

# Prospects of feed additive incorporation in laying hen diets: a narrative review of principal biological effects and recent developments



Received: Sep 25, 2025  
 Revised: Oct 21, 2025  
 Accepted: Oct 22, 2025

**\*Corresponding author**  
 Jung Min Heo  
 Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 34134, Korea.  
 Tel: +82-42-821-5777  
 E-mail: jmheo@cnu.ac.kr

Copyright © 2026 Korean Society of Animal Science and Technology.  
 This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

**ORCID**  
 Elijah Ogola Oketch  
<https://orcid.org/0000-0003-4364-460X>  
 Jung Min Heo  
<https://orcid.org/0000-0002-3693-1320>

**Competing interests**  
 No potential conflict of interest relevant to this article was reported.

**Funding sources**  
 This research was supported by the Rural Development Administration, Republic of Korea under Project No. RS-2021-RD009516.

**Acknowledgements**  
 Not applicable.

**Availability of data and material**  
 Upon reasonable request, the datasets of this study can be available from the corresponding author.

Elijah Ogola Oketch, Jung Min Heo\*

Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 34134, Korea

## Abstract

Selected feed additives (probiotics, prebiotics, synbiotics, postbiotics, phytogenics, feed enzymes, and organic acids) are reviewed for reported biological responses, and some recent developments when incorporated into laying hen diets. Several feed enzymes (phytase, carbohydrase, protease, and multi-enzymes) have been adopted to improve the nutritive quality of feedstuffs by mitigating inherent digestive function inefficiencies, complementing endogenous enzyme activity, and cleaving anti-nutritional factors abundant in vegetable-based poultry diets. Phytase use is targeted at hydrolyzing phytate to liberate phosphorus and possibly other encapsulated nutrients, with widely reported environmental and economic benefits. Proteases often improve the hydrolysis of amino acids and protein complexes to improve dietary protein digestibility and utilization, potentially restoring performance losses and maintaining the egg quality of hens fed low-protein diets whose CP level has been further reduced. The digestibility-enhancing effects of fiber-degrading carbohydrases are associated with the reduction of intestinal viscosity and improved energy utilization through depolymerization of soluble non-starch polysaccharides. Considering that nutrients exist in a complex matrix involving starch and non-starch polysaccharides, protein, lipids, minerals, and vitamins, laying hens could also benefit from potential additive and synergistic effects accrued from adopting defined feed enzyme combinations. The incorporation of gut-health-promoting feed additives (pre, pro, syn- and postbiotics, phytogenics, organic acids) optimizes feed nutrient utilization by inducing immuno-stimulatory, antimicrobial, and antioxidant activities, modulating gut immune function, and microbial balance and population. Hen responses to feed additives are context-dependent and highly variable due to various factors, including rearing system, age, breed, health status, environmental factors, feed composition and quality, and management. However, on balance, feed additive products with proven efficacy and financial value are recommended for inclusion in laying hen diets; and could potentiate optimized performance and egg quality, reduced feed costs, improved animal welfare and skeletal health, and reduced environmental stress due to nutrient excretion, thereby improving the economic and environmental sustainability of hen egg production. Strategic application of feed additive combinations could potentiate additive and synergistic responses.

**Keywords:** Biological responses, Egg quality, Feed additives, Laying hen, Performance

**Authors' contributions**

Conceptualization: Oketch EO, Heo JM.  
 Writing - original draft: Oketch EO.  
 Writing - review & editing: Oketch EO, Heo JM.

**Ethics approval and consent to participate**

This article does not require IRB/IACUC approval because there are no human and animal participants.

## INTRODUCTION

Increasing human food production is essential in line with the global population increase and food demand. As the fastest-growing animal-based food sector, the poultry industry is well-positioned to address rising consumer expectations and sustainability concerns [1]. The avian egg, beyond being the reproductive unit for the domestic fowl, is an encapsulated nutrient-dense, highly digestible, and reasonably priced food, packing proteins, vitamins, micronutrients, and bioactive substances [2,3]. Accordingly, global egg production has increased significantly by more than 69% from 2000 to 2021 [3], coinciding with improvements in egg demand and hen productivity. Asia is showing the greatest production growth, followed by the Americas, Europe, Africa, and Oceania [4]. With a 34 percent share, China retained its position as the largest hen egg producer; the other main producers (India, the United States of America, Indonesia, Brazil, Mexico, Japan, and Russia) each accounted for 3 to 8 percent of the global production [4]. The combined share of the main producers accounted for more than 69% of the global production by 2021.

The entire layer industry is dependent on the productive efficiency of the hen to lay approximately one sound egg within 24–26 hours, making consistent productivity and egg quality the cornerstone of any successful commercial laying hen enterprise. Modern laying hens exhibit enhanced reproductive performance and could sustain egg laying further beyond 68–70 weeks of age to reach approximately 100 weeks of age and yield almost 500 eggs of acceptable quality [5,6]. Achieving and sustaining this high productivity expectation relies heavily on strategic hen nutrition aimed at maximizing the genetic potential of modern hens, supporting consistent egg production, maintaining egg quality, and ensuring overall health and welfare. Early-life nutrition to achieve optimal body weight and composition at sexual maturity mitigates potential delays in the onset of lay, and optimizing pullet diets potentiates laying persistency [7]. At the same time, the layer industry faces pressure to optimize limited feed resources and phase out antibiotic growth promoters (AGPs) [8]. Since feed constitutes the largest input cost, optimizing nutrient utilization and digestive efficiency is paramount with potential beneficial outcomes on animal performance, health, and welfare, as well as economic and environmental sustainability.

The poultry industry has had to adapt in line with growing pressures revolving around AGP use and public health, environmental pollution, animal welfare, changing consumer expectations, and rising food and feed costs. Notable advances in hen nutrition and modern biotechnology have made it possible to implement several nutritional approaches aimed at reducing feed costs; maintaining hen health, modulating gut microbiome population and balance; improving performance; and optimizing feed nutrient utilization. Improved nutrient utilization reduces nutrient excretion and alleviates environmental stress, promoting sustainable poultry production [9]. Improved nutrient utilization could additionally improve egg quality characteristics for functional value in human nutrition. These nutritional strategies often target gut health, which is defined as the dynamic balance between the diet, commensal microbiome, intestinal mucosa, and immune system essential for maintaining physiological functions, homeostasis, and resilience against stressors [8].

One such strategy is the incorporation of feed additives into hen feeding regimens. Feed additives are typically defined as compounds added to a diet in low amounts (usually 50–500 g/tonne) to elicit targeted responses, independent of the hen's nutritional requirements [10,11]. Several feed additives have been mainstreamed in hen diets to improve feed ingredient quality, performance, and gut health. Feed additive choice depends on regulatory authorization, availability, and most importantly, economic justification [12]. Selected feed additives (probiotics, prebiotics, synbiotics, postbiotics, phytogenics, feed enzymes, and organic acids) are reviewed for their reported effects and recent developments when incorporated into hen diets. Notably, large

amounts of research have been generated on the biological responses of laying hens to feed additive incorporation, and it is beyond our scope to summarize the amount of information in this field. This review explores selected feed additives and their role in modulating productivity, egg quality, and gut health of laying hens while emphasizing key concepts and suggesting critical areas warranting further exploration.

## SELECTED FEED ADDITIVES

### Exogenous enzymes as feed additives

The availability of exogenous feed enzymes with affordable pricing and established efficacy has given nutritionists a viable tool to improve feed ingredient quality [11]. Increased knowledge on target feed constituents and modern biotechnology have made it possible for exogenous feed enzymes to be one of the most extensively researched and widely adopted feed additive and could arguably be the most impactful development in modern poultry nutrition. The growth in the feed enzyme industry is driven by the ban on AGP use to enhance performance and feed utilization efficiency; increased cost and erratic supply of conventional feed ingredients, reinforcing the need to maximize nutrient extraction and reduce wastage through excretion. Feed enzymes targeting various substrates (Table 1) have been mainstreamed in poultry diets to correct the inherent nutrient utilization inefficiency and mitigate antinutritional factors [13]. Feed enzymes work through multiple mechanisms to improve nutrient digestibility, including disruption of cell wall integrity, shifting digestion sites, reducing endogenous secretions, modulating gut microbiota, and the degradation of specific bonds and antinutritional factors, as illustrated in Fig. 1 [11,14,15]. Enhanced nutrient utilization deprives harmful bacteria of nutrients in the lower gut (mainly ceca) and is likely to result in improved performance, health status, and environmental sustainability. Enzymes are either i) added “over the top” to adequately formulated rations for additional improvements or ii) incorporated into nutrient-reduced formulations to restore the nutritional value and compensate for any potentially reduced performance responses.

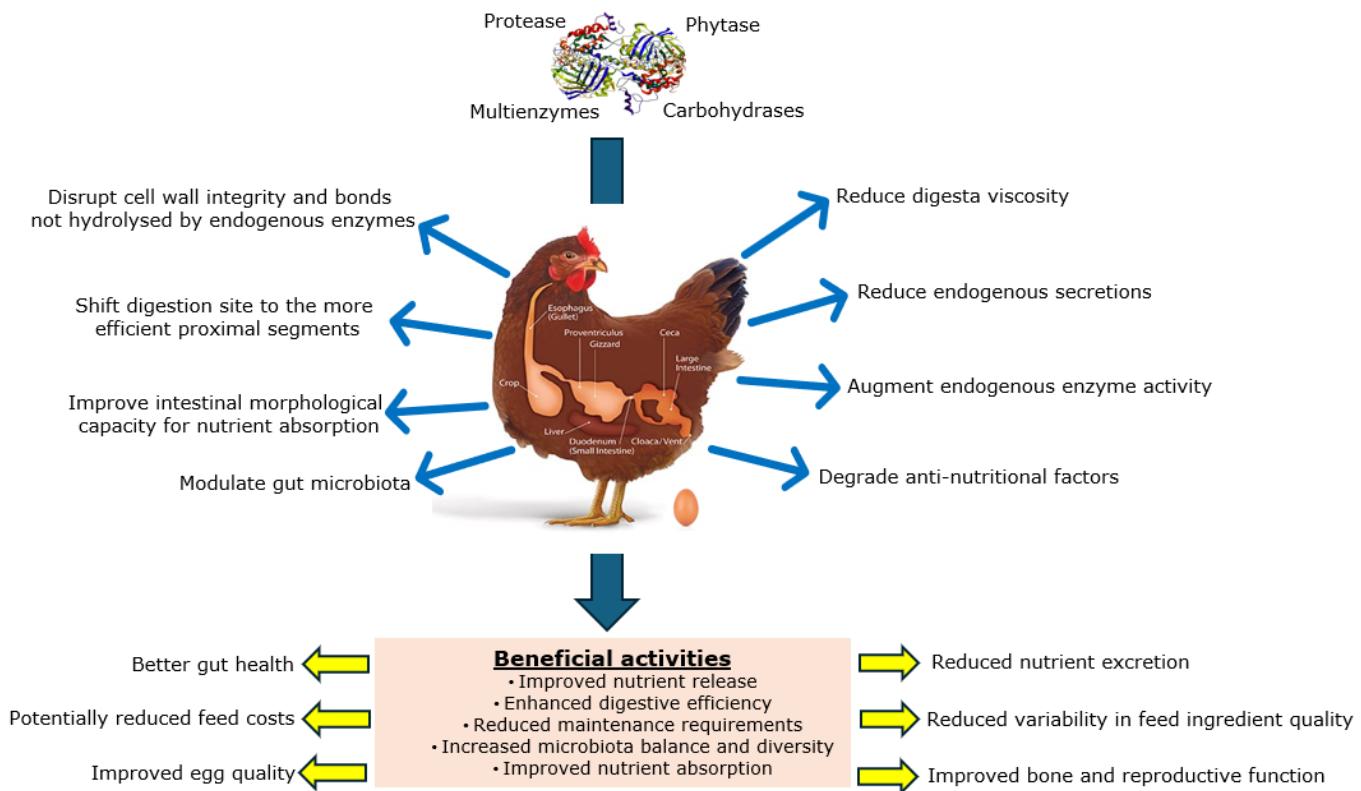
### Phytase

Phosphorus is the third most expensive nutrient in poultry diets, following energy and protein. However, more than 65% of phosphorus in common plant-based feed ingredients is bound to phytate (myo-inositol hexa-phosphate; IP6), rendering it biologically unavailable without enzyme-

**Table 1.** Enzyme types and target substrates

| Enzyme type                         | Target substrate                 | Target feedstuff                                       |
|-------------------------------------|----------------------------------|--|
| Phytases                            | Phytate                          | All plant-derived ingredients                          |
| Proteases                           | Proteins                         | All plant protein sources                              |
| Carbohydrases                       |                                  |  |
| Xylanase                            | Arabinoxylan                     | Wheat, rye, triticale, barley, fibrous plant materials |
| Pectinase                           | Pectin                           | Plant-derived ingredients                              |
| β-Glucanase                         | β-Glucan                         | Barley, oats, and rye                                  |
| α-Galactosidase                     | Oligosaccharides                 | Oilseed meals and grain legumes                        |
| Amylase                             | Amylose                          | Cereal grains, grain legumes                           |
| Mannanase, cellulase, hemicellulase | Mannan, cellulose, hemicellulose | Plant-derived ingredients, fibrous plant materials     |
| Lipase                              | Lipids                           | Lipids in feed ingredients                             |

Data from Kiarie et al. [10], Perera and Ravindran [11], Ravindran [15].



**Fig. 1.** Mode of action, beneficial activities and impacts of incorporating exogenous enzymes in laying hen diets.

induced dephosphorylation. Phytase inclusion has become a common strategy to catalyze phytate and release phosphorus, with well-documented environmental and economic benefits [14]. Particularly, supplemental phytase improves phytate P utilization and reduces P excretion, thereby mitigating environmental pollution. Phytase supplementation in diets low in available phosphorus (avP) could save on feed costs by decreasing the need for inorganic P supplementation [16]. Beyond phosphorus release, phytase could exert 'extra-phosphoric effects' by improving the bioavailability of other encapsulated nutrients, including minerals, energy, and amino acids [17].

Supplemental phytase in hen diets is associated with improved production performance and egg quality, including enhanced shell quality attributed to improved mineral digestibility [18,19]. Beyond production, phytase-mediated improvements in nutrient utilization are linked to improved tibia quality in terms of higher breaking strength and Mg contents [20]. Furthermore, heat stress, known to disrupt the physiological function and reduce mineral absorption and retention [21,22], exacerbates performance losses, compromises immune response and welfare, and could even result in mortalities, causing unnecessary economic losses [23]. Phytase supplementation under heat stress conditions may alleviate these negative responses in laying hens, potentially improving performance and egg quality [24]. Moreover, phytase has been reported to mitigate the stress response induced by low avP diets, as evidenced by reductions in circulating stress hormone levels [20].

The widespread adoption of phytase has generated potential interest in its super-dosing effects at higher than recommended levels [25]. Super-dosing is aimed at greater phytate hydrolysis and liberating as much phosphorus as possible by generating lower esters of IP6. It was previously reported that supplemental phytase at 1,500 FTU/kg resulted in increased inositol phosphate breakdown and bone quality; however, performance and egg quality were unaffected from 40 to 60

weeks of age [17]. Furthermore, Lima et al. [19] reported that optimal performance and egg quality were observed at 1,500 FTU/kg, and further supplementation at 3,000 FTU/kg did not result in extra improvements from 44 to 64 weeks of age. Notably, lower phytase levels (500 and 1,000 FTU/kg) were sufficient to maintain the hens' physical and physiological status [19]. Despite the promising findings, it is the authors' observation that phytase super-dosing in laying hens remains largely unexplored. The next step would be to conduct further research on phytase super-dosing to determine the optimal inclusion levels that will save on feed cost while optimizing performance, egg quality, gut health, and bone function, without adversely affecting the hens' physiological balance.

### **Protease**

The supply of protein (amino acids) occupies the second most expensive component of poultry diets after energy. Strikingly, significant quantities of feed protein (around 18%–20%) are known to escape complete digestion in the avian gastrointestinal tract [26], leading to undesirable hindgut fermentation and elevated nitrogen excretion, with associated negative effects on bird health and environmental sustainability [27]. Exogenous protease supplementation has emerged as a promising strategy to optimize protein digestibility and utilization, particularly in low protein and amino acids (CP/AA) diets [28]. The rationale behind this approach is to provide enough room for protease-mediated improvements in amino acid metabolism that could restore potential performance deficits with reduced CP/AA diets [29]. By improving protein digestibility and utilization, protease adoption also allows for partial displacement of expensive protein ingredients, thereby supporting hen performance at relatively reduced costs.

When incorporated into laying hen diets, protease has been reported to supplement endogenous protease activity and enhance the digestibility of protein and amino acids [29–31]. These improvements are attributed to potential additive effects on gut function, including shifting the site of digestion to more proximal segments [32], reducing endogenous losses [33], and enhancing amino acid availability for mucin synthesis [34]. Additionally, supplemental protease has been associated with enhanced gut morphology [29]; stabilized gut pH [26]; upregulated expression of intestinal amino acid transporters [35]; suppression of pathogenic microorganisms [36]; and mitigation of anti-nutritional factors in plant-based diets [37]. The benefits of protease extend beyond amino acid utilization, thereby improving the digestibility of metabolizable energy (ME), net energy, fat, and starch [33].

The digestibility-enhancing effect of protease is linked to improved performance metrics, including egg mass, weights, and feed conversion ratios [29–31], effectively restoring reported performance losses from feeding low-protein diets [28]. We previously investigated the effects of supplementing a multiprotease combining acid (pepsin-type protease), neutral (metallo-endopeptidase), and alkaline (serine endopeptidase) proteases, produced by *Aspergillus niger*, *Bacillus subtilis*, and *Bacillus licheniformis*, respectively [29]. Multiprotease supplementation led to improved productive performance (feed conversion efficiency, egg weights, egg mass), and egg quality (Haugh units and egg-breaking strength [29]). Improved internal egg quality, especially elevated Haugh units, indicating enhanced egg freshness and protein content [31]. Improved eggshell breaking strength suggests an “extra-proteinaceous” influence on mineral absorption and utilization, potentially reducing egg breakage during transport and handling [29,38].

Furthermore, reducing dietary protein often increases the dietary energy-protein ratio, potentially leading to higher fat deposition, particularly abdominal fat [39]. Although short-term increases in carcass fat may indicate sufficient energy status [40], excessive fat accumulation, as could be the case with longer laying cycles, could decrease egg production and quality [41]. Interestingly, supplemental protease in low CP/AA diets may potentially counteract these effects. For instance, Yi et al. [42] reported that broilers that were fed alkaline protease extracted from *Bacillus licheniformis*

exhibited decreased fat accumulation, likely mediated by a shift in gut microbiota, specifically increased *Bacteroidetes* and reduced *Firmicutes*. These findings warrant further investigation in laying hens to elucidate potential interactions between protease, microbiome composition, and fat metabolism.

Varied results have also been reported [43] and could be explained by differences in diet (protein quality, feed ingredient type) and bird-related factors (age and genotype). Furthermore, excessive CP reduction can compromise performance due to inadequate non-essential amino acids, disrupted electrolyte balance, and lowered potassium levels [44]. Protease products with proven efficacy in improving amino acid digestibility should be considered for inclusion in low-CP diets to restore performance losses, eliciting both economic and environmental benefits. Mineral digestibility and utilization are integral to laying hen performance. It is the author's observation that not much has been done to understand the effects of protease on mineral digestibility and utilization, bone mineralization, and egg quality. Future studies should address these gaps to improve the current understanding of the broader impacts of protease inclusion in laying hen diets.

### ***Carbohydrases***

Energy, a property derived from nutrient metabolism, is known to be the most expensive dietary requirement in feed formulations. As monogastrics, poultry inherently lack the endogenous enzymes to degrade the complex structures of plant cell walls, especially non-starch polysaccharides (NSPs) present in common feedstuffs [45,46]. NSPs are a diverse group of complex carbohydrates that differ in structure, size, and water solubility. They include cellulose, hemicelluloses such as arabinoxylans,  $\beta$ -glucans, and fructans [47,48]. In common grain-based poultry diets, cellulose, arabinoxylans, and  $\beta$ -glucans make up the bulk of the fiber content [49]. NSPs impair nutrient utilization by increasing digesta viscosity, inhibiting intestinal peristalsis, prolonging digesta passage rate, disrupting microbiota balance, and reducing endogenous enzyme activity [47–49]. It is becoming increasingly important to consider the role of NSPs, particularly  $\beta$ -mannans, in triggering what is known as a feed-induced immune response [50]. This response causes birds to expend additional energy to sustain an unnecessary immune activation, ultimately diverting resources away from growth and productive purposes [51]. Collectively, these digestive disturbances reduce nutrient digestibility and performance [48,49]. To address the rising energy supply cost and improve energy utilization, exogenous carbohydrases such as xylanases,  $\beta$ -mannanases, and  $\beta$ -glucanases are increasingly being adopted to catalyze specific substrates, as illustrated in Table 1 [15,49,52]. Increased energy utilization efficiency may partially compensate energy requirements and allow the inclusion of relatively inexpensive and mostly fibrous ingredients, reducing feed costs without compromising performance [53].

Today, nearly all diets that are wheat or barley-based incorporate xylanase and  $\beta$ -glucanase enzymes to improve nutrient digestion and feed efficiency. Increasing evidence shows that carbohydrases improve nutrient digestibility by depolymerizing soluble NSPs, reducing intestinal viscosity, and enhancing nutrient availability [46,47,54]. Supplemental beta 1–4, endo-xylanase was reported to modulate gut viscosity, caecal pH, digesta transit, NSP degradation, and microbiota composition, leading to improved energy utilization and lower excreta moisture [55]. Lowered excreta moisture is correlated with reduced incidence of dirty eggs [56], even though conflicting results have also been reported [53,57]. Xylanase supplementation has also been associated with improved feed conversion ratio, egg mass, egg production, and egg quality traits such as yolk color, shell thickness, albumen height, and Haugh unit [45,53,58]. Similarly, supplemental  $\beta$ -mannanase restored the performance losses of energy-reduced diets by modulating gut morphology, reducing inflammation, improving energy utilization, and promoting beneficial cecal microbiota [47].

Supplemental carbohydrase effects on hen performance and egg quality are variable and inconsistent. For instance, Cufadar et al. [59] reported that the laying performance of White Leghorn Lohmann Selected Leghorn (LSL) laying hens was unaffected by a bacterial endo 1,4- $\beta$ -xylanase supplemented from 52 to 64 weeks of age. These observations suggest the high degree of complexity in the development and application of carbohydrases in laying hen diets, presenting both challenges and opportunities for optimizing carbohydrase enzyme utilization. Variability in response is primarily attributed to differences in NSP type and concentration [48], alongside other factors such as hen age and strain, enzyme source and dose, and feed ingredient composition and batch variation [45,57]. Carbohydrase products with proven efficacy in nutrient digestibility should be considered for inclusion in laying hen diets and could potentiate improved productive performance and egg quality.

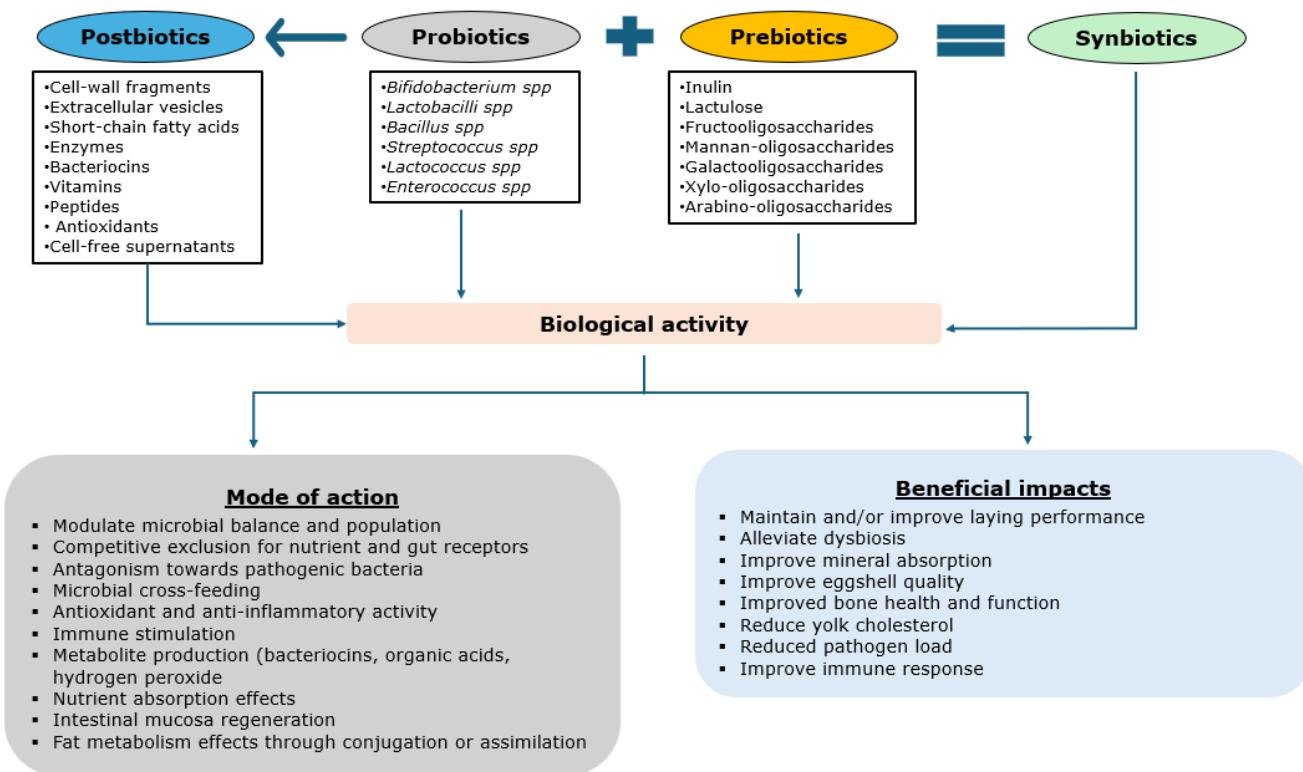
### ***Multienzymes***

Poultry diets constitute multiple ingredients (corn, wheat, soybean meal, by-products) that are structurally complex and could each contain different antinutritional factors (NSPs, phytates, and protease inhibitors). Supplemental multienzymes have been investigated as a strategy to enhance complementary and additive effects across various feed components and are postulated to be more effective than single enzyme approaches in greater substrate hydrolysis and reducing the antinutritive effects on overall nutrient utilization [52]. Gunawardana et al. [60] demonstrated that a multienzyme blend containing xylanases,  $\beta$ -glucanases, mannanases, pectinases, and proteases improved energy and protein utilization, effectively improving egg production, body weight, egg mass, feed conversion, and albumen and yolk solids. Concomitantly, Scheideler et al. [61] reported that a multi-enzyme combining xylanase, protease, and amylase influenced protein and mineral (calcium and phosphorus) retention without affecting feed intake, feed conversion efficiency, egg production, egg weight, or egg mass. Furthermore, a non-starch polysaccharide-targeting enzyme blend containing xylanase,  $\beta$ -glucanase, galactosidase, and galactomannanase increased nitrogen digestibility and reduced excreta ammonia emissions with no adverse effects on egg quality or productive performance [62]. Further evidence reported synergistic effects on laying performance and egg quality of hens, which are attributed to the modulation of gut health [63,64]. These findings support the strategic adoption of multienzymes as a promising approach to optimize nutrient utilization, enhance production efficiency, and improve environmental sustainability for laying hens.

### ***Biotics as feed additives***

Chicken gut harbors a highly complex and dynamic microbial ecosystem that constitutes an integral part of the gut health nexus with definitive impacts on the overall health and productivity [65–67]. Several feed-related approaches targeted at modulating the gut microbiome are available, including probiotics, prebiotics, synbiotics, and postbiotics. Probiotics are defined as single or mixed cultures of non-pathogenic, live microbes that could exert health and productive benefits to the host when supplied in adequate amounts. For optimal efficacy, probiotic microbes must be non-pathogenic, improve gut function and health, adhere to the intestinal epithelium, survive and thrive in the prevailing acidic environment in the gut, and retain viability during storage, processing, and transportation [68]. Some commonly used probiotic bacteria species include *Bacillus*, *Streptococcus*, *Lactobacillus*, *Lactococcus*, *Saccharomyces*, *Aspergillus*, and *Enterococcus* [69]. Probiotic bacteria could be delivered as single or multi-strain formulations via feed or water in the form of either granules, powder, liquid, paste, or gel [69].

Even though the probiotic mode of action is complex (Fig. 2), probiotics are suggested to be most effective following a disturbance. The beneficial effects of live microbial feed supplements



**Fig. 2.** Classification, mode of action and some beneficial activities associated with biotic feed additives in laying hen diets.

are mediated through multiple pathways including the microbiota–gut–brain, microbiota–gut–immune, and the microbiota–gut–bone axes. Probiotic bacteria enhance microbial communities through competitive exclusion and antagonism towards pathogenic bacteria [70,71]. By improving and/or maintaining intestinal microbial diversity and balance, probiotics are reported to enhance colonization resistance against stressors; catalyze immune responses; promote gut integrity; and improve laying hen performance [71]. Probiotic-mediated improvements in laying hen performance, egg quality, and physiological responses are often linked to metabolites such as short-chain fatty acids (SCFAs) and bile acids [66].

We previously reported that multi-strain probiotic supplementation of *Bacillus subtilis* PB6, *B. subtilis* FXA, and *B. licheniformis* G3 at  $3 \times 10^8$  CFU/kg of feed improved egg quality, several tibia traits, and populations of beneficial cecal bacteria while reducing egg yolk cholesterol [67]. Suggesting the impact of the microbiota–gut–liver axis in bile acid enterohepatic circulation, the capacity of probiotics to lower yolk cholesterol has been corroborated by Li et al. [72] using a dried *Bacillus subtilis* culture. Reduced yolk cholesterol levels are attributed to several mechanisms, including bile salt hydrolase-mediated deconjugation of conjugated bile acids into free bile acids, which are less efficiently reabsorbed in the ileum and more readily excreted via the feces [67]. Additionally, probiotic bacteria can directly assimilate cholesterol for their metabolism. Furthermore, SCFAs, particularly propionate, may inhibit hepatic cholesterol synthesis by downregulating 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), the rate-limiting enzyme in the mevalonate pathway [73]. These probiotic-mediated processes in reducing yolk cholesterol align with health-conscious consumer demands.

As breeding targets are increasingly focused on extended laying cycles, bone health becomes increasingly critical for maintaining performance and egg quality [74]. Laying hens possess three

primary bone types- cortical, trabecular, and medullary. The medullary bone, which develops at the onset of sexual maturity, acts as a labile calcium reservoir buffering against dietary calcium insufficiency during eggshell formation [6]. In contrast, cortical bone provides structural support for the skeleton and is produced through osteoblastic activity till the onset of sexual maturity and egg production. Long-term laying behavior leads to supplemental calcium extraction from structural bone (cortical and trabecular), occasioning net loss of mineral mass, and may lead to osteoporosis (cage fatigue syndrome) and reduced egg quality over time [75]. Improvement of tibia traits is linked to probiotic modulation of mineral absorption and bone mineralization through various mechanisms, including stimulating intestinal epithelial cell proliferation and differentiation [66], and lowering gut pH [67], potentially mitigating age-related skeletal deterioration and eggshell defects.

Prebiotics, on the other hand, are selectively fermented feed ingredients that stimulate the growth and activity of beneficial gut bacteria, indirectly exerting beneficial effects on host health [71]. Prebiotics could fuel the growth of beneficial gut microbes while limiting the establishment of foodborne pathogens, thereby improving host microbial balance [76]. Prebiotic fermentation could also inhibit hindgut protein fermentation, effectively decreasing toxic secondary nitrogen metabolites, improving nitrogen balance, and promoting gut health function [77]. Common prebiotic compounds include oligosaccharides such as inulin, lactulose, fructooligosaccharides (FOS), mannan-oligosaccharides (MOS), galactooligosaccharides (GOS), and xylo-oligosaccharides [70]. Prebiotics may exert effects similar to probiotics by enhancing gut health, modulating immune responses, and improving host performance (Fig. 2). For instance, dietary inclusion of MOS has been reported to enhance productive performance and reproductive function in laying hens [78]. Conversely, productive performance, immune response, and blood parameters of laying hens were unaffected by FOS [79].

Furthermore, it is also reasoned that synergistic effects could be drawn from both prebiotics and probiotics in a combined form as synbiotics. The justification for synbiotics is that a fermentable prebiotic substrate could increase the number, survival, and establishment of beneficial probiotic bacteria [71]. In laying hens, synbiotics are increasingly explored as nutritional strategies to optimize gut health, nutrient digestibility, productive performance, egg quality, immune modulation, and pathogen suppression [71]. However, it has been noted that consistent additive or synergistic impacts are rare. In many cases, either prebiotics or probiotics could effectively improve laying performance, egg quality, nutrient absorption, and host physiological responses [80].

Recently, attention has been drawn to postbiotics, which are soluble and non-viable metabolites from microorganisms that have biological activity when supplied in adequate amounts. These include cell wall fragments, extracellular vesicles, short-chain fatty acids, enzymes, bacteriocins, vitamins, and other metabolic by-products with the potential to suppress pathogens, strengthen gut barrier function, and modulate immunity and gut microbiome [81]. Several other names have been used for postbiotics, including pseudo-probiotics, ghost probiotics, paraprobiotics, metabiotics, abiotics, cell-free supernatants, and biogenics [82]. Postbiotics are reported to overcome some problems associated with probiotics, including viability during storage, the temporary nature of colonization, and the possible transfer of virulent genes to pathogenic bacteria. Postbiotics are ascribed to be stable and safe with reduced impact on feed nutrient components [82]. Choe et al. [83] reported that metabolite combinations of the *Lactobacillus plantarum* RI11, RG14, and RG11 improved egg production, modulated fecal microbiota and pH, triggered intestinal morphological changes, and reduced plasma and yolk cholesterol concentrations.

Collectively, dietary approaches targeting gut microbiota can regulate the delicate balance between microbiota, diet, mucosa, and immune function, with definite influences on hen health and overall productivity [8,84]. Nevertheless, variabilities in responses remain a challenge, suggesting

the complex nature of the development and application of these microbiota-modulating approaches in laying hen diets. For instance, Mahdavi et al. [85] found no significant effects of a multi-strain probiotic containing *B. subtilis* and *B. licheniformis* on laying performance or egg quality. The observed variabilities stress the species and/or strain specificity of probiotic bacteria and are attributed to the differences in microbial strains, dosages of administration, administration methods, environmental stress, and diet composition [67]. These variabilities present an exciting opportunity for the continued evaluation of probiotics, prebiotics, synbiotics, and postbiotics, when used singly or in combination, to determine their specific efficacy when incorporated into laying hen diets.

### Organic acids as feed additives

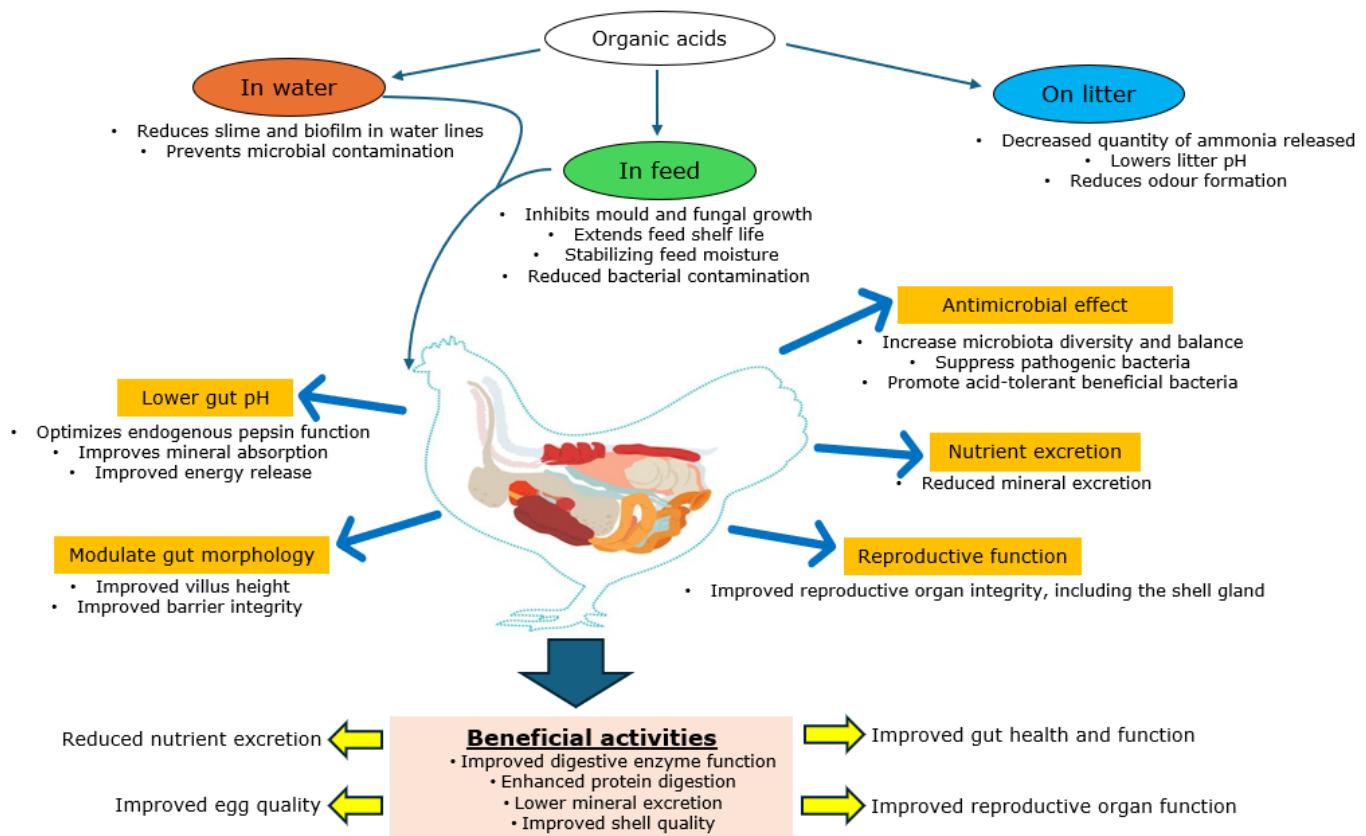
Organic acids (OAs) are original constituents of plant and animal tissues and include a variety of acids such as lactate, acetate, propionate, butyrate, and tannic acids, among others [70,86]. They are also produced via microbial fermentation of carbohydrates in the ceca. According to Pham et al. [87], OAs are chemically classified according to their carbon chain length into SCFAs (1–6 carbon atoms), medium-chain fatty acids (MCFAs; 7–12 carbon atoms), or long-chain fatty acids (LCFAs; 13–21 carbon atoms). Singular or combined forms of OAs are supplied in water, sprayed in litter, or mixed in feed to exert several beneficial activities related to energy metabolism, antimicrobial control, and feed quality preservation [88]. Regarding energy metabolism, acetate is utilized as an energy substrate for muscle tissue, propionate supports the gluconeogenic pathway to generate glucose, and butyrate fuels ceco-colonic epithelial cells [86].

Beyond metabolic functions, OAs exert antimicrobial effects against pH-sensitive bacteria; lower gut pH; promote gut development, maturation, and integrity; and enhance nutrient utilization, health, and productivity as illustrated in Fig. 3 [71,88]. Their ability to lower chyme pH increases pepsin activity and calcium uptake, promoting protein degradation and mineral uptake [86]. These improvements can translate to improved internal egg and eggshell quality [89–91]. Improved eggshell qualities are attributed to improved integrity of reproductive organs, particularly the shell gland [92]. Improvements in egg production and weight have also been reported [90,93], though a lack of significant effects on both egg production and quality has also been observed [94].

The variability in responses is attributed to differences in OA type, dosage used, hen age, and diet composition. The effectiveness of OAs depends on their ability to change from the undissociated to the dissociated form, their pKa value, and hydrophobicity. Of interest, the activity and concentration of OAs could be reduced in the distal gut segments unless they are protected by encapsulation [10,95]. Furthermore, blending different OAs is recommended to cater to variations in membrane permeability [70]. Notably, excessive supplementation can be detrimental, leading to reduced villus height and width, and crypt depth [96]. Administration of low dosages [70] or adherence to proper dosage [86] is crucial to maintain overall gut health as previously defined [8]. Continuous testing to determine the optimal dosage that supports performance and gut health function without adversely affecting the hens' physiological and metabolic balance is recommended.

### Phylogenics as feed additives

Phylogenetic feed additives (PFAs) are a wide variety of natural bioactive compounds derived from plants. They include complex secondary constituents, such as terpenoids (linalool, menthol, borneol, geraniol,  $\alpha$ -terpineol), phenolics (tannins), and low molecular weight aliphatic hydrocarbons (thymol, eugenol, carvacrol, cinnamaldehyde) [97]. PFAs are broadly classified based on their biological origin, formulation, chemical description, and purity into herbs, botanicals, essential oils, and oleoresins [98]. Phylogenics are thought to be natural, less toxic, and residue-free, and thus have been widely applied in both human and animal industries in the form of oregano, green tea,

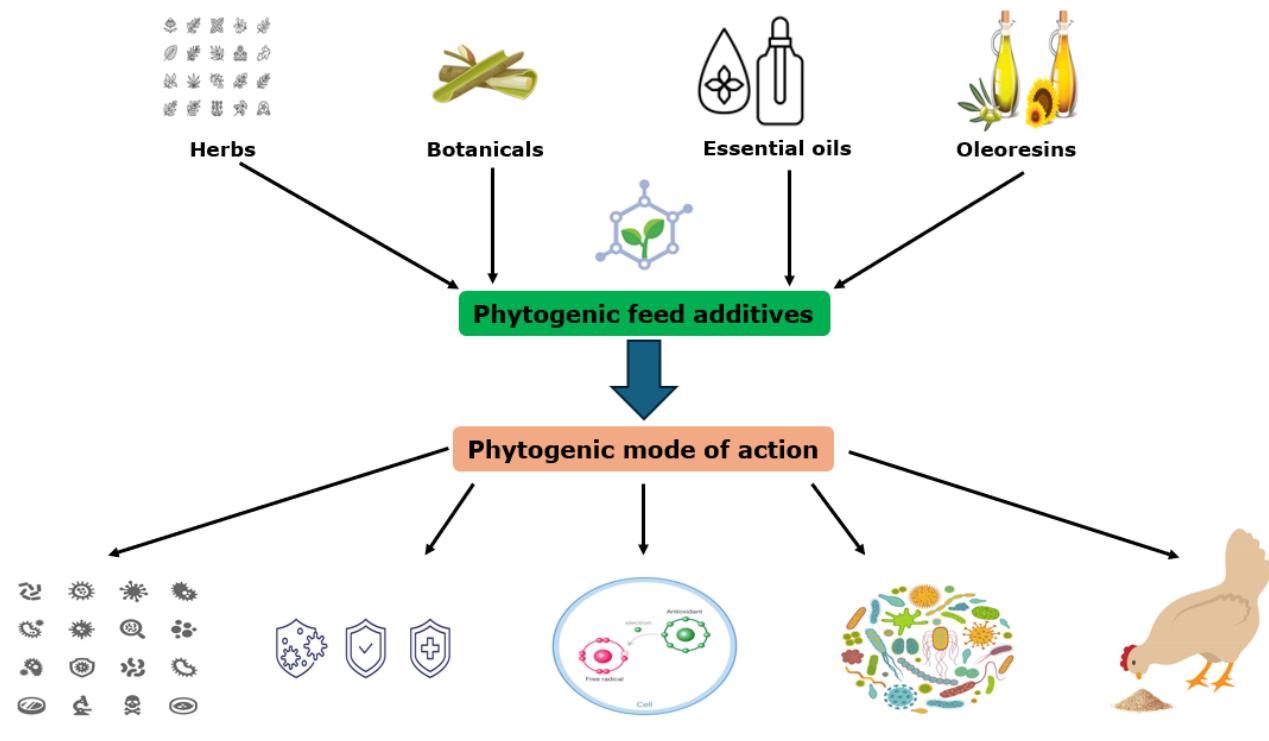


**Fig. 3.** Different forms of application and possible mode of action of organic acids.

peppermint, aloe vera, moringa, cinnamon, garlic, thyme, turmeric, rosemary, and coriander, among many others [13,99]. Phytogenics are widely reported to possess strong antimicrobial, immune-enhancing, anti-inflammatory, and antioxidant activities [13].

Although the phytogenic mechanism of action is complex and not completely elucidated, it is suggested that PFA activate the aryl hydrocarbon receptor (AhR) and nuclear factor-erythroid-derived 2-like 2 (Nrf2) signaling pathways, which induce cytoprotective, homeostatic, and immune-protective effects, as illustrated in Fig. 4 [100,101]. Specifically, AhR regulates the expression of genes responsible for the detoxification and elimination of xenobiotic compounds, while Rrf2 regulates antioxidant response and inflammatory modulation [100]. Additionally, PFA may induce antipathogenic effects by damaging bacterial membranes, promoting the colonization of beneficial gut microbiota, or modulating immune responses [13]. Strikingly, essential oils (steam-distilled extracts of volatile plant compounds) are ascribed to be of higher biological activity and have been widely tested in poultry diets [102,103].

The beneficial phytogenic-mediated effects on production performance and egg quality have been widely reported [103,104]. Reported improvements include enhanced eggshell thickness, higher egg weight and mass, improved tibia traits, and protein digestibility [105–107]. Additionally, supplemental PFA-induced modulation of cecal microbiota and gut morphology (longer villus heights and higher villus heights to crypt depth ratio) could translate to improved internal egg nutrient content (riboflavin, thiamine, selenium, and phosphorus) and thicker eggshells [108]. Dietary PFA were reported to down-regulate AhR-associated gene expression while up-regulating the Nrf2-related genes, leading to improved gut cytoprotection and performance [100]. Notably,



**Fig. 4.** Overview of phytogenic feed additives, including classifications and potential modes of action.

inconsistent results regarding supplemental phytogenics have also been documented [105].

A wide variety of PFAs are available for dietary utilization across different geographical areas of the world, but the efficacy of PFA supplementation is debatable due to their complexity, instability, lack of full understanding of their modes of action, and hence, the commonly reported variabilities in laying hen responses to PFAs. Variability in phytogenic efficacy is attributed to the differences in source and composition of the active components, method of preparation and storage, feed inclusion levels, bird genetics and age (laying phase), and overall diet composition [70,101,109]. When incorporating PFAs into diets, consideration should be paid to accreditation, potential contamination, mode of action, experimental testing, quality, feed matrix interactions, and economic value [12]. Strikingly, a negative effect of higher essential oil dosages was observed on biomechanical properties and mineral contents of the tibia [106], highlighting the importance of appropriate dosing used to avoid undesired physiological responses. More effort is still needed to determine the appropriate inclusion levels of phytogenic feed additives and to fully expound their mode of action on gut microbiota, function, and immunology, and bird performance when incorporated into laying hen diets.

### Combination of feed additives

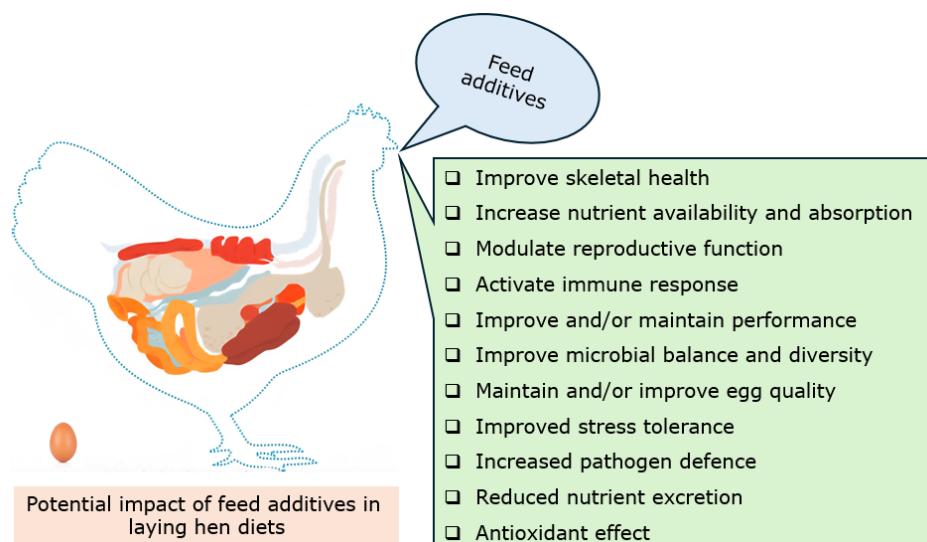
Nutrients exist in a complex matrix involving starch and non-starch polysaccharides, protein, lipids, minerals, and vitamins [33]. It is reasoned that feed additive combinations may yield synergistic and additive effects, especially in AGP-free systems [9,110]. Organic acids may synergize with

probiotics (by lowering pH favoring *Lactobacilli*) and with enzymes (improving digestibility). While antinutritional factors in PFAs could limit their utility, co-supplementation with exogenous enzymes could improve their bioavailability and effectiveness [99]. Furthermore, NSP depolymerization by carbohydrases generates oligosaccharides that may exert a prebiotic effect, supporting live in-feed microbials [57,111]. Supplemental  $\beta$ -mannanase derived from *Paenibacillus latus* bacteria combined with a multi-strain probiotic (*Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Enterococcus faecium*, and *Streptococcus thermophilus*) improved the laying rate, egg weight, and egg mass while modulating intestinal morphology [112].

Recently, attention has been drawn to stimbiotics, defined as non-digestible but fermentable additives that accelerate fiber-degrading microbiome establishment in the gastrointestinal tract (GIT) [113]. Stimbiotic dosages are usually low and enhance the fermentation of dietary fiber already present in the feed, rather than serving as a direct fermentable substrate as in the case of prebiotics [113]. While stimulating probiotic bacteria without providing adequate substrates can induce detrimental effects on microbiota balance, xylanase inclusion when feeding high levels of prebiotic xylo-oligosaccharides exerted stimbiotic effects, optimizing probiotic bacteria diversity [114]. Furthermore, combined incorporation of compound acidifiers (fumaric, sorbic, citric, and malic acids at 1.5 g/kg) and plant essential oils (cinnamaldehyde, carvacrol, and thymol at 100 mg/kg) improved egg quality, alleviated inflammatory responses, increased digestive enzyme activities, and enhanced serum antioxidant capacity [97]. However, excessive supplementation of the compound acidifiers increased serum Malonyldialdehyde (MDA) levels in the Wang et al. [97] study, indicating enhanced oxidative stress. The need to determine the optimal dosage and ratio of combined feed additives that will maximize benefits without compromising the physiological functions of the birds is stressed. Future development of feed additives should consider combinations to determine potential synergistic and additive effects in laying hen responses.

## FUTURE DIRECTIONS AND SUMMARY

Given the considerable advances in the understanding of laying hen nutrition and modern biotechnology, several feed additives are routinely incorporated into the feeding program of laying hens. Despite being used in low amounts (usually 50–500 g/tonne), the selected feed additives (probiotics, prebiotics, synbiotics, postbiotics, phytogenics, feed enzymes, and organic acids) showed immense potential to maintain productive performance and egg quality and could also improve gut and musculoskeletal health and function. The strategic application of feed additive combinations could also potentiate several additive and synergistic responses. Optimized nutrient utilization through feed additives is aimed at reducing feed costs, maintaining animal health and welfare, improving productivity, and reducing nutrient excretion to the environment. Reduced nutrient excretion could alleviate environmental stress and contribute to the sustainability of poultry production. Furthermore, improved nutrient utilization could enrich egg quality characteristics for their functional value in human nutrition. Feed additive products with proven efficacy are recommended for inclusion in laying hen diets and could potentiate several responses, as summarised in Fig. 5. A pragmatic future for layer nutrition lies in strategies that could integrate enzymes to unlock maximal feed nutrient value; biotics to stabilize microbiota; organic acids to exert antimicrobial effects against pH-sensitive bacteria and optimize gut function; and phytogenics to enhance antioxidant status, immune function and gut resilience. However, while many effects are described, deeper understanding of gut microbiome shifts, mucosal immunity pathways, and systemic metabolic effects in layers is incomplete. It is also evident that laying hen responses seem to



**Fig. 5. Possible impacts of the dietary incorporation of the selected feed additives in laying hen diets.**

be largely dependent on breed, hen age, health status, feed composition and quality, environmental factors, and management. These variabilities in laying hen responses are not uncommon, suggesting that it may not always be economically beneficial to supplement feed additive compounds, especially under commercial conditions. The reported variabilities present an opportunity for further research on the specific efficacy of these feed additives on the performance, egg quality, and gut health of laying hens under varied experimental conditions that could favorably mimic commercial conditions with larger flock sizes and extended laying cycles. Practical implementation will require problem diagnosis (poor shell strength, inconsistent egg weight, pathogen load); defining expected outcomes; evidence-based product selection; careful formulation (matrix adjustments for enzymes); consideration of additive compatibility when combinations are adopted; accounting for strain- and product-specific variation (particularly for probiotics and phytogenics); monitoring performance; and economic assessment to gauge if the returns justify the expense. Ultimately, we wish to maintain egg quality and safety; achieve breeding objectives of longer laying cycles and persistence in laying performance; and improve skeletal, immune, and gut health under commercial conditions.

## REFERENCES

1. Kleyn FJ, Ciacciariello M. Future demands of the poultry industry: will we meet our commitments sustainably in developed and developing economies? *World's Poult Sci J.* 2021;77:267-78. <https://doi.org/10.1080/00439339.2021.1904314>
2. Lee JH, Lee Y, Paik HD, Park E. Antioxidant and immune-modulating activities of egg yolk protein extracts. *Food Sci Anim Resour.* 2022;42:321-31. <https://doi.org/10.5851/kosfa.2022.e3>
3. Oketch E, Yu M, Nawarathne S, Chaturanga N, Lee J, Park H, et al. Evaluating phenotypic correlations between internal and external egg quality indicators of Hy-Line Brown laying hens at 35 weeks of age. *J Anim Sci Technol.* 2025. <https://doi.org/10.5187/jast.2025.e24>
4. FAO (Food and Agriculture Organization of the United Nations). Production, trade and prices of commodities. *World Food and Agriculture: Statistical Yearbook 2023.* FAO; 2023.
5. Bain MM, Nys Y, Dunn IC. Increasing persistency in lay and stabilising egg quality in longer

laying cycles. What are the challenges? *Br Poult Sci.* 2016;57:330-8. <https://doi.org/10.1080/00071668.2016.1161727>

- 6. Hanlon C, Takeshima K, Kiarie EG, Bédécarrats GY. Bone and eggshell quality throughout an extended laying cycle in three strains of layers spanning 50 years of selection. *Poult Sci.* 2022;101:101672. <https://doi.org/10.1016/j.psj.2021.101672>
- 7. Kiarie EG, Cheng V, Tan Z, Chen W, Xu X, Peng Y, et al. Comparative impact of bacitracin and select feed additives in the feeding program of Lohmann LSL-Lite pullets at the onset of lay through to 31 weeks of age. *Trans Anim Sci.* 2024;8:txae013. <https://doi.org/10.1093/tas/txae013>
- 8. Oketch EO, Wickramasuriya SS, Oh S, Choi JS, Heo JM. Physiology of lipid digestion and absorption in poultry: an updated review on the supplementation of exogenous emulsifiers in broiler diets. *J Anim Physiol Anim Nutr.* 2023;107:1429-43. <https://doi.org/10.1111/jpn.13859>
- 9. Heo JM, Opapeju FO, Pluske JR, Kim JC, Hampson DJ, Nyachoti CM. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. *J Anim Physiol Anim Nutr.* 2013;97:207-37. <https://doi.org/10.1111/j.1439-0396.2012.01284.x>
- 10. Kiarie E, Walsh MC, Nyachoti CM. Performance, digestive function, and mucosal responses to selected feed additives for pigs. *J Anim Sci.* 2016;94 suppl 3:169-80. <https://doi.org/10.2527/jas.2015-9835>
- 11. Perera WNU, Ravindran V. Role of feed additives in poultry nutrition: historical, current and future perspectives. *Anim Feed Sci Technol.* 2025;326:116371. <https://doi.org/10.1016/j.anifeedsci.2025.116371>
- 12. Kleyn R, Roosendaal B. The use of new-era feed additives to enhance gut health, immune status, nutrient absorption and broiler performance. Presented at 7th International Broiler Nutritionists' Conference, Poultry Beyond 2030; 2024 Sep 16-20; Queenstown, New Zealand.
- 13. Abd El-Hack ME, El-Saadony MT, Salem HM, El-Tahan AM, Soliman MM, Youssef GBA, et al. Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. *Poult Sci.* 2022;101:101696. <https://doi.org/10.1016/j.psj.2022.101696>
- 14. Kiarie E, Romero LF, Nyachoti CM. The role of added feed enzymes in promoting gut health in swine and poultry. *Nutr Res Rev.* 2013;26:71-88. <https://doi.org/10.1017/S0954422413000048>
- 15. Ravindran V. Feed enzymes: the science, practice, and metabolic realities. *J Appl Poult Res.* 2013;22:628-36. <https://doi.org/10.3382/japr.2013-00739>
- 16. Yao JH, Han JC, Wu SY, Xu M, Zhong LL, Liu YR, et al. Supplemental wheat bran and microbial phytase could replace inorganic phosphorus in laying hen diets. *Czech J Anim Sci.* 2007;52:407-13. <https://doi.org/10.17221/2321-CJAS>
- 17. Waters CA, Wamsley KGS, Elliot MA, Bedford M, Wyatt C, Kim WK, et al. The response of laying hen production, performance, bone health, and inositol levels to limestone particle size ratios and phytase levels supplemented during the post-peak period (40–60 wk of age). *J Appl Poult Res.* 2024;33:100407. <https://doi.org/10.1016/j.japr.2024.100407>
- 18. Gao CQ, Ji C, Zhang JY, Zhao LH, Ma QG. Effect of a novel plant phytase on performance, egg quality, apparent ileal nutrient digestibility and bone mineralization of laying hens fed corn–soybean diets. *Anim Feed Sci Technol.* 2013;186:101-5. <https://doi.org/10.1016/j.anifeedsci.2013.09.011>
- 19. Lima GS, Cavalcante DT, Guerra RR, Vilar da Silva JH, de Brito AB, Gomes GA, et al.

Dosing of bacterial phytase (EC 3.1.3.26) in laying hens' diets. *Animals*. 2024;14:3219. <https://doi.org/10.3390/ani14223219>

20. Park J, Heo YJ, Kim DH, Kim YB, Kwon BY, Song JY, et al. Nutritional and physiological responses to dietary phosphorus levels and phytase in pullets and laying hens. *Poult Sci*. 2024;103:103886. <https://doi.org/10.1016/j.psj.2024.103886>
21. Lee SA, Nagalakshmi D, Raju MVLN, Rao SVR, Bedford MR, Walk CL. Phytase as an alleviator of high-temperature stress in broilers fed adequate and low dietary calcium. *Poult Sci*. 2019;98(5):2122-32. <https://doi.org/10.3382/ps/pey566>
22. Oluwagbenga EM, Fraley GS. Heat stress and poultry production: a comprehensive review. *Poult Sci*. 2023;102:103141. <https://doi.org/10.1016/j.psj.2023.103141>
23. Kim DH, Lee KW. An update on heat stress in laying hens. *World's Poult Sci J*. 2023;79:689-712. <https://doi.org/10.1080/00439339.2023.2239769>
24. Mohebbifar A, Torki M, Ghasemi HA. Effect of phytase supplementation of diets with different levels of rice bran and non-phytate phosphorus on productive performance, egg quality traits, leukocytes profile and serum lipids of laying hens reared indoor under high environmental temperatures. *Anim Feed Sci Technol*. 2015;207:222-33. <https://doi.org/10.1016/j.anifeedsci.2015.06.024>
25. Ogola OE, Choi JS, Hong JS, Kim YB, Nawarathne SR, Yu M, et al. Determination of dietary Ca and P levels and their equivalence values of phytase and vitamin D<sub>3</sub> for improved growth performance in weanling pigs. *Korean J Agric Sci*. 2021;48:397-412. <https://doi.org/10.7744/kjoas.20210030>
26. Stefanello C, Dalmoro YK, Rosa DP, Teixeira L, Sorbara JOB, Cowieson AJ, et al. Effects of dietary digestible amino acids and a novel exogenous sfericase protease on growth performance of broilers. *Heliyon*. 2024;10(5):e26098. <https://doi.org/10.1016/j.heliyon.2024.e26098>
27. Elling-Staats ML, Gilbert MS, Smidt H, Kwakkel RP. Caecal protein fermentation in broilers: a review. *World's Poult Sci J*. 2022;78:103-23. <https://doi.org/10.1080/00439339.2022.2003170>
28. Poudel I, Hodge VR, Wamsley KGS, Roberson K, Adhikari PA. Effects of protease enzyme supplementation and varying levels of amino acid inclusion on productive performance, egg quality, amino acid digestibility, and economics of egg production in Hy-Line W-36 laying hen from 50 to 70 wk of age. *J Appl Poult Res*. 2024;33:100452. <https://doi.org/10.1016/j.japr.2024.100452>
29. Oketch EO, Yu M, Nawarathne SR, Chaturanga NC, Maniragaha 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;104:104977. <https://doi.org/10.1016/j.psj.2025.104977>
30. Chen X, Ma W, Hu N, Yan Y, Zhu Y, Wang Z, et al. Effects of alkaline protease on the production performance, egg quality, and cecal microbiota of hens during late laying period. *Anim Sci J*. 2021;92:e13658. <https://doi.org/10.1111/asj.13658>
31. Poudel I, Hodge VR, Wamsley KGS, Roberson KD, 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;102:102465. <https://doi.org/10.1016/j.psj.2022.102465>
32. Liu SY, Selle PH, Court SG, Cowieson AJ. Protease supplementation of sorghum-based broiler diets enhances amino acid digestibility coefficients in four small intestinal sites and accelerates their rates of digestion. *Anim Feed Sci Technol*. 2013;183:175-83. <https://doi.org/10.1016/j.anifeedsci.2013.05.006>

33. Cowieson AJ, Roos FF. Toward optimal value creation through the application of exogenous mono-component protease in the diets of non-ruminants. *Anim Feed Sci Technol.* 2016;221:331-40. <https://doi.org/10.1016/j.anifeedsci.2016.04.015>
34. Cowieson AJ, Roos FF. Bioefficacy of a mono-component protease in the diets of pigs and poultry: a meta-analysis of effect on ileal amino acid digestibility. *J Appl Anim Nutr.* 2013;2:e13. <https://doi.org/10.1017/jan.2014.5>
35. Park JH, Lee SI, Kim IH. The effect of protease on growth performance, nutrient digestibility, and expression of growth-related genes and amino acid transporters in broilers. *J Anim Sci Technol.* 2020;62:614-27. <https://doi.org/10.5187/jast.2020.62.5.614>
36. de Cesare A, Faria do Valle I, Sala C, Sirri F, Astolfi A, Castellani G, et al. Effect of a low protein diet on chicken ceca microbiome and productive performances. *Poult Sci.* 2019;98:3963-76. <https://doi.org/10.3382/ps/pez132>
37. Peñuela-Sierra LM, Aragão-Neto VL, Lozano-Cruz P, Mejia-Abaunza JN, Ali M, Cabañas-Ojeda J, et al. Multiprotease improves amino acid release in vitro, energy, and nutrient utilization in broilers fed diets varying in crude protein levels. *Poult Sci.* 2024;103:103546. <https://doi.org/10.1016/j.psj.2024.103546>
38. Cai P, Liu S, Tu Y, Fu D, Zhang W, Zhang X, et al. Effects of different supplemental levels of protease DE200 on the production performance, egg quality, and cecum microflora of laying hens. *J Anim Sci.* 2024;102:skae086. <https://doi.org/10.1093/jas/skae086>
39. Heo YJ, Park J, Kim YB, Kwon BY, Kim DH, Song JY, et al. Effects of dietary protein levels on performance, nitrogen excretion, and odor emission of growing pullets and laying hens. *Poult Sci.* 2023;102:102798. <https://doi.org/10.1016/j.psj.2023.102798>
40. Lyons AM, Granghelli CA, Persia ME. Estimating energy utilization in laying hens: what are the best response criteria? *J Appl Poult Res.* 2023;32:100357. <https://doi.org/10.1016/j.japr.2023.100357>
41. Fouad AM, El-Senousey HK. Nutritional factors affecting abdominal fat deposition in poultry: a review. *Asian-Australas J Anim Sci.* 2014;27:1057-68. <https://doi.org/10.5713/ajas.2013.13702>
42. Yi W, Liu Y, Fu S, Zhuo J, Wang J, Shan T. Dietary novel alkaline protease from *Bacillus licheniformis* improves broiler meat nutritional value and modulates intestinal microbiota and metabolites. *Anim Microbiome.* 2024;6:1. <https://doi.org/10.1186/s42523-023-00287-z>
43. Filho JAV, Geraldo A, Machado LC, de Brito JÁG, Bertechini AG, Murakami ESF. Effect of protease supplementation on production performance of laying hens. *Acta Sci Anim Sci.* 2015;37:29-33. <https://doi.org/10.4025/actascianimsci.v37i1.22830>
44. Applegate TJ, Angel R. Protein and amino acid requirements for poultry. Purdue University Extension Publication AS-584-W; 2008.
45. Lei XJ, Lee KY, Kim IH. Performance, egg quality, nutrient digestibility, and excreta microbiota shedding in laying hens fed corn-soybean-meal-wheat-based diets supplemented with xylanase. *Poult Sci.* 2018;97:2071-7. <https://doi.org/10.3382/ps/pey041>
46. Maniraguha V, Hong JS, Yu M, Oketch EO, Yi YJ, Yun H, et al. Feeding dietary non-starch polysaccharides supplemented with xylanase could improve the performance of broilers. *J Anim Sci Technol.* 2024;66:999-1009. <https://doi.org/10.5187/jast.2023.e114>
47. Liu H, Liu W, Ai M, Hao X, Zhang Q, Ren J, et al. Effects of  $\beta$ -mannanase supplementation on productive performance, inflammation, energy metabolism, and cecum microbiota composition of laying hens fed with reduced-energy diets. *Poult Sci.* 2024;103:103521. <https://doi.org/10.1016/j.psj.2024.103521>
48. Ryu MH, Hosseindoust A, Kim JS, Choi YH, Lee SH, Kim MJ, et al.  $\beta$ -Mannanase derived

from *Bacillus subtilis* WL-7 improves the performance of commercial laying hens fed low or high mannan-based diets. *J Poult Sci.* 2017;54:212-7. <https://doi.org/10.2141/jpsa.0160021>

49. Raza A, Bashir S, Tabassum R. An update on carbohydrases: growth performance and intestinal health of poultry. *Heliyon.* 2019;5:e01437. <https://doi.org/10.1016/j.heliyon.2019.e01437>
50. Kiarie EG, Steelman S, Martinez M, Livingston K. Significance of single  $\beta$ -mannanase supplementation on performance and energy utilization in broiler chickens, laying hens, turkeys, sows, and nursery-finish pigs: a meta-analysis and systematic review. *Transl Anim Sci.* 2021;5:txab160. <https://doi.org/10.1093/tas/txab160>
51. Yu M, Oketch EO, Nawarathne SR, Chathuranga NC, Maniraguha V, Sta. Cruz BG, et al. Metabolizable energy and amino acid-deficient diets supplemented with  $\beta$ -mannanase in response to growth performance, intestinal health, and immune response in broilers. *Poult Sci.* 2025;104:105222. <https://doi.org/10.1016/j.psj.2025.105222>
52. Sta. Cruz BG, Yu M, Oketch EO, Nawarathne SR, Chathuranga NC, Maniraguha V, et al. A study of solely used phytase or in combination with multi-carbohydrase on growth performance along with tibia mineralization, and carcass traits in broilers fed nutrient-deficient diets. *J Anim Sci.* 2024;102:skae299. <https://doi.org/10.1093/jas/skae299>
53. Bobeck EA, Nachtrieb NA, Batal AB, Persia ME. Effects of xylanase supplementation of corn-soybean meal-dried distiller's grain diets on performance, metabolizable energy, and body composition when fed to first-cycle laying hens. *J Appl Poult Res.* 2014;23:174-80. <https://doi.org/10.3382/japr.2013-00841>
54. Sta. Cruz BG, Hong JS, Yu M, Oketch EO, Yun H, Jayasena DD, et al. Xylanase supplementation in energy-deficient corn-based diets: impact on broiler growth, nutrient digestibility, chyme viscosity and carcass proximates. *Anim Biosci.* 2024;37:1246-54. <https://doi.org/10.5713/ab.23.0340>
55. Nguyen HT, Wu SB, Bedford MR, Nguyen XH, Morgan NK. Dietary soluble non-starch polysaccharide level and xylanase influence the gastrointestinal environment and nutrient utilisation in laying hens. *Br Poult Sci.* 2022;63:340-50. <https://doi.org/10.1080/00071668.2021.2003754>
56. Roberts JR, Choct M. Effects of commercial enzyme preparations on egg and eggshell quality in laying hens. *Br Poult Sci.* 2006;47:501-10. <https://doi.org/10.1080/00071660600834175>
57. Nguyen XH, Nguyen HT, Morgan NK. Dietary soluble non-starch polysaccharide level and xylanase supplementation influence performance, egg quality and nutrient utilization in laying hens fed wheat-based diets. *Anim Nutr.* 2021;7:512-20. <https://doi.org/10.1016/j.aninu.2020.05.012>
58. Senkoylu N, Samli HE, Akyurek H, Okur AA, Kanter M. Effects of whole wheat with or without xylanase supplementation on performance of layers and digestive organ development. *Ital J Anim Sci.* 2009;8:155-63. <https://doi.org/10.4081/ijas.2009.155>
59. Cufadar Y, Yildiz AÖ, Olgun O. Effects of xylanase enzyme supplementation to corn/wheat-based diets on performance and egg quality in laying hens. *Can J Anim Sci.* 2010;90:207-12. <https://doi.org/10.4141/CJAS09081>
60. Gunawardana P, Roland Sr. DA, Bryant MM. Effect of dietary energy, protein, and a versatile enzyme on hen performance, egg solids, egg composition, and egg quality of Hy-Line W-36 hens during second cycle, phase two. *J Appl Poult Res.* 2009;18:43-53. <https://doi.org/10.3382/japr.2008-00047>
61. Scheideler SE, Beck MM, Abudabos A, Wyatt CL. Multiple-enzyme (avizyme) supplementation of corn-soy-based layer diets. *J Appl Poult Res.* 2005;14:77-86. <https://doi.org/10.3382/japr.2005-00014>

org/10.1093/japr/14.1.77

- 62. Sun HY, Kim IH. Effects of multi-enzyme on production performance, egg quality, nutrient digestibility, and excreta noxious gas emission of early phase Hy-Line Brown hens. *Poult Sci.* 2019;98:4889-95. <https://doi.org/10.3382/ps/pez237>
- 63. Huang Q, Yi W, Fan J, Chen R, Ma X, Chen Z, et al. Effects supplementation of novel multi-enzyme on laying performance, egg quality, and intestinal health and digestive function of laying hens. *Poult Sci.* 2024;103:104461. <https://doi.org/10.1016/j.psj.2024.104461>
- 64. Juan JJ, Martin EA, Mingala CN, Wigley P, Allison HE, Williams NJ, et al. The effect of multicarbohydrase-phytase complex supplementation on the egg production performance, egg quality, and economics of laying hens. *Cogent Food Agric.* 2024;10:2398122. <https://doi.org/10.1080/23311932.2024.2398122>
- 65. 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:e00600-20. <https://doi.org/10.1128/AEM.00600-20>
- 66. Dai D, Qi G, Wang J, Zhang H, Qiu K, Wu S. Intestinal microbiota of layer hens and its association with egg quality and safety. *Poult Sci.* 2022;101:102008. <https://doi.org/10.1016/j.psj.2022.102008>
- 67. Oketch EO, Yu M, Hong JS, Chaturanga NC, Seo E, Lee H, et al. Laying hen responses to multi-strain *Bacillus*-based probiotic supplementation from 25 to 37 weeks of age. *Anim Biosci.* 2024;37:1418-27. <https://doi.org/10.5713/ab.23.0495>
- 68. Gaggia F, Mattarelli P, Biavati B. Probiotics and prebiotics in animal feeding for safe food production. *Int J Food Microbiol.* 2010;141:S15-28. <https://doi.org/10.1016/j.ijfoodmicro.2010.02.031>
- 69. Yaqoob MU, Wang G, Wang M. An updated review on probiotics as an alternative of antibiotics in poultry: a review. *Anim Biosci.* 2022;35:1109-20. <https://doi.org/10.5713/ab.21.0485>
- 70. Sugiharto S. Role of nutraceuticals in gut health and growth performance of poultry. *J Saudi Soc Agric Sci.* 2016;15:99-111. <https://doi.org/10.1016/j.jssas.2014.06.001>
- 71. Salem HM, Saad AM, Soliman SM, Selim S, Mosa WFA, Ahmed AE, et al. Ameliorative avian gut environment and bird productivity through the application of safe antibiotics alternatives: a comprehensive review. *Poult Sci.* 2023;102:102840. <https://doi.org/10.1016/j.psj.2023.102840>
- 72. Xu CL, Ji C, Ma Q, Hao K, Jin ZY, Li K. Effects of a dried *Bacillus subtilis* culture on egg quality. *Poult Sci.* 2006;85:364-8. <https://doi.org/10.1093/ps/85.2.364>
- 73. Liong MT, Lee BH, Choi SB, Lew LC, Lau ASY, Daliri EBM. Cholesterol-lowering effects of probiotics and prebiotics. In: Venema K, do Carmo AP, editors. *Probiotics and prebiotics: current research and future trends.* Caister Academic Press; 2015. p. 429-46.
- 74. Alfonso-Carrillo C, Benavides-Reyes C, de los Mozos J, Dominguez-Gasca N, Sanchez-Rodríguez E, Garcia-Ruiz AI, et al. Relationship between bone quality, egg production and eggshell quality in laying hens at the end of an extended production cycle (105 weeks). *Animals.* 2021;11:623. <https://doi.org/10.3390/ani11030623>
- 75. Toscano MJ, Dunn IC, Christensen JP, Petow S, Kittelsen K, Ulrich R. Explanations for keel bone fractures in laying hens: are there explanations in addition to elevated egg production? *Poult Sci.* 2020;99:4183-94. <https://doi.org/10.1016/j.psj.2020.05.035>
- 76. Ricke SC. Prebiotics and alternative poultry production. *Poult Sci.* 2021;100:101174. <https://doi.org/10.1016/j.psj.2021.101174>
- 77. Yang X, Bist RB, Subedi S, Guo Y, Chai L. The application of probiotics and prebiotics in poultry production and impacts on environment: a review. *Encyclopedia.* 2025;5:35. <https://doi.org/10.5187/jast.2500369>

doi.org/10.3390/encyclopedia5010035

78. 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. <https://doi.org/10.1093/tas/txae123>
79. Alsherify SM, Hassanabadi A. Effects of adding different levels of fructooligosaccharide to diet on productive performance, egg quality traits, immune response and blood metabolites in commercial laying hens. *Vet Med Sci.* 2024;10:e1550. <https://doi.org/10.1002/vms3.1550>
80. Obianwuna UE, Qiu K, Wang J, Zhang H, Qi G, Huang L, et al. Effects of dietary Clostridium butyricum and fructooligosaccharides, alone or in combination, on performance, egg quality, amino acid digestibility, jejunal morphology, immune function, and antioxidant capacity of laying hens. *Front Microbiol.* 2023;14:1125897. <https://doi.org/10.3389/fmicb.2023.1125897>
81. Ikram A, Safdar SZ, Arshad MT, Rasheed A, Gnedeka KT. An overview of postbiotics: unveiling their distinct role in gut health. *Food Agric Immunol.* 2024;35:2434463. <https://doi.org/10.1080/09540105.2024.2434463>
82. Waqas M, Nastoh NA, Çinar AA, Farooq MZ, Salman M. Advantages of the use of postbiotics in poultry production: a new concept. *Braz J Poult Sci.* 2024;26:eRBCA-2024-1939. <https://doi.org/10.1590/1806-9061-2024-1939>
83. Choe DW, Loh TC, Foo HL, Hair-Bejo M, Awis QS. Egg production, faecal pH and microbial population, small intestine morphology, and plasma and yolk cholesterol in laying hens given liquid metabolites produced by *Lactobacillus plantarum* strains. *Br Poult Sci.* 2012;53:106-15. <https://doi.org/10.1080/00071668.2012.659653>
84. Wickramasuriya SS, Park I, Lee K, Lee Y, Kim WH, Nam H, et al. Role of physiology, immunity, microbiota, and infectious diseases in the gut health of poultry. *Vaccines.* 2022;10:172. <https://doi.org/10.3390/vaccines10020172>
85. Mahdavi AH, Rahmani HR, Pourreza J. Effect of probiotic supplements on egg quality and laying hen's performance. *Int J Poult Sci.* 2005;4:488-92. <https://doi.org/10.3923/ijps.2005.488.492>
86. Yadav S, Jha R. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. *J Anim Sci Biotechnol.* 2019;10:2. <https://doi.org/10.1186/s40104-018-0310-9>
87. Pham VH, Abbas W, Huang J, He Q, Zhen W, Guo Y, et al. Effect of blending encapsulated essential oils and organic acids as an antibiotic growth promoter alternative on growth performance and intestinal health in broilers with necrotic enteritis. *Poult Sci.* 2022;101:101563. <https://doi.org/10.1016/j.psj.2021.101563>
88. Abd El-Ghany WA. Applications of organic acids in poultry production: an updated and comprehensive review. *Agriculture.* 2024;14:1756. <https://doi.org/10.3390/agriculture14101756>
89. Kadim IT, Al-Marzooqi W, Mahgoub O, Al-Jabri A, Al-Waheebi SK. Effect of acetic acid supplementation on egg quality characteristics of commercial laying hens during hot season. *Int J Poult Sci.* 2008;7:1015-21.
90. Azizollahi M, Ghasemi HA, Foroudi F, Hajkhodadadi I. Effect of guanidinoacetic acid on performance, egg quality, yolk fatty acid composition, and nutrient digestibility of aged laying hens fed diets with varying substitution levels of corn with low-tannin sorghum. *Poult Sci.* 2024;103:103297. <https://doi.org/10.1016/j.psj.2023.103297>
91. Świątkiewicz S, Koreleski J, Arczewska A. Laying performance and eggshell quality in laying hens fed diets supplemented with prebiotics and organic acids. *Czech J Anim Sci.*

2010;55:294-306. <https://doi.org/10.17221/207/2009-CJAS>

92. Park KW, Rhee AR, Um JS, Paik IK. Effect of dietary available phosphorus and organic acids on the performance and egg quality of laying hens. *J Appl Poult Res.* 2009;18:598-604. <https://doi.org/10.3382/japr.2009-00043>

93. Gama NMSQ, de Oliveira MBC, Santin E, Berchieri Junior Â. Supplementation with organics acids in diet of laying hens. *Cienc Rural.* 2000;30:499-502. <https://doi.org/10.1590/S0103-84782000000300022>

94. Güll M, Tunç MA, Cengiz S, Yıldız A. Effect of organic acids in diet on laying hens' performance, egg quality indices, intestinal microflora, and small intestinal villi height. *Eur Poult Sci.* 2014;78:1-9. <https://doi.org/10.1399/eps.2013.5>

95. Nguyen DH, Kim IH. Protected organic acids improved growth performance, nutrient digestibility, and decreased gas emission in broilers. *Animals.* 2020;10:416. <https://doi.org/10.3390/ani10030416>

96. Smulikowska S, Czerwiński J, Mieczkowska A. Effect of an organic acid blend and phytase added to a rapeseed cake-containing diet on performance, intestinal morphology, caecal microflora activity and thyroid status of broiler chickens. *J Anim Physiol Anim Nutr.* 2010;94:15-23. <https://doi.org/10.1111/j.1439-0396.2008.00876.x>

97. Wang Z, Zhang C, Wang H, Gu T, Tian Y, Gu Z, et al. Effects of combined use of compound acidifiers and plant essential oils in feed on the reproductive performance and physiological status of Xianjv chickens. *Poult Sci.* 2025;104:104710. <https://doi.org/10.1016/j.psj.2024.104710>

98. Yang Y, Iji PA, Choct M. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World's Poult Sci J.* 2009;65:97-114. <https://doi.org/10.1017/S0043933909000087>

99. Mnisi CM, Mlambo V, Gila A, Matabane AN, Mthiyane DMN, Kumanda C, et al. Antioxidant and antimicrobial properties of selected phytogenics for sustainable poultry production. *Appl Sci.* 2023;13:99. <https://doi.org/10.3390/app13010099>

100. Anagnostopoulos EC, Mountzouris KC. Gut detoxification and antioxidant responses in laying hens supplemented with phytogenics. *J Appl Anim Nutr.* 2025;13:1-13. <https://doi.org/10.1163/2049257x-20251009>

101. Biswas S, Kim IH. A thorough review of phytogenic feed additives in non-ruminant nutrition: production, gut health, and environmental concerns. *J Anim Sci Technol.* 2025;67:497-519. <https://doi.org/10.5187/jast.2025.e26>

102. Puvača N, Stanaćev V, Glamočić D, Lević J, Perić L, Stanaćev V, et al. Beneficial effects of phytoadditives in broiler nutrition. *World's Poult Sci J.* 2013;69:27-34. <https://doi.org/10.1017/S0043933913000032>

103. Marume U, Mokagane JM, Shole CO, Hugo A. *Citrullus lanatus* essential oils inclusion in diets elicit nutraceutical effects on egg production, egg quality, and physiological characteristics in layer hens. *Poult Sci.* 2020;99:3038-46. <https://doi.org/10.1016/j.psj.2020.01.029>

104. Sharma MK, Dinh T, Adhikari PA. Production performance, egg quality, and small intestine histomorphology of the laying hens supplemented with phytogenic feed additive. *J Appl Poult Res.* 2020;29:362-71. <https://doi.org/10.1016/j.japr.2019.12.001>

105. Abdelli N, Solà-Oriol D, Pérez JF. Phytogenic feed additives in poultry: achievements, prospective and challenges. *Animals.* 2021;11:3471. <https://doi.org/10.3390/ani11123471>

106. Olgun O. The effect of dietary essential oil mixture supplementation on performance, egg quality and bone characteristics in laying hens. *Ann Anim Sci.* 2016;16:1115-25. <https://doi.org/10.1515/aoas-2016-0038>

107. Ding X, Yu Y, Su Z, Zhang K. Effects of essential oils on performance, egg quality, nutrient

digestibility and yolk fatty acid profile in laying hens. *Anim Nutr.* 2017;3:127-31. <https://doi.org/10.1016/j.aninu.2017.03.005>

108. Xian L, Wang Y, Peng D, Zang L, Xu Y, Wu Y, et al. Dietary oregano oil supplementation improved egg quality by altering cecal microbiota function in laying hens. *Animals.* 2024;14:3235. <https://doi.org/10.3390/ani14223235>

109. Yang C, Chowdhury MAK, Huo Y, Gong J. Phytopreparative compounds as alternatives to in-feed antibiotics: potentials and challenges in application. *Pathogens.* 2015;4:137-56. <https://doi.org/10.3390/pathogens4010137>

110. Sugiharto S, Raza MA. Optimization of poultry physiological condition in the post-antibiotic era through nutritional intervention: a review. *J Med Vet.* 2024;7:388-406. <https://doi.org/10.20473/jmv.vol7.iss2.2024.388-406>

111. van Hoeck V, Somers I, Abdelqader A, Wealleans AL, Van de Craen S, Morisset D. Xylanase impact beyond performance: a microbiome approach in laying hens. *PLoS ONE.* 2021;16:e0257681. <https://doi.org/10.1371/journal.pone.0257681>

112. Carvalho CL, Andretta I, Galli GM, Stefanello TB, Camargo NOT, Mendes RE, et al. Dietary supplementation with  $\beta$ -mannanase and probiotics as a strategy to improve laying hen performance and egg quality. *Front Vet Sci.* 2023;10:1229485. <https://doi.org/10.3389/fvets.2023.1229485>

113. Cho HM, González-Ortiz G, Melo-Durán D, Heo JM, Cordero G, Bedford MR, et al. Stimbiotic supplementation improved performance and reduced inflammatory response via stimulating fiber fermenting microbiome in weaner pigs housed in a poor sanitary environment and fed an antibiotic-free low zinc oxide diet. *PLOS ONE.* 2020;15:e0240264. <https://doi.org/10.1371/journal.pone.0240264>

114. Morgan NK, Wallace A, Bedford MR, González-Ortiz G. Impact of fermentable fiber, xylo-oligosaccharides and xylanase on laying hen productive performance and nutrient utilization. *Poult Sci.* 2022;101:102210. <https://doi.org/10.1016/j.psj.2022.102210>