiota, in which beneficial microbes outcompete harmful bacteria via mechanisms such as competitive exclusion and production of inhibitory metabolites[38,39].

In laying hens, dietary prebiotic supplementation has been associated with increased egg production rates, improved feed conversion ratios, and enhanced egg quality parameters compared with unsupplemented controls[40]. The MOS, in particular, has been shown to improve productive performance and gastrointestinal function by modulating intestinal microbial activity, enhancing nutrient digestibility, and improving gut morphology and absorptive capacity[38]. Under high-stress conditions, including thermal stress and pathogenic challenge, prebiotic supplementation has been shown to enhance resilience in laying hens by supporting intestinal integrity, stabilizing gut microbial communities, and reducing enteric pathogen load[13,37].

### *4.3. Synbiotics*

Probiotics and prebiotics can create a synbiotic effect when used together, enhancing microbial viability, functional activity, and stable colonization of the gastrointestinal tract[25,26]. Synbiotics supplementation has consistently been linked to increased SCFA production, enhanced mucosal immune function, improved nutrient digestibility, and strengthened intestinal barrier integrity in laying hens[22,25,26]. Increased SCFA production, particularly acetate and butyrate, supports epithelial cell energy metabolism, modulates inflammatory signaling pathways, and promotes intestinal homeostasis under both normal physiological conditions and during environmental or nutritional stress[13,22,37]. From a practical poultry nutrition standpoint, incorporating probiotics and prebiotics into layer diets represents a sustainable strategy for enhancing intestinal health, productivity, and egg quality while reducing dependence on antibiotic growth promoters[25,26]. Synbiotics facilitate villus development, SCFA synthesis, and mucosal defense systems through complementary mechanisms[22,25,26].

**Table 1.** Influence of probiotics and prebiotics on gut microbiome, health, and productivity in laying hens

| Probiotic / Prebiotic | Influence on gut microbiome, health, and productivity effects | Reference |
|---|---|---|
| *Lactobacillus* spp. | Outcompetes pathogens and increases villus height, produces SCFAs, which improve gut morphology, nutrient absorption, and eggshell quality | (28) |
| *Bacillus* spp. | Modulates the microbiota-gut-brain axis and then enhances laying performance and stress tolerance | (29-32) |
| *Saccharomyces* spp. | Ferments indigestible substrates and supports gut health, and immunity | (32-34) |
| Fructo-oligosaccharides (FOS) | Supports beneficial bacteria, produce SCFA, improves egg quality, and reduces cholesterol level | (36) |
| Glycans | Modulates microbial composition and supports microbial balance | (37) |
| Mannan-oligosaccharides (MOS) | Substrate for *Lactobacillus*/*Bifidobacterium*, maintains gut health, and disease resistance | (38) |

SCFAs = Short-chain fatty acid

### *4.4. Organic acids*

Organic acids, including formic, acetic, lactic, and citric acids, along with their respective salts, are increasingly recognized as effective alternatives to antibiotic growth promoters in poultry diets[44]. These acids serve multiple functions, including modulation of the gut microbiome, enhancement of nutrient utilization, and improvement of overall productivity[43-47]. In the gut microbiome, organic acids primarily lower gastrointestinal pH, creating an environment unfavorable for acid-sensitive pathogens such as *Salmonella spp.* and *Escherichia coli*, while promoting beneficial populations of *Lactobacillus* and *Bifidobacterium*[43,45,46]. Lactic acid, whether directly supplemented or produced by lactic acid bacteria, enhances microbial fermentation, suppresses coliforms, and supports SCFA production[45]. Citric acid lowers intestinal pH and chelates minerals, thereby enhancing microbial balance[45-50]. Additionally, citric acid increases goblet cell density, reinforcing mucin production and improving intestinal barrier function[48-50]. Regarding intestinal morphology and nutrient absorption, organic acids that modulate microbial populations and pH have been shown to increase the villus height-to-crypt depth ratio, thereby improving absorptive surface area and nutrient absorption[45,50,51]. Organic acid supplementation has been associated with improved feed conversion efficiency, egg mass, and laying rate due to enhanced nutrient utilization and reduced pathogen-associated energy costs[47-52].

### *4.5. Exogenous enzymes*

In laying hens, exogenous enzymes such as xylanase, phytase, β-glucanase, α-galactosidase, and protease help degrade non-starch polysaccharides and other anti-nutritional components in plant-based feeds[53-55]. This enzymatic hydrolysis reduces digest viscosity, increases release of encapsulated nutrients, and enhances nutrient digestibility and absorption[53-55]. These effects supported a healthier gut environment and can contribute to improved gut integrity and immune competence[53,56]. Dietary protease supplementation improved protein hydrolysis and amino acid availability, reducing undigested protein in the hindgut and limiting substrates for pathogenic bacteria[56-58].

### *4.6. Vitamins*

Vitamins A, D, E[59,60], and the B-complex[61,62] are essential for maintaining a healthy gut microbiome, supporting immune function, promoting nerve health, facilitating metabolic processes, and improving calcium/phosphorus absorption[63,64]. Some evidence from poultry studies, including broiler chickens, suggested that vitamin B6 supplementation may alleviate intestinal stress and modulate gut microbiota[61]. Vitamin B12 supplementation has been linked to beneficial shifts in SCFA-producing bacteria and improved eggshell quality[62]. Vitamins A and C support intestinal immunity and help stabilize microbial balance[59]. The gut microbiota, including that of chickens, contributes to vitamin K2 biosynthesis[65], and distinct forms of vitamin K (K1 and K2) have been shown to play different roles in health and disease[66] (Table 2).

**Table 2.** Impact of vitamins on gut microbiome, health, and productivity in laying hens

| Vitamin | Microbiome influence | Health and productivity effects | Reference |
|---|---|---|---|
| A, C | Enhances *Lactobacillus*, *Bifidobacterium* and reduces opportunistic pathogens | Supports gut integrity, egg production, and mucosal immunity | (59) |
| B6 | Promotes immune-related metabolism | Prevents dysbiosis and supports immunity | (61) |
| B12 | Improves *Butyricicoccus*, *Ruminococcaceae and enhance* SCFA-producing bacteria | Improves nutrient utilization, eggshell quality, and gut fermentation | (62) |
| D, E | Supports beneficial bacteria and reduces oxidative stress-related dysbiosis | Enhances mineral absorption, immunity and stress tolerance | (60, 64) |

SCFAs = Short-chain fatty acid

### *4.7. Minerals*

Minerals such as calcium, magnesium, zinc, copper, iron, selenium, phosphorus, sodium, iodine, and potassium are vital for laying hens' health[67-69] (Table 3). In addition to their structural and metabolic roles, these minerals significantly affect the gut microbiome, intestinal health, immune response, and overall egg quality[69-71]. Calcium plays a crucial role in regulating gut pH, although excessive levels can lead to increased *Clostridium* populations[68]. Magnesium helps maintain stable gut microbial communities and enhances fermentation efficiency[69,72]. Zinc strengthens the gut barrier and increases villus height, while organic or nano-zinc improves the balance of gut microbiota and enhances zinc content in eggs[73,74]. Selenium promotes the growth of beneficial lactic acid bacteria while reducing harmful strains, thus maintaining a balanced gut microbiome[67,75,76]. Phosphorus enhances microbial diversity and boosts the production of SCFA[68]. Sodium and potassium are essential for maintaining osmotic balance and regulating gut pH, thereby supporting stable microbial populations[67,77]. Additionally, iodine regulates thyroid hormone levels, contributing to microbial stability and immune function[78,79]. In laying hens, zinc, selenium, and copper are critical for immune modulation[67,81,82]. Calcium and magnesium are essential for eggshell thickness[68,72,83]. Organic mineral supplementation, even at lower levels, may outperform inorganic forms by improving egg mass, laying rates, shell quality, and feed efficiency due to higher bioavailability[69,74,84].

**Table 3.** Impact of minerals on gut microbiome, health, and productivity in laying hens

| Mineral | Gut microbiome role | Health and productivity effects | Reference |
|---|---|---|---|
| Ca, Mg | Maintain gut pH and microbial balance | Eggshell formation, gut integrity | (68, 72, 83) |
| Fe, Na, K | Supports beneficial bacteria colonization and osmotic balance | Improves egg production | (78, 80, 67, 77) |
| Zn, Cu, Se | Support immune modulation | Disease resistance, egg quality | (67, 73-76, 81, 82) |
| P | Enhances microbial diversity | Nutrient utilisation | (68) |

Ca: Calcium, Mg: Magnesium, Fe: Iron, Na: Sodium, K: Potassium, Zn: Zinc, Cu: Copper, Se: Selenium, and P: Phosphorus.

### *4.8. Amino acids*

Amino acids are vital for shaping the gut microbiome by maintaining mucosal integrity, regulating immune responses, and influencing metabolite production such as histamine, which affects gut motility and immunity[85-87]. Methionine exerts significant immunomodulatory effects and serves as a precursor to glutathione[88-90]. Histidine and tryptophan play essential roles in immune regulation and intestinal immunological tolerance[85-87]. Interactions between amino acids and the microbiota significantly affect nitrogen utilization efficiency and intestinal health[87,90,91]. Synergistic interactions between minerals and amino acids further enhance nutrient bioavailability and microbial stability[84,87]. Calcium, phosphorus, zinc, copper, and iron influence gut microbial function by regulating enzymes[92]. Organic and chelated minerals enhance bioavailability and regulate microbial composition more efficiently than inorganic sources[85].

## 5. Conclusion

The present study demonstrated that a focus on dietary interventions, including probiotics, prebiotics, organic acids, exogenous enzymes, vitamins, minerals, and functional amino acids, can influence the gut microbiota and its metabolic processes in laying hens. The evidence indicated that these interventions are associated with increased SCFA synthesis, improved intestinal morphology, enhanced mucosal and systemic immune responses, optimized mineral absorption, and reduced pathogen colonization. Correspondingly, improvements were observed in egg production rate, eggshell quality, antioxidant status, and overall hen health resilience. The current findings underscore the importance of nutritional interactions, particularly between amino acids and trace minerals in modulating microbial ecology, oxidative balance, and optimal life expectancy. Precision nutrition that integrates microbiome-aware feeding regimens offers an appealing option for sustainable, reduced-antibiotic egg production systems. The present study was limited by heterogeneity of study designs, intervention types, dosages, and outcome measures across the included studies, which precluded meta-analysis. Additionally, most studies were conducted under controlled experimental conditions, which may not fully reflect commercial production environments. Future studies should focus on understanding how specific microbes and their byproducts influence productivity, as well as on long-term studies of nutrition across different housing systems and breeds. Furthermore, studies on the interactions between multiple dietary additives and their synergistic effects on gut health and performance would provide valuable insights for precision feeding strategies.

## Declarations

### *Competing interests*

The authors declared no competing interests.

### *Authors' contributions*

Emeka Austin Anyigor drafted and wrote the manuscript. Eleleh Oluchi Jecinta, Ifeanyi Princewell Ogbuewu, and Edeheudim Bassey Etuk revised the manuscript critically for important intellectual content. All authors read and approved the final edition of the manuscript.

### *Availability of data and materials*

All collected data and related studies are prepared for publication and no new data were generated in support of the present study.

### *Ethical considerations*

This manuscript is original submitted exclusively to the Journal of World's Poultry Science and is not under consideration elsewhere. The authors affirmed that all content has been checked carefully to prevent plagiarism, data fabrication, data falsification, duplicate publication, and other breaches of publication ethics, in accordance with standard ethical guidelines. The authors acknowledged the use of OpenAI, ChatGPT 4 for language editing and formatting restrictidly. All content was critically reviewed and validated by the authors, who take full responsibility for the final edition of the manuscript.

### *Funding*

No external and internal funding was received for the present study.

## References


1. Food and agriculture organization of the united nations (FAO). Agribusiness handbook: Poultry meat and eggs. Food And Agriculture Organizations of The United Nations, 2023. Available at: https://openknowledge.fao.org/bitstreams/c548c19f-b615-45fe-a55c-5fb7404715d3/download
2. Korver DR. Review: Current challenges in poultry nutrition, health, and welfare. Animal. 2023; 17(Suppl 2): 100755. DOI: 10.1016/j.animal.2023.100755
3. Dai D, Qi GH, Wang J, Zhang HJ, Qiu K, and Wu SG. Intestinal microbiota of layer hens and its association with egg quality and safety. Poult Sci. 2022; 101(9): 102008. DOI: 10.1016/j.psj.2022.102008
4. Desbruslais A, Wealleans A, Gonzalez-Sanchez D, and di Benedetto M. Dietary fibre in laying hens: A review of effects on performance, gut health and feather pecking. World's Poult Sci J. 2021; 77(4): 797-823. DOI: 10.1080/00439339.2021.1960236
5. Mulder ND. Global egg industry outlook 2035: Long-term egg market and investment perspectives. World Egg Organisation, 2025. Available at: https://www.rabobank.com/knowledge/q011497178-global-egg-industry-outlook-2035-long-term-egg-market-and-investment-perspectives
6. ISA brown management guide. Boxmeer, Netherlands: Institute for Animal Breeding; 2019. Available at: https://www.scribd.com/document/513752045/isa-brown-product-guide-cage-production-systems-vs1408a
7. Lohmann breeders. Outstanding performances: LOHMANN layers above the standards. Lohmann Breeders, 2025. Available at: https://lohmann-breeders.com/outstanding-performances-lohmann-layers-above-the-standards/
8. Kidd MT, and Anderson KE. Laying hens in the U.S. market: An appraisal of trends from the beginning of the 20th century to present. J Appl Poult Res. 2019; 28(4): 771-784. DOI: 10.3382/japr/pfz043
9. Aruwa CE, and Sabiu S. Interplay of poultry-microbiome interactions - influencing factors and microbes in poultry infections and metabolic disorders. Br Poult Sci. 2024; 65(5): 523-537. DOI: 10.1080/00071668.2024.2356666
10. Feng J, Lu M, Wang J, Zhang HJ, Qiu K, Qi GH, et al. Dietary oregano essential oil supplementation improves intestinal functions and alters gut microbiota in late-phase laying hens. J Anim Sci Biotechnol. 2021; 12: 1-15. DOI: 10.1186/s40104-020-00529-3
11. Singh AK, and Kim WK. Effects of dietary fiber on nutrients utilization and gut health of poultry: A review of challenges and opportunities. Animals. 2021; 11(1): 181. DOI: 10.3390/ani11010181
12. El Jeni R, Dittoe DK, Olson EG, Lourenco J, Corcionivoschi N, Ricke SC, et al. Probiotics and potential applications for alternative poultry production systems. Poult Sci. 2021; 100(7): 101156. DOI: 10.1016/j.psj.2021.101156
13. Kers JG, Velkers FC, Fischer EAJ, Hermes GDA, Stegeman JA, and Smidt H. Host and environmental factors affecting the intestinal microbiota in chickens. Front Microbiol. 2018; 9: 235. DOI: 10.3389/fmicb.2018.00235
14. Pin Viso N, Redondo E, Díaz Carrasco JM, Redondo L, Garcia JS, Miyakawa MF, et al. Geography as non-genetic modulation factor of chicken cecal microbiota. PLOS ONE. 2021; 16(1): e0244724. DOI: 10.1371/journal.pone.0244724
15. Khan S, Moore RJ, Stanley D, Chousalkar KK. The gut microbiota of laying hens and its manipulation with prebiotics and probiotics to enhance gut health and food safety. Appl Environ Microbiol. 2020; 86(13): e00600-20. DOI: 10.1128/aem.00600-20
16. Bernard M, Lecoeur A, Coville JL, Bruneau N, Jardet D, Lagarrigue S, et al. Relationship between feed efficiency and gut microbiota in laying chickens under contrasting feeding conditions. Sci Rep. 2024; 14: 8210. DOI: 10.1038/s41598-024-58374-3
17. Ballou AL, Ali RA, Mendoza MA, Ellis JC, Hassan HM, Croom WJ, et al. Development of the chick microbiome: How early exposure influences future microbial diversity. Front Vet Sci. 2016; 3: 2. DOI:10.3389/fvets.2016.00002
18. Nafady AA, Ghoneim SS, Behour TS, Akhtar M, Youssef IM, Abd-Elkareem M, et al. Comparative study of gut microbiota profiles and reproductive traits in high- and low-laying chickens. Poult Sci. 2025; 104(12): 105991. DOI: 10.1016/j.psj.2025.105991
19. Ding J, Dai R, Yang L, He C, Xu K, Liu S, et al. Inheritance and establishment of gut microbiota in chickens. Front Microbiol. 2017; 8: 1967. DOI: 10.3389/fmicb.2017.01967
20. Lee S, La TM, Lee HJ, Choi IS, Song CS, Park SY, et al. Characterization of microbial communities in the chicken oviduct and the origin of chicken embryo gut microbiota. Sci Rep. 2019; 9(1): 6838. DOI: 10.1038/s41598-019-43280-w
21. Kogut MH. The effect of microbiome modulation on the intestinal health of poultry. Anim Feed Sci Technol. 2019; 250: 32-40. DOI: 10.1016/j.anifeedsci.2018.10.008
22. Zhang JM, Sun YS, Zhao LQ, Chen TT, Fan MN, Jiao HC, et al. SCFAs-induced GLP-1 secretion links the regulation of gut microbiome on hepatic lipogenesis in chickens. Front Microbiol. 2019; 10: 2176. DOI: 10.3389/fmicb.2019.02176
23. Kim HR, Ryu C, Lee SD, Cho JH, and Kang H. Effects of heat stress on the laying performance, egg quality, and physiological response of laying hens. Animals. 2024; 14(7): 1076. DOI: 10.3390/ani14071076
24. Shehata AA, Yalçın S, Latorre JD, Basiouni S, Attia YA, Abd El-Wahab A, et al. Probiotics, prebiotics, and phytogenic substances for optimizing gut health in poultry. Microorganisms. 2022; 10(2): 395. DOI: 10.3390/microorganisms10020395
25. Markowiak P, and Śliżewska K. The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut Pathog. 2018; 10(1): 21. DOI: 10.1186/s13099-018-0250-0
26. Pineda-Quiroga C, Borda-Molina D, Chaves-Moreno D, Ruiz R, Atxaerandio R, Camarinha-Silva A, et al. Microbial and functional profile of the ceca from laying hens affected by feeding prebiotics, probiotics, and synbiotics. Microorganisms. 2019; 7: 123. DOI: 10.3390/microorganisms7050123
27. Onbaşılar EE, Yalçın S, Bakıcı C, Batur B, Kartal YK, Ahlat O, et al. Comprehensive evaluation of probiotic effects on laying hen physiology: From performance to bone and gut morphology. Animals. 2025; 15(16): 2408. DOI: 10.3390/ani15162408
28. Liu L, Zhang G, Qu G, Liu B, Zhang X, Li G, et al. Effects of dietary *Lactobacillus rhamnosus* GG supplementation on the production performance, egg quality, eggshell ultrastructure, and lipid metabolism of late-phase laying hens. BMC Vet Res. 2023; 19(1): 150. DOI: 10.1186/s12917-023-03719-9
29. Dangsawat O, Rattanawut J, Srisawat T, Sowanpreecha R, Tang Phuc Khang L, Srinual O, et al. *Bacillus aryabhattai* CKNJh11 as a promising probiotic improves growth performance and egg quality in laying hens. Sci Rep. 2025; 15: 13659. DOI: 10.1038/s41598-025-97553-8
30. Tajudeen H, Ha SH, Hosseindoust A, Mun JY, Park S, Park S, et al. Effect of dietary inclusion of *Bacillus*-based probiotics on performance, egg quality, and the faecal microbiota of laying hen. Anim Biosci. 2024; 37(4): 689-696. DOI: 10.5713/ab.23.0299
31. Neijat M, Shirley RB, Barton J, Thiery P, Welsher A, and Kiarie E. Effect of dietary supplementation of *Bacillus subtilis* DSM29784 on hen performance, egg quality indices, and apparent retention of dietary components in laying hens from 19 to 48 weeks of age. Poult Sci. 2019; 98(11): 5622-5635. DOI: 10.3382/ps/pez324
32. Hakami ZM, Alhotan RA, Al-Sulaiman AR, Aljumaah RS, Palombo V, D'Andrea M, et al. Enhancing laying hen productivity and health: Influence of dietary probiotic *Bacillus* strains and prebiotic *Saccharomyces cerevisiae* yeast cell wall on production performance, egg quality, and inflammatory responses. Animals. 2025; 15(10): 1398.

DOI: 10.3390/ani15101398
33. Fathima S, Shanmugasundaram R, Sifri M, and Selvaraj R. Yeasts and yeast-based products in poultry nutrition. J Appl Poult Res. 2023; 32(2): 100345. DOI: 10.1016/j.japr.2023.100345
34. Abd El-Ghany WA. Yeasts and their derivatives as functional feed additives in poultry nutrition. Agriculture. 2025; 15(9): 1003. DOI: 10.3390/agriculture15091003
35. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol. 2017; 14(8): 491-502. DOI: 10.1038/nrgastro.2017.75
36. Morgan NK. Advances in prebiotics for poultry: Role of the caeca and oligosaccharides. Anim Prod Sci. 2023; 63(18): 1911-1925. DOI: 10.1071/AN23011
37. Petranyi F, Whitton MM, Lobo E, Ramirez S, Radovanović A, Bajagai YS, et al. Precision glycan supplementation: A strategy to improve performance and intestinal health of laying hens in high-stress commercial environments. J Anim Physiol Anim Nutr (Berl). 2024; 108(5): 1498-1509. DOI: 10.1111/jpn.13996
38. Youssef IM, Aldhalmi AK, Felemban SG, Elsherbeni AI, Khalil HA, Hassan MS, et al. Mannan oligosaccharides as a prebiotic for laying hens: Effects on fertility, hatchability, productive performance, and immunity. Transl Anim Sci. 2024; 8: txae123. DOI: 10.1093/tas/txae123
39. Zhou J, Wu S, Qi G, Fu Y, Wang W, Zhang H, et al. Dietary supplemental xylooligosaccharide modulates nutrient digestibility, intestinal morphology, and gut microbiota in laying hens. Anim Nutr. 2021; 7(1): 152-162. DOI: 10.1016/j.aninu.2020.05.010
40. Mahmood T, and Guo Y. Dietary fiber and chicken microbiome interaction: Where will it lead to? Anim Nutr. 2020; 6(1): 1-8. DOI: 10.1016/j.aninu.2019.11.004
41. Dibakoane SR, Mhlongo G, Moonsamy G, Wokadala OC, Mnisi CM, and Mlambo V. Phenomenological and mechanistic insights into potential dietary nucleotide-probiotic synergies in layer chickens: A review. Poult Sci. 2025; 104(5): 105049. DOI: 10.1016/j.psj.2025.105049
42. Naeem M, and Bourassa D. Probiotics in poultry: Unlocking productivity through microbiome modulation and gut health. Microorganisms. 2025; 13(2): 257. DOI: 10.3390/microorganisms13020257
43. Dittoe DK, Ricke SC, and Kiess AS. Organic acids and potential for modifying the avian gastrointestinal tract and reducing pathogens and disease. Front Vet Sci. 2018; 5: 216. DOI: 10.3389/fvets.2018.00216
44. Gerzilov V, and Hristakieva P. Organic acids supplementation in poultry nutrition: A review. Open Vet J. 2025; 15(8): 3448-3458. DOI: 10.5455/OVJ.2025.v15.i8.8
45. Rezaeipour M, Afsharmanesh M, and Bami MK. Evaluation of the effect of short-chain organic acids and probiotics on production performance, egg white quality, and fecal microbiota of laying hens. Comp Clin Pathol. 2022; 31: 621-626. DOI: 10.1007/s00580-022-03360-2
46. Markazi AD, Luoma A, Shanmugasundaram R, Murugesan R, Mohnl M, and Selvaraj R. Effect of acidifier product supplementation in laying hens challenged with *Salmonella*. J Appl Poult Res. 2019; 28(4): 919-929. DOI: 10.3382/japr/pfz053
47. Lee J, Oh S, and Heo JM. Application of dietary organic acids in laying hens production: A comprehensive review. J Anim Sci Technol. 2025; 67(6): 1185-1206. DOI: 10.5187/jast.2500224
48. Zhou Y, Qiu C, Zhou Z, Zhang Y, Zhang D, Cai Y, et al. Baicalein and citric acid modulate intestinal morphology and health status in laying hens. Vet Sci. 2025; 12(8): 706. DOI: 10.3390/vetsci12080706
49. Vargas-Rod LM, Herrera JG, Morales EJ, Arcos-Garc JL, Lopez-Pozo R, and Ruelas G. Effect of citric acid, phytase and calcium in diets of laying hens on productive performance, digestibility and mineral excretion. Int J Poult Sci. 2015; 14(4): 222-228. DOI: 10.3923/ijps.2015.222.228
50. Warsito SH, Lamid M, Al-Arif MA, Hermadi HA, Sabdoningrum EK, Ayuti SR, et al. The results of intestinal villi of laying hens exposed with avian pathogenic *Escherichia coli* (APEC) after giving citric acid and dextrose. Vet Med Int. 2025; 2025: 6623764. DOI: 10.1155/vmi/6623764
51. Abd El-Ghany WA. Applications of organic acids in poultry production: An updated and comprehensive review. Agriculture. 2024; 14(10): 1756. DOI: 10.3390/agriculture14101756
52. Khan SH, and Iqbal J. Recent advances in the role of organic acids in poultry nutrition. J Appl Anim Res. 2016; 44(1): 359-369. DOI: 10.1080/09712119.2015.1079527
53. Macambira GM, Rabello CBV, Oliveira HS de H, Dos Santos MJB, Ribeiro AG, Lins Júnior OS, et al. Effects of xylanase and phytase supplementation in diets containing *Moringa oleifera* leaf meal on intestinal morphology and the relative size and weight of internal organs of laying hens. Front Vet Sci. 2025; 12: 1615214. DOI: 10.3389/fvets.2025.1615214
54. Mun JY, Tajudeen H, Silvestre PN, Ha SH, Hosseindoust A, Lokhande A, et al. Multienzyme supplementation alleviates the performance of hens fed diets with lower crude protein and energy levels during peak to late production phase. Poult Sci. 2025; 104(11): 105823. DOI: 10.1016/j.psj.2025.105823
55. Kayan A, Prasongsook S, and Poeikhampha T. Diet supplementation with phytase and xylanase on laying performance, egg quality, apparent metabolizable energy, and phosphorous use in laying hens. Vet World. 2025; 18(1): 155-161. DOI: 10.14202/vetworld.2025.155-161
56. Poudel I, Hodge VR, Wamsley KGS, Roberson KD, and Adhikari PA. Effects of protease enzyme supplementation and varying levels of amino acid inclusion on productive performance, egg quality, and amino acid digestibility in laying hens from 30 to 50 weeks of age. Poult Sci. 2023; 102(3): 102465. DOI: 10.1016/j.psj.2022.102465
57. Oketch EO, Yu M, Nawarathne SR, Chaturanga NC, Maniraguha V, Sta Cruz BG, et al. Multiprotease supplementation in laying hen diets: Impact on performance, egg quality, digestibility, gut histomorphology, and sustainability. Poult Sci. 2025; 104(4): 104977. DOI: 10.1016/j.psj.2025.104977
58. Walker H, Bauer K, Cvejić D, Keegan JD, Nikodinoska I, Taylor-Pickard J, et al. Allzyme® Spectrum supplementation improves apparent ileal phosphorus digestibility and retention in older laying hens. J Appl Anim Nutr. 2025; 1-9. DOI: 10.1163/2049257x-20241014
59. Chen Y, Li X, Zhang H, Bai X, Ding J, Ye D, et al. Effects of vitamins A and C on intestinal immunity and gut microbiota in poultry. Front Microbiol. 2023; 14: 1180838. DOI: 10.3389/fmicb.2023.1180838
60. Sharma MK, Lee J, Shi H, Ko H, Goo D, Paneru D, et al. Effect of dietary inclusion of 25-hydroxyvitamin $D_3$ and vitamin E on performance, gut health, oxidative status, and immune response in laying hens infected with coccidiosis. Poult Sci. Poult Sci. 2024; 103(9): 104033. DOI: 10.1016/j.psj.2024.104033
61. Ouyang J, Zhang C, Deng C, Wen A, Zhou H, You J, et al. Dietary vitamin B6 supplementation alleviates heat stress-induced intestinal barrier impairment by regulating the gut microbiota and metabolites in broilers. Poult Sci. 2024; 103(11): 104202. DOI: 10.1016/j.psj.2024.104202
62. Wang X, Bai S, Yang F, Wu D, Li L. Comparison of production performance, egg quality, and cecal microbiota in laying hens receiving graded levels of vitamin B12. Poult Sci. 2021; 100(11): 100982. DOI: 10.1016/j.psj.2021.100982
63. Wang C, Shan H, Chen H, Bai X, Ding J, Ye D, et al. Probiotics and vitamins modulate the cecal microbiota of laying hens submitted to induced molting. Front Microbiol. 2023; 14: 1180838. DOI: 10.3389/fmicb.2023.1180838
64. Akter N, Dao TH, Crowley TM, Sukirno, Nawab A, and Moss AF. Afternoon calcium and vitamin D supplementation in water: A targeted approach to improve laying hen nutrition. Animals. 2025; 15(5): 72. DOI: 10.3390/ani15050720
65. Gao ZQ, Su JW, Qin Y, Ye T, Cao H, Yang LH, et al. Metagenomic analysis of vitamins B and K2 biosynthesis in chicken gut microbiota across laying periods. BMC Microbiol. 2025; 25: 553. DOI: 10.1186/s12866-025-04296-7
66. Halder M, Petsophonsakul P, Akbulut AC, Pavlic A, Bohan F, Anderson E, et al. Vitamin K: Double bonds beyond coagulation insights into differences between vitamin K1 and K2 in health and disease. Int J Mol Sci. 2019; 20(4): 896. DOI: 10.3390/ijms20040896
67. Muhammad AI, Mohamed DAA, Chwen LT, Akit H, and Samsudin AA. Effect of sodium selenite, selenium yeast, and bacterial enriched protein on chicken egg yolk color, antioxidant profiles, and oxidative stability. Foods. 2021; 10(4): 871. DOI: 10.3390/foods10040871
68. Sinclair-Black M, Garcia RA, and Ellestad LE. Physiological regulation of calcium and phosphorus utilization in laying hens. Front Physiol. 2023; 14: 1112499. DOI: 10.5713/ab.22.0086
69. Elnesr SS, Mahmoud BY, da Silva Pires PG, Moraes P, Elwan HAM, El-Shall NA, et al. Trace minerals in laying hen diets and their effects on egg quality. Biol Trace Elem Res. 2024; 202(12): 5664-5679. DOI:

10.1007/s12011-024-04121-8
70. Dong Y, Zhang K, Han M, Miao Z, Liu C, and Li J. Low level of dietary organic trace minerals improved egg quality and modulated the status of eggshell gland and intestinal microflora of laying hens during the late production stage. Front Vet Sci. 2022; 9: 920418. DOI: 10.3389/fvets.2022.920418
71. Wlaźlak S, Pietrzak E, Biesek J, and Dunislawska A. Modulation of the immune system of chickens a key factor in maintaining poultry production - a review. Poult Sci. 2023; 102(8): 102785. DOI: 10.1016/j.psj.2023.102785
72. Belkameh MM, Sedghi M, and Azarfar A. The effect of different levels of dietary magnesium on eggshell quality and laying hen's performance. Biol Trace Elem Res. 2021; 199(4): 1566-1573. DOI: 10.1007/s12011-020-02259-9
73. Ogbuewu IP, and Mbajiorgu CA. Meta-analysis of zinc supplementation on laying performance, egg quality characteristics, and blood zinc concentrations in laying hens. Biol Trace Elem Res. 2022; 200(12): 5188-5204. DOI: 10.1007/s12011-021-03080-8
74. Niknia AD, Vakili R, and Tahmasbi AM. Zinc supplementation improves antioxidant status, and organic zinc is more efficient than inorganic zinc in improving the bone strength of aged laying hens. Vet Med Sci. 2022; 8(5): 2040-2049. DOI: 10.1002/vms3.896
75. Qin K, Cong X, Wang H, Yan M, Xu X, Liu M, et al. Effects of supplementing selenium-enriched Cardamine violifolia to laying hens on egg quality and yolk antioxidant capacity during storage at 4 °C and 25 °C. Foods. 2024; 13(5): 802. DOI: 10.3390/foods13050802
76. Surai PF, and Kochish II. Nutritional modulation of the antioxidant capacities in poultry: The case of selenium. Poult Sci. 2019; 98(10): 4231-4239. DOI: 10.3382/ps/pey406
77. Abbas G, Ahmad F, Saeed M, Ayasan T, Mahmood A, Yasmeen R, et al. Effect of dietary inclusion of sodium bicarbonate on digestibility of nutrients and immune response in caged layers during the summer. Rev Bras Cienc Avic. 2019; 21(2): 1-8. DOI: 10.1590/1806-9061-2018-0915
78. Sarlak S, Tabeidian SA, Toghyani M, Shahraki ADF, Goli M, and Habibian M. Organic iron and iodine in laying hen diet: Performance, egg quality, thyroid hormones, antioxidant status, serum and yolk lipids, and egg iron and iodine contents. J Trace Elem Miner. 2025; 11: 100211. DOI: 10.1016/j.jtemin.2025.100211
79. Słupczyńska M, Jamroz D, Orda J, Wiliczkiewicz A, Kuropka P, and Król B. The thyroid hormone and immunoglobulin concentrations in blood serum and thyroid gland morphology in young hens fed with different diets, sources, and levels of iodine supply. Animals. 2022; 13(1): 158. DOI: 10.3390/ani13010158
80. Cao J, Zhu J, Zhou Q, Zhao L, Zou C, Guo Y, et al. Efficacy evaluation of novel organic iron complexes in laying hens: Effects on laying performance, egg quality, egg iron content, and blood biochemical parameters. Anim Biosci. 2023; 36(3): 498-505. DOI: 10.5713/ab.22.0086
81. da Silva JVC, de Lima AV, de Brito ANEF, Ferreira T da S, Kaneko IN, Pascoal LAF, et al. Effect of supplementation with different sources and levels of zinc, copper, and manganese on the reproductive development of laying hens. Poult Sci. 2025; 104(8): 105306. DOI: 10.1016/j.psj.2025.105306
82. Ma N, Liu M, Song M, Li S, Lin X, Jiao H, et al. The application of copper waterline on laying performance and gut health of aged laying hens. J Poult Sci. 2022; 59(3): 223-232. DOI: 10.2141/jpsa.0210124
83. Gong C, Shi H, Liu S, Gao X, Zhang S, Liu H, et al. Determination of optimal dietary calcium levels under different sources of zinc in Jing tint 6 layer chicks from 15 to 42 d of age. Poult Sci. 2024; 103(11): 104192. DOI: 10.1016/j.psj.2024.104192
84. Umar Yaqoob M, Wang G, Sun W, Pei X, Liu L, Tao W, et al. Effects of inorganic trace minerals replaced by complexed glycinates on reproductive performance, blood profiles, and antioxidant status in broiler breeders. Poult Sci. 2020; 99(5): 2718-2726. DOI: 10.1016/j.psj.2019.11.058
85. Santos MJB, Ludke MCMM, Silva LM, Rabello CBV, Barros MR, Costa FS, et al. Complexed amino acid minerals vs. bis-glycinate chelated minerals: Impact on the performance of old laying hens. Anim Nutr. 2024; 16: 395-408. DOI: 10.1016/j.aninu.2023.11.006
86. Alabi T, and Adedokun S. Amino acid nutrition in poultry: A review. Animals. 2025; 15(22): 3323. DOI: 10.3390/ani15223323
87. Alagawany M, Elnesr SS, Farag MR, Tiwari R, Yatoo MI, Karthik K, et al. Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health - a comprehensive review. Vet Q. 2020; 41(1): 1-29. DOI: 10.1080/01652176.2020.1857887
88. Liu G, and Kim WK. The functional roles of methionine and arginine in intestinal and bone health of poultry: Review. Animals. 2023; 13(18): 2949. DOI: 10.3390/ani13182949
89. Ma M, Geng S, Liu M, Zhao L, Zhang J, Huang S, et al. Effects of different methionine levels in low protein diets on production performance, reproductive system, metabolism, and gut microbiota in laying hens. Front Nutr. 2021; 8: 739676. DOI: 10.3389/fnut.2021.739676
90. Reda FM, Swelum AA, Hussein EOS, Elnesr SS, Alhimaidi AR, and Alagawany M. Effects of varying dietary DL-methionine levels on productive and reproductive performance, egg quality, and blood biochemical parameters of quail breeders. Animals. 2020; 10(10): 1839. DOI: 10.3390/ani10101839
91. Wang C, Liu X, Sun X, Li Y, Yang X, and Liu Y. Dietary betaine supplementation improved egg quality and gut microbes of laying hens under dexamethasone-induced oxidative stress. Poult Sci. 2024; 103(11): 104178. DOI: 10.1016/j.psj.2024.104178
92. Mikulewicz M, Chojnacka K, Kawala B, and Gredes T. Trace elements in living systems: From beneficial to toxic effects. Biomed Res Int. 2017; 2017: 8297814. DOI: 10.1155/2017/8297814