한국가금학회지 제 52권 제 2호, 85～103 (2025)
Korean J. Poult. Sci. Vol.52, No.2, 85～103 (2025)
https://doi.org/10.5536/KJPS.2025.52.2.85

Dietary Additives to Extend Laying Persistency in Aged Hens

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ABSTRACT The laying hen industry plays a vital role in supplying high-quality protein through egg production. As the global population grows, the demand for animal-derived proteins such as eggs continues to rise. However, aging hens experience a decline in egg production performance and quality due to cumulative oxidative stress and physiological deterioration. This review explores the use of feed additives as a nutritional strategy to mitigate age-related challenges and sustain productivity in late-laying hens. Various additives－including probiotics, prebiotics, organic acids, exogenous enzymes, vitamins, and herbal extracts－have shown beneficial effects on gut health, nutrient absorption, immune function, and eggshell quality in older hens. These compounds function by enhancing digestive efficiency, promoting microbial balance, improving antioxidant defenses, and supporting mineral utilization. This review provides a focused synthesis of recent findings and highlights the scientific significance of feed additives in extending laying persistency beyond 70 weeks of age. By offering evidence-based insights, it aims to guide the development of age-specific nutritional interventions that improve both productivity and welfare in commercial laying operations.

(Key words: egg production, egg quality, feed additives, laying hen)

INTRODUCTION

The laying hen industry, a pivotal sector in the global food supply, provides humans with a high-quality protein source through egg production (Réhault-Godbert et al., 2019). With the global population on the rise, the demand for animal-derived protein sources such as eggs is increasing, underscoring the significant role of the laying hen industry. The primary goal of the laying hen industry is to raise healthy hens and consistently produce high-quality eggs. This can be achieved by managing various sectors, such as nutritional strategies, genetic selection, housing management, and disease prevention (Bain et al., 2016). However, as hens age, they face a significant challenge as egg production performance and quality naturally decrease due to the cumulative oxidative stress from continuous laying over time (Park and Sohn, 2018). As hens age beyond their peak production period, a decline in egg production performance and quality is commonly observed, primarily due to reduced secretion of endogenous antioxidant enzymes and reproductive hormones, as well as impaired intestinal morphology (Liu et al., 2013, 2018). During the late laying stage, there is an increase in the production of abnormal eggs, such as speckled or pimpled eggs, and a decrease in eggshell strength, leading to a higher breakage rate (Kim et al., 2018). At 80 weeks, laying hens tend to have relatively poor eggshell thickness and strength compared to their peak laying period (Yilmaz and Bozkurt, 2009). In the late laying period, eggshell quality decreases due to lower calcium availability than peak laying (Roland, 1988). These are the challenges that the laying hen industry faces, and it's essential to manage them to sustain productivity in the field.

In South Korea, the *Enforcement Decree of the Livestock Act* was revised in 2021 to reduce the minimum stocking density for laying hens in battery cage systems, aiming to prevent epidemic diseases and enhance animal welfare by alleviating bird stress (MAFRA, 2021). While this regulation supports welfare improvement, it simultaneously reduces the total number of hens housed, potentially lowering overall egg production at the farm level. As a result, strategies to extend the laying persistency of aged hens have gained importance as a means to offset reduced bird numbers and maintain

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productivity. Improving laying persistency not only helps sustain egg production and quality but also supports health aspects such as immune response, antioxidant capacity, and gut microbial stability during the late-laying period (Arulnathan et al., 2024). By improving laying persistency in the late laying period, it is estimated that approximately 500 eggs might be produced over 90−100 weeks (Son et al., 2020), leading to reduced feed consumption, lower feed costs, and minimized natural resource waste.

The use of feed additives, such as probiotics, prebiotics, organic acids, exogenous enzymes, vitamins, and herb extracts in livestock nutrition can optimize feed utilization, improve growth performance, enhance feed palatability, and provide essential nutrients (Pandey et al., 2019). The demand for animal-derived protein is increasing globally, leading to a rise in the use of feed additives, especially in poultry, due to escalating feed ingredient prices and the ban on antibiotics (Rafiq et al., 2022). Several studies have explored the effects of feed additives on late-laying hens to enhance their health and production performance (Pirgozliev et al., 2019).

Introducing various feed additives into the diet may aid in sustaining the egg production performance and quality of late-laying hens. Numerous studies have evaluated the use of probiotics, prebiotics, organic acids, exogenous enzymes, and vitamins in aged laying hens, as summarized in Table 1. This review aims to outline the impact of feed additives on late-laying hens, with a specific focus on laying persistency.

PROBIOTICS

Probiotics, as live microbial feed additives, play a crucial role in maintaining the balance of gut microbiota, thereby promoting the health condition of the host (Khan and Naz, 2013). They achieve this by competitive exclusion in the gut, thereby reducing the colonization of pathogenic bacteria (Mak et al., 2022). In contrast, disruption in gut microbiota a is often linked to poor production performance in aged hens (Wang et al., 2020). Probiotics have been shown to lower the acidity of the gut through the secretion of beneficial metabolites like lactic acid, creating an unfavorable environment for pathogenic bacteria such as *E. coli* and *Salmonella* spp. (Khan et al., 2020). An ideal gut microbiota balance in the gut is deeply associated with improved nutrient digestibility, immune system, gut health, and production performance (Mak et al., 2022). Probiotics used in the animal industry typically consist of a combination of beneficial bacteria strains such as *Bacillus*, *Lactobacillus*, *Enterococcus*, *Bifidobacterium*, and *Streptococcus*, or individual beneficial bacteria strains (Arsène et al., 2021).

A previous study has shown that supplementing laying hens with *Bacillus subtilis* can significantly enhance their laying performance, as it produces enzymes such as protease, lipase, and amylase, which aid in breaking down carbohydrate complexes in their diet (Gao et al., 2017; Kim et al., 2023). These specific probiotics offer unique benefits to the hens, enhancing their overall health and production performance in the late-laying period. Supplementing hens with *Clostridium butyricum* has been shown to improve feed efficiency by promoting the production of digestive enzymes, especially lipase, in 59-week-old Hy-Line Brown layers (Wang et al., 2020). It also helps in the modulation of gut microbiota composition and the production of organic acids, such as butyric acid, thereby promoting the secretion of endogenous enzymes in the gut (Liu et al., 2017). Additionally, supplementing 64-week-old Hy-line hens with *Clostridium butyricum* and *Bacillus subtilis* has been found to improve intestinal morphology and gut microbiota, reducing the occurrence of abnormal eggs, such as sandpaper-like eggs, which tends to happen in older hens (Khogali et al., 2022). Since hens' gastrointestinal health and immune systems naturally deteriorate with age, supplementing probiotics may help maintain the integrity of their gastrointestinal tract, immune response, and health condition, sustaining consistent egg production even in the late-laying period. Xu et al. (2023) have also demonstrated that supplementing young hens with probiotics consisting of *Firmicutes*, *Desulfobacterota*, and *Actinobacteriota* for 54 weeks may improve egg weight, egg quality, and uterus development in the late laying period. Moreover, supplementing 50-week-old Hy-line Brown hens with probiotics consisting of *Lactobacillus acidophilus* and *Bacillus subtilis* had a beneficial effect on gut microbiota, nutrient metabolism, intestinal integrity, and the immune system (Chen et al., 2023). Specific probiotics such as *Lactobacillus acidophilus* and *Bacillus subtilis* have been shown to improve gut microbiota composition, nutrient meta-

Table 1. Summary of studies on the impact of feed additives in aged hens

Type of feed additives	Age (weeks)	Feed additives	Major observations	References
Probiotics	69−74	*Bacillus subtilis*	↑ Laying performance, egg quality, gut health	Kim et al. (2023)
Probiotics	60−68	*Clostridium butyricum*	↑ Feed efficiency and yolk color	Wang et al. (2020)
Probiotics	64−68	*Clostridium butyricum* and *Bacillus subtilis*	↑ Intestinal structure, gut microbiota composition ↓ Sandpaper−shelled eggs	Khogali et al.(2022)
Probiotics	50−57	*Lactobacillus acidophilus* and *Bacillus subtilis*	↑ Intestinal barrier function and immune homeostasis	Chen et al. (2023)
Prebiotics	74−83	Xylo-oligosaccharides	↑ Laying performance, egg quality	Xiao et al. (2020)
Prebiotics	52−62	Chitooligosaccharide	↑ Laying performance, egg quality, antioxidant activity, immune ability ↓ Serum lipids	Xu et al. (2020)
Prebiotics	69−74/74−79	Mannan-oligosaccharides	↑ Laying performance, feed efficiency, nutrient digestibility ↓ Pathogenic intestinal bacteria	Ghasemian and Jahanian (2016)
Prebiotics	43−51	β-glucan	↑ Eggshell color, fertile egg hatchability, immune function	Zhen et al. (2020)
Prebiotics	64−75	Inulin	↑ Laying performance, egg quality, feed efficiency, tibia traits, small intestine morphology, microflora composition	Abdelqader et al.(2013)
Organic acids	53−70	Blends of formic acid, butyric acid, and lactic acid	↑ Live body weight	Soltan (2008)
Organic acids	67−74	Blends of fumaric acid, butyric acid, propionic acid, and lactic acid	↑ Feed efficiency	Rahman et al. (2008)
Organic acids	67−74	Blends of fumaric acid, butyrate, propionate, and lactic acid	↑ Feed efficiency, eggshell quality	Rahman et al. (2011)
Exogenous enzymes	63−73	Phytase	↑ Laying performance, peak laying period, eggshell quality	Sun and Kim (2021)
Exogenous enzymes	42−47	Phytase	↑ Laying performance	Kim et al. (2017)
Exogenous enzymes	60−69	Protease	↑ Laying performance, feed efficiency, cecal microbiota	Chen et al. (2021)
Vitamin	56−61	Vitamin A	↑ Laying performance, egg weight, feed intake ↓ Oxidative stress	Lin et al. (2002)
Vitamin	90−97	Vitamin A and vitamin K	↑ Yolk color, eggshell quality, antioxidative status	Guo et al. (2021)
Vitamin	42−47/ 44−49/52−57 /72−77	Vitamin D_3	↑ Eggshell quality, vitamin D_3 contents in eggs	Cho et al. (2023)
Vitamin	60−72	Vitamin D_3 and 25-OHD_3	↑ Laying performance, eggshell quality, plasma calcium levels	Jing et al. (2022)
Vitamin	74−81	25-OHD_3	↑ Egg weight	Kakhki et al. (2019)
Vitamin	57−63	Vitamin D_3, vitamin D_2, and 25-OHD_3	↑ Utilization of calcium and phosphorus	Adhikari et al.(2020)

Table 1. Continued

Type of feed additives	Age (weeks)	Feed additives	Major observations	References
Vitamin	66−72	Vitamin E	↑ Laying performance, egg quality, antioxidant capacity	Linghu et al. (2021)
Vitamin	70−80	Vitamin E	↑ Immune function, antioxidant capacity, ovarian functions, estrogen secretion, follicle development	Wang et al. (2024)
Vitamin	46−55	Vitamin E	↑ Laying performance, tocopherol deposition, antioxidant capacity ↓ Serum cholesterol concentration	Zhao et al. (2021)
Vitamin	46−52	Vitamin C	↑ Laying performance	Reyes et al. (2021)
Herbal extracts	70−80	Oregano extract	↑ Laying performance, egg quality, intestinal morphometry	Ramirez et al. (2021)
Herbal extracts	64−72	*Moringa oleifera*	↑ Laying performance, egg quality ↓ Serum cholesterol and triglyceride, excreta ammonia concentrations, uric acid, creatinine	Abdel-Wareth and Lohakare (2021)
Herbal extracts	72−78	*Ligustrum lucidum*	↑ Laying performance, caecal microbial composition	Li et al. (2017)
Herbal extracts	52−64	*Lonicera confusa* and *Astragali Radix*	↑ Egg quality, antioxidant capacity ↓ Systemic inflammation	Xie et al. (2019)
Herbal extracts	60−64	*Mentha piperita* L.	↑ Laying performance, egg quality, immune response, antioxidant capacity, intestinal microbiota	Bai et al. (2023)

bolism, intestinal integrity, and immune responses in older laying hens (Khoder et al., 2019; Zhuang et al., 2019). These effects are largely attributed to their ability to enhance the intestinal mucosal barrier by upregulating tight junction protein expression, thereby strengthening gut health. From previous studies on the effects of probiotics on late-laying hens, it is evident that supplementing aged hens with probiotics may improve gut microbiota, intestinal integrity, immune response, and uterus development, thereby enhancing their egg production performance and quality.

PREBIOTICS

Prebiotics are carbohydrate fragments, such as oligosaccharides composed of mannose, fructose, and galactose, that cannot be digested by endogenous enzymes but are fermented by gut microbiota in the hindgut to produce beneficial metabolites like short-chain fatty acids (Remesy et al., 1993). Thereby, prebiotics can influence animal health and production performance by promoting the growth of beneficial bacteria in the gut (Singh and Kim, 2021). They can also enhance the immune system directly or through the increased beneficial bacteria in response to the dietary supplementation of prebiotics. To improve the production performance of hens, the effects of supplementing dietary oligosaccharides, including inulin, chito-oligosaccharides, fructo-oligosaccharides, xylo-oligosaccharides, mannan-oligosaccharides, and β-glucan have been studied so far.

Supplementing xylo-oligosaccharides to 74-week-old hens has improved laying performance and egg quality by generating short-chain fatty acids in the hindgut, which are regulated by xylo-oligosaccharides (Xiao et al., 2020). Fatty acids produced as fermentation metabolites of beneficial bacteria in the hindgut reduce intestinal pH and inhibit the growth of pathogenic bacteria (Liu et al., 2021). Besides, chickens lack the endogenous enzymes required to degrade the β-1,4-glycosidic bonds between xylose units, so xylo-oligosaccharides may pass through the stomach and small intestine and be utilized by the hindgut microbiota (Zhou et al., 2021). Xylo-oligosaccharides have been studied for their roles in promoting mucosal regeneration and butyrate production in the hindgut (De Maesschalck et al., 2015).

It was reported that supplementing chitooligosaccharides to 52-week-old laying hens for 10 weeks improved laying performance, egg quality, immune function, antioxidant capacity, and health condition by reducing serum lipid concentration (Xu et al., 2020). Chito-oligosaccharides, derived from chitosan (Lodhi et al., 2014), have low molecular weight and viscosity (Chae et al., 2005). They bind to bile acids, which help digest fats, then decrease fatty acid synthesis in the liver due to lower levels of triglycerides in the blood (Herzberg and Rogerson, 1988; Swiatkiewicz et al., 2015). Additionally, increased antioxidant capacity due to supplementing chitooligosaccharides may improve production performance in aged hens by reducing cumulative oxidative stress.

Mannan-oligosaccharides, derived from yeast cell walls, are considered good alternatives to antibiotic compounds in poultry feed (Ferket et al., 2002). A study examined the effect of mannan-oligosaccharides supplementation on production performance, immunological responses, and bacterial communities in 68-week-old hens, reporting that dietary mannan-oligosaccharides could increase production performance and feed efficiency in the late-laying period (Ghasemian and Jahanian, 2016). The improvements were attributed to the increased nutrient digestibility and reduced intestinal pathogenic bacteria resulting from dietary supplementation of mannan-oligosaccharides. A previous study suggested that beneficial bacterial colonies established by dietary mannan-oligosaccharides could improve nutrient digestibility and feed efficiency (Liu et al., 2002). Additionally, mannan-oligosaccharides serve as an attachment site for gram-negative bacteria, preventing their attachment to the gut epithelial cells and promoting intestinal health in the host (van der Wielen et al., 2002).

Zhen et al. (2020) investigated the effects of dietary supplementation of β-glucan extracted from *Saccharomyces cerevisiae* cell wall on 43-week-old Hy-Line Brown breeders over 8 weeks. This study found that β-glucan supplementation improved eggshell color, hatchability of fertile eggs, and immune function. Improvement of aged hens' gut health and immune system by dietary supplementation of β-glucan led to enhancement of eggshell color and nutrient absorption in this study. Also, Zhen et al. (2020) suggested that the improved immune system may be linked to the increased hatchability of fertile eggs, although further research is needed to confirm the exact mechanism. This study concluded that dietary supplementation of β-glucan extracted from *Saccharomyces cerevisiae* cell wall may help sustain production performance in aged hens. Furthermore, the combination of probiotics and prebiotics in the diet of aged hens may help sustain egg production and quality by supporting gut health and mineral absorption. Abdelqader et al. (2013) demonstrated that supplementing Bacillus subtilis and inulin in 64-week-old Lohmann White hens improved laying performance, egg quality, tibia mineralization, and gut morphology and microbiota, possibly due to enhanced calcium absorption by inulin (Younes et al., 2001; Abrams et al., 2005). Improved tibia mineralization may contribute to stronger eggshell formation by supplying sufficient calcium (Chowdhury and Smith, 2001). Additionally, synbiotic supplementation promotes the growth of beneficial gut bacteria and the production of metabolites like volatile fatty acids, which reduce gut pH and increase calcium ionization and availability.

ORGANIC ACIDS

Organic acids, also known as acidifiers, are crucial for improving intestinal health by reducing the activity of harmful bacteria in the gut (Pearlin et al., 2020). These consist of individual or blended acids to create antimicrobial effects (Wang et al., 2009). Commonly used organic acids in the animal industry include formic, acetic, propionic, and butyric acids, as well as short-chain carboxylic acids such as citric, sorbic, and fumaric acids (Khan and Iqbal, 2016). Numerous studies have examined the impact of organic acids on animals, particularly in terms of their effect on growth performance, gut microbiota, nutrient utilization, and hygiene, through the reduction of pathogens (Ndelekwute et al., 2016; Hajati, 2018; Nguyen et al., 2020). Based on their beneficial roles, supplementing dietary organic acids in the diet may effectively improve gut health as well as production performance in poultry (Broom, 2015; Khan and Iqbal, 2016; Haq et al., 2017).

Dietary supplementation with various organic acids, including formic, butyric, lactic, fumaric, and propionic acids, has been shown to enhance laying performance, egg mass, and eggshell quality in aged hens (Rahman et al., 2008; Soltan, 2008). This is significant as nutrient availability in aged hens is typically low due to the damage to intestinal cell lining and morphology, leading to poor production performance (Nowaczewski et al., 2021). However, organic acids have been found to promote the regeneration of intestinal epithelial cells, improving nutrient utilization and production performance in hens (Rahman et al., 2008; Soltan, 2008). Another study investigated the effect of volatile fatty acids and saponin supplementation on laying performance, egg quality, and body composition in 67-week-old hens (Rahman et al., 2011). Rahman et al. (2011) demonstrated that supplementing volatile fatty acids has improved feed efficiency and eggshell quality in late-laying hens, which is achieved by promoting microbial balance and repairing damaged cells in the digestive wall. In this study, eggshell thickness has increased due to the higher absorption rate of minerals or protein in the diet (Soltan, 2008). This is followed by a low acidity environment in the gut, which results from supplementing volatile fatty acids in the diet. Moreover, supplementing organic acids, fructan, and inulin to 26-week-old laying hens for 34 weeks has improved eggshell quality from 46-week-old to 70-week-old (Świątkiewicz et al., 2010). This study suggested that supplementation of organic acids in aged hens enhanced egg quality, intestinal morphology, acidity in the foregut environment, and the availability of calcium and phosphorus (Al-Batshan et al., 1994; Garcia et al., 2007). In the late laying period, eggshell quality decreases following the low availability of calcium due to aging (Al-Batshan et al., 1994). However, calcium is easily solubilized at lower pH, negatively correlated to pH in the gut (Guinotte et al., 1995), so supplementation of organic acids may increase the calcium availability in aged hens. Moreover, it is speculated that providing hens with short-chain fatty acids may help prevent skeletal diseases such as osteoporosis, which is common in the late-laying period, producing eggs over an extended period (Hanna, 2019; Zaiss et al., 2019).

EXOGENOUS ENZYMES

In the animal industry, exogenous enzymes are commonly added to animal feed to improve nutrient availability. This enables a more efficient breakdown of feed ingredients in the diet compared to the impact of sole endogenous enzymes in animals (Velázquez-De Lucio et al., 2021). Relying solely on endogenous enzymes in hens can make it challenging to achieve optimal production performance in hens. Thus, supplementing exogenous enzymes to a hen's diet may enhance production performance by improving nutrient availability (Abu, 2019). Additionally, as hens age, a decrease in endogenous enzyme secretion is considered one of the main reasons for reducing egg production (Usman et al., 2014). Thus, supplementing a hen diet with exogenous enzymes may improve nutrient utilization and production performance, especially during the later stages of laying. The use of various exogenous enzymes in hens' diets in the laying hen industry aims to improve egg production performance. Applying appropriate enzymes based on the feed ingredients maximizes nutrient utilization, egg production performance, and even laying persistency as the hens age.

Phosphorus is essential for hens, particularly in intestinal metabolism processes, maintaining skeletal health, as well as eggshell synthesis (Zhang et al., 2023). However, about 60－70% of the phosphorus in hen's diet is present as phytate, resulting in low phosphorus availability due to the low ability to secrete endogenous phytase in chickens (Singh, 2008). Phytate, an anti-nutritional factor in animal feed, forms an insoluble complex after combining with nutrients in the diet. It reduces the availability of nutrients in the diet, increases phosphorus excretion, and stimulates environmental pollution (Guo et al., 2009). So far, previous studies have been carried out to enhance the availability of phosphorus in the diet due to the low secretion of endogenous phytase in hens. However, only a few studies have reported the effect of phytase on aged hens. According to Sun and Kim (2021), supplementing the diet of 63-week-old Hy-Line Brown hens with phytase for 10 weeks has decreased the occurrence of damaged eggs and improved hen-day egg production due to the improvement in nutrient utilization. Furthermore, Kim et al. (2017) reported that super-dosing of phytase to 42-week-old Hy-line Brown hens for 6 weeks improved hen-day egg production without impacting egg quality. The addition of phytase to the hens' feed improves egg production performance by breaking down phytate in the feed, resulting in better access to phosphorus, energy, amino acids, and minerals, leading to an improvement in nutrient availability (Gehring et al., 2013).

Protease is an exogenous enzyme crucial for the degradation of protein sources in animal feed, resulting in improvement in feed efficiency, health conditions, as well as reproduction systems in animals (Gifre et al., 2017). Supplementing exogenous proteases may enhance the rate of intestinal protein degradation (Mahmood et al., 2017), efficiently meeting the requirement for amino acids in animals (Isaksen et al., 2010). However, research has shown that alkaline protease is more effective than other protease types, such as acidic or neutral protease (Sharma et al., 2019). Chen et al. (2021) showed that supplementing alkaline protease in the diet of 60-week-old hens improved feed efficiency and ceca microbiota. This study found that supplementing exogenous protease has improved the availability of amino acids in the diet, resulting in enhanced feed efficiency (Lahaye et al., 2018). Additionally, alkaline protease improved the composition of microorganisms in the ceca, improving the production performance of aged hens. A balanced gut microbiota in the ceca can break down polysaccharides that the endogenous enzymes cannot, and then use the polysaccharides as an energy source (Choi et al., 2015). Therefore, the production of additional energy from the ceca may contribute to consistent egg production even in the later stages of laying. However, only a few studies have been reported on the effects of supplementing exogenous enzymes in the aged hens diet so far.

VITAMIN

Vitamins are organic compounds required only in trace amounts but are vital for proper metabolism and production in animals (McDowell, 2000). They are essential for enhancing the health condition of poultry through biological functions such as anti-inflammatory, antimicrobial, and antioxidant properties (Shojadoost et al., 2021). A vitamin deficiency can lead to inflammation or infection, resulting in poor productivity in hens (Koutsos et al., 2006). Therefore, hen diet should contain a vitamin premix to meet the nutritional requirements for maintaining body function and productivity (Ao et al., 2019).

Vitamin A, a fat-soluble micronutrient, plays several roles in vision, reproductive system, and immune function in animals (Shastak and Pelletier, 2023). Its various roles include antioxidant effects, gene expression, and cellular differentiation, contributing positively to overall physiological function in the body (Shastak and Pelletier, 2024). A deficiency of vitamin A in the diet leads to various health issues, particularly in the immune and reproductive systems (Green and Fascetti, 2016). To our knowledge, only a few studies have been reported about the effects of additional vitamin A in a late-laying hen diet. A previous study demonstrated that supplementing additional vitamin A (9,000 IU) in the diet of 56-week-old laying hens that are raised in hot climates (31.5℃) has beneficial effects on feed intake and laying performance (Lin et al., 2002). This study also reported that egg weight was increased by the high concentration of vitamin A (6,000 and 9,000 IU) in the diet. According to Lin et al. (2002), supplementing additional vitamin A in the diet of aged hens may reduce oxidative stress followed by aging and heat stress, improving production performance. Additionally, Guo et al. (2021) demonstrated that dietary supplementation with both vitamin A and vitamin K_3 for 8 weeks in 87-week-old laying hens improved yolk color, eggshell quality, and the antioxidative status in the eggshell gland, although laying performance remained unaffected. However, since the vitamins were administered simultaneously, the individual contributions of each vitamin cannot be distinguished. It has been speculated that dietary vitamin A may be converted into β-carotene, which is deposited in the egg yolk and contributes to enhanced yolk pigmentation (Toomer et al., 2019). Similarly, Zhu et al. (2020) reported that supplementing both vitamin A and vitamin E improved the antioxidant status of the eggshell gland mucosa, resulting in better eggshell quality in late-laying hens. These findings suggest that while supplemental vitamin A may support egg quality and antioxidative function, further studies are needed to isolate its effects from those of other vitamins.

Vitamin D is a fat-soluble micronutrient closely related to the metabolism of calcium and phosphorus and the maintenance of skeletal integrity in animals (Bikle, 1994). Vitamin D also has a significant role in bone mineralization and parathyroid hormone regulation (Garcia et al., 2013). Especially, vitamin D is crucial in the diet of laying hens as it regulates calcium levels in the blood, supporting continuous eggshell production during the laying period (Mattila et al., 2003). In nature, animals synthesize vitamin D when their skin is exposed to ultraviolet light exposure from sunlight, which transforms 7-dehydrocholesterol into vitamin D_3 (MacLaughlin and Holick, 1985). In the case of hens raised indoors, such as in battery cage systems, it is hard to synthesize vitamin D for hens due to the absence of exposure to sunlight (Mattila et al., 2003). According to previous studies, vitamin D deficiency is closely linked to rickets, a classic metabolic bone disease in animals (Pettifor, 2005). Hens experience skeletal issues such as keel bone deformities, cage layer fatigue, and osteoporosis due to a lack of vitamin D especially in the laying period (Whitehead et al., 2003). Thus, the diet of laying hens should meet the nutritional requirements of vitamin D. Moreover, the ability of vitamin D metabolism in the kidneys and calcium utilization decreases as the hen ages (Abe et al., 1982; Diana et al., 2021). Vitamin D is essential to maintain production performance by modulating calcium utilization, especially in the late-laying period. Joh et al. (2023) reported that including additional vitamin D_3 in the diet of 42, 44, 52, and 72-week-old laying hens in commercial farms enhanced eggshell thickness. This study suggests that vitamin D enhances the absorption of calcium and phosphorus in the diet, promoting superior eggshell quality (Adhikari et al., 2020). In the liver, vitamin D is converted into 25-hydroxyvitamin D_3 before being transformed into calcitriol, the hormone form of vitamin D (Wyatt et al., 1990). Since 2006, 25-hydroxyvitamin D_3 (25-OHD) has been permitted to be included in poultry feed as a vitamin D source (Mattila et al., 2011). The structure of 25-hydroxyvitamin D_3 is much more stable than calcitriol, the hormone form of vitamin D_3, and has fewer disadvantages than vitamin D_3 (Soares et al., 1995). A previous study examined the impacts of supplemental 125 μg/kg doses of vitamin D_3 or 25-OHD_3 on 60-week-old laying hens and found improvement in plasma calcium concentration, eggshell quality, and laying performance (Jing et al., 2022). Effects of 125 μg/kg doses of vitamin D_3, or 25-OHD_3, on eggshell quality and laying performance were identical in this study. However, Kakhki et al. (2019) examined the interactive effects of calcium and top-dressed 25-OHD_3 on 74-week-old Lohmann LSL-lite for 7 weeks, then reported that additional 25-OHD_3 linearly enhanced egg weight at various calcium intakes without negative impact on bone health and eggshell quality. Furthermore, Adhikari et al. (2020) reported that extra vitamin D supplementation on hens, regardless of forms (vitamin D_3, vitamin D_2, and 25-hydroxyvitamin D_3), had no impact on either egg quality or bone mineralization. In this study, supplementing different isoforms of vitamins in the diets improved only the utilization of calcium and phosphorus. According to the development of genetic selection, hens' nutritional requirements, such as vitamin D_3, may not be enough to achieve the best production performance. Based on the results of studies, including additional vitamin D_3 in the diet may prevent osteoporosis and sustain egg production performance for an extended period.

Vitamin E is a fat-soluble molecule that can transfer signals between cells and modulate animal immune systems (Meydani and Blumberg, 2020). It is also a potent antioxidant in cell membranes and slows down the aging process in the body by preventing free radical chain reactions from cell membrane oxidation (Miyazawa et al., 2019). A previous study demonstrated that supplementing additional vitamin E in 66-week-old Hy-line Brown improved the laying performance, egg quality, and antioxidant effect (Linghu et al., 2021). The author reported that supplemental additional vitamin E in the diet of late-laying hens may sustain the immune function and productivity for an extended period. Moreover, Wang et al. (2024) investigated the impact of vitamin E on laying performance, vitamin E deposition, immunity, antioxidant capacity, estrogen secretion, follicle development, cecal microbiota, and ovary metabolome of 70-week-old hens. As a result, vitamin E supplementation in aged laying hens improved laying performance, immunity, antioxidant capacity, ovarian functions, estrogen secretion, follicle development, and even regulated ovary metabolites and gut microbiota by enhancing antioxidant capacity. The ovary is a major reproductive organ for egg production; its role in follicle formation and development directly affects the egg production performance in hens (Apperson et al., 2017). Thus, ovarian function is closely related to egg-laying persistency, as the ovary is crucial in determining hens' egg production performance. Furthermore, estrogen secretion decreases as ovary function deteriorates, reducing the number of developing follicles in hens (Colella et al., 2021). In this study, supplementing additional vitamin E improved ovarian functions, estrogen secretion, and follicle development, leading to an improvement in production performance in the late-laying period. Moreover, Zhao et al. (2021) evaluated the impact of dietary vitamin E supplementation on laying performance, egg quality, antioxidant capacity, serum biochemical indices, and tocopherol deposition in 46-week-old hens. In this research, additional vitamin E supplementation enhanced laying performance and antioxidant capacity while promoting tocopherol deposition of the liver and yolk. However, vitamin E may improve antioxidant capacity by directly reducing malondialdehyde content and improving total antioxidant capacity (Sahin et al., 2002). Based on the limited studies, it is speculated that supplementing vitamin E to hens in the late-laying period may improve production performance by enhancing antioxidant capacity.

Vitamin C has antioxidant effects that protect cells from reactive oxygen species produced by stress (Shakeri et al., 2020). A previous study demonstrated that supplementing additional vitamin C to broilers raised in hot weather has reduced oxidative stress from hot ambient temperatures (Khan et al., 2012). As hens' ability to generate vitamin C decreases with age, older hens are much more susceptible to oxidative stress than young hens (Abe et al., 1982). Therefore, supplementing vitamin C in the diet of old hens may help sustain their production performance due to its antioxidant properties. Limited studies have been conducted on the impact of supplementing additional vitamin C on aged hens. One study found that supplementing the diet of 46-week-old hens with vitamin C enhanced laying performance and egg quality while decreasing eggshell breakage and the occurrence of shell-less eggs (Reyes et al., 2021). Another study revealed that excessive vitamin D and C supplementation in the diet of 12-week-old hens for 60 weeks improved bone characteristics in hens (Ogunwole et al., 2018). Bone diseases such as osteoporosis can result from continuous laying for an extended period (Randall and Duff, 1988). Vitamin D_3 is essential for calcium utilization in skeleton formation (Mattila et al., 2003), while vitamin C is necessary for synthesizing procollagen and activating vitamin D_3 in the body (Tillman, 1993), so supplementing both vitamin D_3 and vitamin C may help to sustain skeletal health in aged hens.

HERBAL EXTRACTS

These days, antibiotic growth promoters are prohibited in animal feed due to the rise of antibiotic-resistant microbial species (Casewell et al., 2003). Therefore, the poultry industry needs to find alternatives to antibiotic growth promoters to improve production performance in the future. Herbal extracts, such as oleoresins, herbs, and essential oils, known as phytobiotics, are considered one of the alternatives to antibiotic growth promoters in the poultry industry (Kumar et al., 2018). Plant-derived products play crucial roles due to their antioxidant, anti-inflammatory, and antimicrobial properties (Alagawany et al., 2019). Studies have investigated the effects of supplementing various types of herbal extracts to hens, particularly in the late-laying period to sustain egg production performance for an extended period.

Oregano extract, a representative natural herbal extract from herbaceous Mediterranean species of the Lamiaceae family, is rich in phenolic compounds including thymol and carvacrol. It has various antioxidant, antibacterial, anti-inflammatory, and anti-proliferative effects (Zhang et al., 2014; Pezzani et al., 2017; Oniga et al., 2018). Several studies have shown that the supplementation of oregano extract to hens has positive effects, particularly in enzyme secretion, nutrient utilization, as well as egg production (Hernandez et al., 2004; Windisch et al., 2008; Bozkurt et al., 2016; Reshadi et al., 2020). A previous study also demonstrated that supplementing dietary oregano extract has improved the laying performance, egg quality, and intestinal morphology of 70-week-old ISA Brown hens (Ramirez et al., 2021). In this study, oregano extract enhanced the uterine health of hens by improving the composition of gut microbiota (Zou et al., 2016; Emmanuel et al., 2022). Additionally, it interfered with the activity of intestinal pathogenic bacteria (Kers et al., 2018a) and improved nutrient utilization necessary for egg formation, ultimately leading to improved egg production performance during the late-laying period (Kers et al., 2018b; Diaz Carrasco et al., 2019). Additionally, oregano extract improved feed efficiency through the impact of thymol and carvacrol with antimicrobial and anti-inflammatory properties, resulting in the improvement of gut health in late-laying hens (Feng et al., 2021). Studies also reported that feeding oregano extract to hens improved the eggshell thickness (Feng et al., 2021; Ramirez et al., 2021), by enhancing intestinal morphology and calcium utilization in the diet for eggshell synthesis (Emmanuel et al., 2022). Furthermore, thymol in oregano extract has a positive effect on the activity of gut microbiota and the utilization of minerals such as calcium and magnesium, increasing eggshell thickness (Kim et al., 2021). Moreover, oregano extract may increase eggshell strength by promoting the metabolism of intestinal microbiota, resulting in an improvement in calcium utilization. Oregano extract may improve the albumen height and Haugh unit by promoting the secretion of albumin protein in the uterus (Ramirez et al., 2021). In this way, oregano extract containing polyphenol molecules exhibits antioxidant effects, improves the intestinal gut health of late-laying hens, and sustains laying persistency through its antimicrobial properties and immune-modulating action (Darmawan et al., 2022).

Moringa oleifera is an herbal extract that improves the immune function, antimicrobial properties, and blood cholesterol levels in chickens (Marrufo et al., 2013). A previous study reported that supplementing 3, 6, and 9 g/kg of *Moringa oleifera* extract to 64-week-old Hy-Line Browns for 8 weeks has increased laying performance, eggshell quality, and Haugh unit while reducing feed conversion ratio, ammonia emission in urine, and serum cholesterol due to the active substances of *Moringa oleifera*, which has antimicrobial and antioxidant properties (Abdel-Wareth and Lohakare, 2021). The substances β-sitosterol and 4-[α-(L-rhamnosyloxy) benzyl]-o-methyl thiocarbamate (trans) present in *Moringa oleifera* are known to improve the overall health condition of chickens (Mahfuz and Piao, 2019). The active substances in *Moringa oleifera* may reduce the ammonia emission from the feces by promoting the secretion of endogenous enzymes and hampering the activity of intestinal pathogenic bacteria (Sethiya, 2016).

Rosemary powder is a spice produced by dehydrating the leaves of the rosemary herb. It contains numerous phenolic compounds, including carnosic acid, the most potent antioxidant in rosemary powder with strong antioxidant properties (Labban et al., 2014). In a study by Alagawany and Abd El-Hack (2015), the effects of dietary rosemary herb on the laying performance, egg quality, serum biochemical parameters, and oxidative status in 36-week-old hens until 52-week-old were investigated. The findings highlighted that supplementing with rosemary improved hens' egg production performance, immune response, and antioxidant status. The author noted that the biological activity of phenolic compounds found in rosemary led to higher feed efficiency, thereby enhancing production performance (Bozin et al., 2006). Moreover, phytogenic compounds found in rosemary, such as flavonoids, have been shown to enhance the biological activity of ascorbic acid, act as antioxidants, and potentially improve immune function (Acamovic and Brooker, 2005).

Ligustrum lucidum is a Chinese herb known for its rich content of phenolic glucosides, flavones, ursolic acid, and oleanolic acid. A study by Li et al. (2017) examined the impact of *Ligustrum lucidum* on laying performance, egg quality, and caecal microbiota in 72-week-old Hy-Line Brown hens. The study indicated that dietary supplementation of *Ligustrum lucidum* led to improved laying performance and influenced the caecal microbial community structure of hens during the late laying period. It was suggested that including *Ligustrum lucidum* in the diet may promote enhanced egg production due to the beneficial effects of its bioactive components, which improve feed efficiency and overall production performance. Furthermore, it was noted that herbs can affect the gut microbiota through favorable stimulation of the gut microbiota's aerobiosis or antimicrobial activity (Wenk, 2003).

Lonicera confusa has therapeutic functions for detoxification in humans and has been commonly used as a traditional prescription. It contains chlorogenic acid as its main component, which has various beneficial effects such as anti-inflammatory, anti-tumor, antibacterial, and antioxidant properties (Wang et al., 2015). *Astragali Radix* is an herbal medicine used in traditional East Asian medicine to treat fever, weakness, loss of appetite, and anemia in humans (Li et al., 2014). The primary constituent of *Astragali Radix* is astragalus polysaccharide, which has several beneficial effects in animals, such as anti-inflammatory, antibacterial, and antioxidant properties (He et al., 2012). A previous study examined the impact of supplementing *Lonicera confusa* and *Astragali Radix* extracts in the diet on laying performance, egg quality, antioxidative status, and sensory evaluation in 52-week-old Lohmann pink-shell hens (Xie et al., 2019). The results show that supplementing *Lonicera confusa* and *Astragali Radix* in a diet improved albumen quality and yolk color. Chlorogenic acid increased the strength of albumen gels and the content of β-ovomucin in the thick albumin, enhancing albumen quality (Hatanaka et al., 2009). The improvement of yolk color is related to increased antioxidative status, which could inhibit carotenoid oxidation in the yolk (Mine, 2008).

Peppermint (*Mentha piperita* L.) is a well-known medicinal plant with recognized antibacterial and anti- inflammatory properties attributed to its bioactive component (Dorman et al., 2003). Flavonoid, the main component of peppermint, has been found to have advantageous effects on gut microbiota by reducing harmful bacteria and promoting the growth of beneficial bacteria in the gut (Han et al., 2023). A previous study has reported that adding peppermint to the diet of aged hens improved laying performance, egg quality, antioxidant capacity, and cecal microbiota (Bai et al., 2023). In this study, the inclusion of peppermint into the diet of hens has improved antioxidant capacity, metabolism, gut microbiota as well as egg production performance. The bioactive components such as linalool, menthone, and menthol found in peppermint have beneficial effects on intestinal health, antioxidant status, and nutrient utilization. Moreover, the flavonoids and phenols in peppermint are effective at scavenging free radicals and safeguarding body tissues from oxidative stress by aging. The study also revealed that dietary peppermint supplementation improved gut microbiota by reducing the presence of Proteobacteria, which is known to be linked to the development of inflammatory bowel diseases (Mukhopadhya et al., 2012). The author suggested that dietary peppermint extract could potentially decrease the occurrence of diseases in aged hens by inhibiting harmful bacteria in the gut.

CONCLUSION

The decline in laying performance and egg quality in aged hens presents a significant challenge to the commercial egg industry. This review highlights the potential of feed additives to counteract age-related physiological deterioration. Probiotics and prebiotics can restore gut microbiota balance and enhance nutrient absorption in hens with compromised digestive function. Organic acids improve mineral absorption and gut barrier integrity, while exogenous enzymes aid nutrient utilization in birds with reduced endogenous enzyme activity. Vitamins such as A, D, and E support immune and antioxidant functions, and herbal extracts offer antimicrobial benefits that may compensate for immunosenescence in late-laying hens.

To support laying persistency beyond 70 weeks, future studies should focus on optimizing additive combinations, dosages, and timing of administration. Research targeting the late-laying phase is critical for maintaining shell quality, production performance, and overall hen health.

ACKNOWLEDGMENTS

This research study was funded by the project (RS-2024-00400914) of the Rural Development Administration, Republic of Korea.

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Received Jun. 4, 2025, Revised Jun. 16, 2025, Accepted Jun. 16, 2025