REVIEW

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Application of dietary organic acids in laying hens production: a comprehensive review

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Abstract
The removal of antibiotic growth promoters from poultry diets has driven research into alternative feed additives to optimize production performance in laying hens. Among these alternatives, dietary organic acids (OAs) have gained significant attention due to their antimicrobial properties, ability to modulate intestinal acidity, and role in enhancing nutrient utilization. This review comprehensively examines the effects of OAs supplementation on egg production and quality, blood parameters, intestinal morphology, fecal and intestinal microbiota, and bone health in laying hens. Studies indicate that various OAs supplementation improves egg production and quality. Additionally, OAs contribute to superior eggshell quality by enhancing calcium and protein absorption. The physiological benefits of OAs supplementation include improved intestinal morphology, gut microbiota, and immune and physiological responses. On the other hand, the effectiveness of OAs varies depending on the type of acid, dosage, environmental conditions, and interactions with feed ingredients or additives. This review consolidates current findings to provide practical insights into the application of OAs as a viable alternative to AGPs in commercial laying hen production.
Keywords: Egg production, Egg quality, Laying hen, Organic acids, Physiological responses

INTRODUCTION

Modern commercial laying hens possess an exceptionally high genetic potential for egg production, necessitating precise nutritional strategies to maximize productivity while maintaining optimal egg quality. The global egg industry has witnessed continuous growth, driven by the rising demand for high-quality eggs as a sustainable protein source. Historically, antibiotic growth promoters (AGPs) have been widely employed to enhance growth performance, improve feed efficiency, and mitigate disease challenges in poultry production. However, concerns regarding antimicrobial resistance and potential antibiotic residues in poultry products have raised significant public health concerns. In response, the European Union (EU) implemented a phased ban on AGPs in animal feed, restricting certain antibiotics in 1999 and fully prohibiting their inclusion in livestock diets by January 2006 (Regulation 1831/2003/EC). This regulatory shift has prompted extensive research into alternative feed additives with antimicrobial and performance-enhancing properties, including OAs, plant extracts, enzymes, probiotics, prebiotics, herbs, and essential oils [1,2].

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

Authors' contributions
Conceptualization: Lee J, Oh S, Heo JM.
Data curation: Lee J, Oh S, Heo JM.
Formal analysis: Lee J, Heo JM.
Methodology: Lee J.
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Validation: Oh S
Investigation: Lee J, Oh S.
Writing - original draft: Lee J, Oh S, Heo JM.
Writing - review & editing: Lee J, Oh S, Heo JM.

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

Among these alternatives, OAs have emerged as highly promising feed additives, owing to their well-documented antimicrobial effects, capacity to modulate intestinal pH, and ability to enhance nutrient digestibility [3]. OAs are characterized by their carboxyl (-COOH) functional group and are classified into short-chain fatty acids (SCFAs; C1–C7) and medium-chain fatty acids (MCFAs; C6–C12). These acids occur naturally in plant and animal tissues and are also produced through microbial fermentation in the gastrointestinal tract [3]. Notable examples include formic acid, acetic acid, propionic acid, butyric acid, and citric acid, each exhibiting distinct biological roles in gut health and nutrient metabolism [4]. SCFAs, including acetate, propionate, and butyrate, are produced via microbial fermentation of dietary fibers in the hindgut. These acids contribute to gut homeostasis by modulating microbial populations, enhancing epithelial integrity, and regulating host metabolism [5,6]. Butyrate, in particular, has been reported to promote villi development, stimulate epithelial proliferation, and exert anti-inflammatory effects, while also serving as an energy source for colonic epithelial cells [7–9]. Additionally, OAs have been shown to prevent or limit the proliferation of pathogenic bacteria, such as *Escherichia coli*, *Salmonella* spp., and *Campylobacter* spp., by lowering intestinal pH and disrupting microbial cell membranes [10]. In addition to SCFAs, MCFAs such as caproic (C6), caprylic (C8), capric (C10), and lauric (C12) acids exhibit potent antimicrobial effects, rapid absorption kinetics, and superior metabolic efficiency compared to long-chain fatty acids (LCFAs) [11]. While LCFAs require re-esterification and lymphatic transport, MCFAs are directly absorbed and rapidly utilized for energy, making them a valuable component of antimicrobial feeding strategies [12]. Beyond their antimicrobial properties, OAs play a crucial role in enhancing nutrient utilization. Citric acid, for instance, improves calcium bioavailability by chelating calcium ions and preventing the formation of insoluble calcium-phytate complexes [13]. Similarly, ascorbic acid functions as an antioxidant, supporting stress resilience in laying hens, particularly under high-temperature conditions [14]. Other OAs, such as benzoic acid and humic acid, have demonstrated beneficial effects in feed preservation, detoxification, and growth stimulation [15,16].

Acidifiers, including OAs and their salts, have been extensively studied in poultry nutrition for their ability to regulate gastrointestinal pH, promote beneficial microbiota, and improve nutrient digestibility [17,18]. By reducing gut pH, OAs create an unfavorable environment for pathogenic bacteria while fostering the growth of beneficial microbes. Moreover, OAs supplementation enhances the activity of digestive enzymes, increases microbial phytase activity, and stimulates pancreatic secretion, ultimately improving the absorption of proteins, amino acids, and minerals [19,20]. However, despite their potential health benefits, the effects of OAs in laying hens remain highly variable across studies, with inconsistencies arising due to differences in OAs sources, dosage levels, supplementation timing, environmental conditions, diet composition, and gut microbiota heterogeneity [19,21,22]. Moreover, factors such as the buffering capacity of dietary ingredients and interactions with other feed additives may influence the efficacy of OAs in production performance and physiological responses. Given these discrepancies, a comprehensive review is warranted to consolidate current findings and identify optimal supplementation strategies for OAs in laying hen production.

This review aims to evaluate the role of OAs in laying hen nutrition, with a particular focus on their effects on egg production performance and physiological responses. By reviewing recent findings, this paper seeks to provide practical insights into the application of OAs as a viable alternative to AGPs in commercial poultry production.

EFFECTS OF ORGANIC ACIDS ON EGG PRODUCTION

Egg production is a primary determinant of profitability in the layer industry, influenced by factors

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such as nutrient digestibility, mineral utilization, gut health, and environmental stress. OAs enhance laying performance by improving nutrient absorption, stabilizing the intestinal microbiota, and reducing gut pH, which supports better feed efficiency and egg production, even under stress conditions. Studies that investigated the effects of OAs on egg production are summarized in Table 1.

Formic acid and blends

Formic acid and salts of butyric, propionic, and lactic acids supplemented in 53-week-old White Lohmann LSL hens for 16 weeks increased body weight and HDEP, alongside increases in EM and better FCR, while FI remained unchanged [5]. Similarly, formic acid supplementation in drinking water for 68-week-old Hy-Line W-98 White Leghorns under heat stress increased HDEP and EW and improved FCR [23]. The positive effects were attributed to enhanced digestive enzyme activity, microbial phytase activity, and pancreatic secretions [19,24]. A multi-acid mixture containing formic, lactic, malic, citric, and tartaric acids in 32-week-old W-36 layers improved EW [25]. Another mixture (15% formic acid, 14% acetic acid, 7% propionic acid, etc.) in 73-week-old Shaver 579 hens improved HDEP and FCR when provided via drinking water [26]. Acidifier blends containing formic, propionic, and acetic acids with cinnamaldehyde improved BW and weekly HDEP in long-term trials [27,28]. Overall, formic acid and multi-acid blends consistently improve HDEP, EW, and FCR, especially under heat stress, by enhancing enzyme activity, nutrient absorption, and gut health.

Acetic acid

Acetic acid supplementation in drinking water (200, 400, and 600 ppm) in 30-40-week-old Brown Leghorn hens under heat stress (35℃) increased egg production and significantly improved EW at higher inclusion levels [29]. Since heat stress reduces serum and tissue mineral concentrations, thereby impairing egg production [30], acetic acid likely enhanced nutrient digestibility and helped maintain microbial balance. Additionally, silicic acid powder containing bamboo vinegar (rich in acetic acid), supplemented at 2-4 g/kg for 16 weeks in 25-week-old ISA Brown hens, significantly improved HDEP during the later weeks of lay [31]. Bamboo vinegar-derived acetic acid inhibits pathogenic bacteria and promotes beneficial microbiota [32], while silicic acid supports skeletal development [33] and collagen biosynthesis [34]. Collectively, acetic acid is particularly effective under heat stress conditions, improving HDEP and EW through enhanced mineral absorption and modulation of gut microbiota.

Propionic acid and salts

Dietary supplementation of 0.1% propionic acid, 0.2% butyric acid, and 0.3% acetic acid in 47-week-old Bowens hens increased FI [35], although no detailed mechanism was provided. Youssef et al. [36] evaluated the impact of an OAs mixture (0.06% fumaric acid, calcium formate, calcium propionate, potassium sorbate, and hydrogenated vegetable oil) on 27-week-old Hy-Line Brown hens over 12 weeks and reported improved HDEP and EM. A mixture of calcium-formate, calcium-propionate, calcium-lactate, and citric acid added to the diet of 75-week-old Hy-Line Browns reduced soft-shell and broken eggs while improving FCR [4]. These findings suggest propionic acid and its calcium salts support better nutrient solubility and gut balance, sustaining HDEP and reducing egg defects under stress conditions.

Butyric acid and salts

Sodium butyrate supplementation (0.05%, 0.10%, 0.20%) in 65-week-old Hy-Line Brown layers for 8 weeks significantly reduced broken egg percentage, with the highest inclusion showing the

Table 1. Effects of organic acids on egg production in laying hens

Breed	Age (weeks)	Composition of OAs	Dosage	Production performance							References
				BWG	FI	FCR	HDEP	EW	EM	Soft-shell or broken egg production	
Hybrid Hysex Brown hens	36–52	Humic acid (85% polymeric polyhydroxy acid)	30, 60 g/ton		↓	↓	↑				[46]
Shaver 579	67–74	2% Fumaric acid, 50% calcium butyrate-calcium propionate and calcium lactate, 48% carrier	0.5, 1.0, 1.5 kg/ton (260, 520, 780 ppm, respectively)			↓	↑				[93]
White Lohmann LSL	53–70	Formic acid and salt of butyric, propionic, and lactic acids	0.5, 1.0, 1.5 kg/ton (260, 520, 780 ppm, respectively)	↑		↓	↑		↑		[5]
Brown Leghorn (heat stress conditions)	30–40	Acetic acid in drinking water	200, 400, 600 ppm				↑	↑			[29]
Gimmizah strain	20–44	Humic acid (85% polymeric polyhydroxy acid, 10% phophorus, 2% magnesium, 2% sulfur, 1% trace minerals)	100, 200 mg/kg				↑	↑			[47]
Hy-Line Brown	75–80	17% Calcium-formate, 5% ca-propionate, 15% calcium-lactate, 27% citric acid, 36% carrier	0.2% (1280 ppm)			↓				↓	[4]
ISA Brown	36–41	Phenyllactic acid	0.1, 0.2, 0.3%				↑				[94]
Shaver 579	73–78	OAs mixture (15% formic acid, 14% acetic acid, 7% propionic acid, 2.5% sorbic acid, 2% citric acid, 2% lauric acid, 1% ascorbic acid, 24% ammonium formate, 7% ammonium propionate, 5% propyline glycol, 18.5% water) in drinking water	0.5, 1.0, 1.5 mL/liter of drinking water			↓	↑				[26]
Brown Nick layers	23–43	400 mg propionic acid, 542 mg formic acid, 100 mg acetic acid, 100 mg sorbic acid, 300 mg ammonium propionate, 200 mg ortofosforic acid, 800 mg almond favour in each kg feed	1.0%		↑	↓	↑				[57]
Laying hens (heat stress conditions)	53–61	Sodium formate	0.1, 0.2, 0.3%			↓	↑				[60]
Hy-line Brown	27–39	Fumaric acid, calcium formate, calcium propionate, potassium sorbate, hydrogenated vegetable oil	0.06%				↑		↑		[36]
Hy-Line W-98 commercial White Leghorn (heat stress conditions)	68–76	Formic acid	0.5, 1.0, 1.5 mL/ liter of drinking water			↓	↑	↑	↑		[23]
W-36 laying hens	32–42	38% OAs (formic, lactic, malic, citric, tartaric, ortho phosphoric acids), 62.0% silicate as carriers	3 kg/ton					↑			[25]
Hy-Line Brown	65–73	A sodium salt of butyrate	0.05, 0.10, 0.20%							↓	[37]

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Table 1. Continued

Breed	Age (weeks)	Composition of OAs	Dosage	Production performance							References
				BWG	FI	FCR	HDEP	EW	EM	Soft-shell or broken egg production	
Lohman layers	26–38	70% propionic acid, 5% citric acid, 25% modified ligonosulphonic acid	1.5, 3.0, 4.5 kg/ton		↑	↓		↑			[66]
Hy-Line brown	25–35	17% fumaric acid, 13% citric acid, 10% malic acid, 1.2% capric and caprylic acid, a carrier	0.05, 0.10, 0.20%				↑				[42]
Hyline-White	59–75	Boron acid (17.5% boron) / ascorbic acid / combination of boric acid and ascorbic acid	200 mg/kg / 120 mg/kg / 200 mg/kg ascorbic acid + 120 mg/kg boric acid		↑		↑				[65]
White-leghorn	24–40	Sodium-butyrate / calcium-propionate	0.5, 1.0, 1.5% sodium-butyrate, 0.5, 1.0, 1.5% calcium-propionate			↓	↑		↑		[40]
ISA Brown	25–41	Silicic acid powder containing bamboo vinegar liquid	2, 4, 6 g/kg				↑		↑		[31]
Hy-Line W-36 laying hens	34–44	Citric, butyric, fumaric acid supplementation in 2 different NPP levels (60 or 100% of Hy-Line W-36 recommended values)	5 g/kg			↓	↑		↑		[43]
Shaver laying hens	24–40	Acetic acid	2.5, 5g/kg			↓	↑		↑		[95]
Hy-Line W-36 commercial layers	32–42	A combination of formic, lactic, malic, citric, tartaric, and orthophosphoric acids containing 38% OAs and 62% silicates as carriers	0.005%			↓					[96]
Layers	0–28	Formic, propionic, and acetic acids in ammonium salt form, and cinnamaldehyde	1 g/kg	↑			↑				[27]
Lohman pink-shell laying hens	45–61	Benzoic acid	1000, 2000 mg/kg					↓			[48]
ISA Brown strain laying hens	26–30	Acidifier-dextrose combination (citric acid)	1 g/1.25, 1 g/2.5, 1 g/3.75 liters of drinking water			↓	↑				[45]
Lohmann Brown hens	24–36	Caprylic acid	500, 1000, 2000 mg/kg	↑		↓	↑		↑		[51]
Isa Brown hens	70–78	Calcium propionate, calcium butyrate (The calcium content in calcium propionate and calcium butyrate was 21.48% and 18.69%, respectively)	0.5%		↓						[41]
Commercial strain of Bowens	47–58	Propionic acid / butyric acid / acetic acid	0.1% / 0.2% / 0.3%		↑						[35]
Hy-Line Brown layers	43–51	*B. subtilis* + 25% Formic acid, 10% fumaric acid, and 10% sorbic acid	0.15%				↑		↑		[97]

OAs, organic acids; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; HDEP, hen-day egg production; EW, egg weight; EM, egg mass; NPP, non-phytate phosphorus.

lowest breakage rates [37]. Butyrate supports mineral utilization, improving eggshell integrity, especially in older hens [38,39]. Calcium propionate and calcium butyrate supplementation (0.5%) in 24-week-old White Leghorns for 16 weeks significantly reduced FCR and increased EM [40]. However, in 70-week-old ISA Browns, FI decreased with the addition of calcium propionate or butyrate, while HDEP remained unaffected [41]. A microencapsulated OAs containing MCFAs (capric and caprylic acid) and butyric acid in 25-week-old Hy-Line Browns improved HDEP [42], likely due to antimicrobial effects that selectively alter gut microbiota. In summary, butyric acid enhances nutrient utilization and eggshell quality, while microencapsulated blends with MCFAs further improve laying performance via antimicrobial effects.

Citric and fumaric acids

A mixture of citric, butyric, and fumaric acids under NPP-deficient conditions in 34-week-old Hy-Line W-36 hens improved HDEP and maintained EM, showing significant OAs×NPP interactions [43]. Citric acid can chelate calcium and prevent the formation of insoluble calcium-phytate complexes, thereby enhancing mineral bioavailability [13,27,44]. Similarly, fumaric acid inclusion with calcium formate/propionate in 27-week-old Hy-Line Browns increased HDEP and EM [36]. Citric acid-dextrose supplementation also increased HDEP and reduced FCR in APEC-infected ISA Browns [45], mitigating pathogen-induced performance decline. Together, citric and fumaric acids enhance mineral utilization, maintain laying performance under phosphorus-deficient diets, and mitigate the effects of pathogenic stress.

Humic, benzoic, and caprylic acids

Kucukersan et al. [46] reported that supplementing humic acid (85% polymeric polyhydroxy acid at 30 and 60 g/ton) to 36-week-old Hybrid Hysex Brown hens for 16 weeks significantly enhanced HDEP, EW, and feed efficiency by stabilizing intestinal microbiota. Similarly, humic acid supplementation (100 or 200 mg/kg) for 24 weeks in 20-week-old Gimmizah hens increased HDEP and EW, while reducing the age at first egg, suggesting improved sexual maturity [47]. Benzoic acid (1,000–2,000 mg/kg) in 45-week-old Lohmann Pink-Shell hens for 16 weeks decreased EW at high doses [48], illustrating variability in acidifier responses due to dietary buffering and gut microbiota differences [49,50]. In contrast, caprylic acid (500–2,000 mg/kg) in 24-week-old Lohmann Browns improved BW, HDEP, EM, and feed efficiency by reducing bacterial competition for nutrients and minimizing toxic metabolites [6,51]. Overall, humic and caprylic acids enhance nutrient utilization and microbial stability, thereby improving overall laying performance. In contrast, benzoic acid exhibits dose-dependent effects on laying performance.

EFFECTS OF ORGANIC ACIDS ON EGG QUALITY

Egg quality has a direct impact on the economic value of eggs and hatchability. Key traits such as eggshell strength and thickness protect eggs from breakage and contamination, while the HU reflects albumen freshness and protein quality. OAs can enhance mineral absorption and protein metabolism, thereby improving shell integrity and internal quality. Previous studies that investigated the effects of OAs on egg quality are summarized in Table 2.

Formic acid and blends

Formic acid and salts of butyric, propionic, and lactic acids were examined in 53-week-old White Lohmann LSL hens over 16 weeks, improving shell thickness and reducing the proportion of thin and broken shells at later stages of lay [5]. These improvements were attributed to enhanced

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Table 2. Effects of organic acids on egg quality in laying hens

Breed	Age (weeks)	Composition of OAs	Dosage	Egg quality										References
				Yolk index	Albumen index	Albumen height	HU	Yolk color	Eggshell thickness	Eggshell strength	Percent of eggshell	Percent of albumen	Percent of yolk	
Shaver 579	67–74	2% Fumaric acid, 50% calcium butyrate-calcium propionate and calcium lactate, 48% carrier	0.5, 1.0, 1.5 kg/ton (260, 520, 780 ppm, respectively)		↓				↑			↑	↑	[93]
White Lohmann LSL	53–70	Formic acid and salt of butyric, propionic, and lactic acids	0.5, 1.0, 1.5 kg/ton (260, 520, 780 ppm, respectively)						↑					[5]
Brown Leghorn (heat stress conditions)	30–40	Acetic acid in drinking water	200, 400, 600 ppm			↑	↑					↑	↓	[29]
Gimmizah strain	20–44	Humic acid (85% polymeric polyhydroxy acid, 10% phophorus, 2% magnesium, 2% sulfur, 1% trace minerals)	100, 200 mg/kg						↑		↑			[47]
ISA Brown	36–41	Phenyllactic acid	0.1, 0.2, 0.3%				↑			↑				[94]
Bovans Brown hens	26–70	Volatile fatty acids (formic acid, propionic acid, acetic acid) / caproic acid and capric acid	0.50% SCFAs, 0.25% MCFAs / 0.30% SCFAs + 0.20% MCFAs							↑	↑			[59]
Shaver 579	73–78	OAs mixture (15% formic acid, 14% acetic acid, 7% propionic acid, 2.5% sorbic acid, 2% citric acid, 2% lauric acid, 1% ascorbic acid, 24% ammonium formate, 7% ammonium propionate, 5% propyline glycol, 18.5% water) in drinking water	0.5, 1.0, 1.5 mL/liter of drinking water						↑		↑	↑		[26]
Hy-line Brown	27–39	Fumaric acid, calcium formate, calcium propionate, potassium sorbate, hydrogenated vegetable oil	0.06%					↑	↑					[36]
Laying hens (heat stress conditions)	53–61	Sodium formate	0.1, 0.2, 0.3%				↑		↑		↑			[60]
Hy-Line W-98 commercial White Leghorn (heat stress conditions)	68–76	Formic acid	0.5, 1.0, 1.5 mL/liter of drinking water				↑		↑					[23]

Table 2. Continued

Breed	Age (weeks)	Composition of OAs	Dosage	Egg quality										References
				Yolk index	Albumen index	Albumen height	HU	Yolk color	Eggshell thickness	Eggshell strength	Percent of eggshell	Percent of albumen	Percent of yolk	
Lohmann Brown Commercial hens	45–55	60% formic acid, 20% propionic acid, 20% lignosulphonic acid / 70% propionic acid, 5% citric acid, 25% lignosulphonic acid	2.5 kg per ton					↑						[52]
Hy-Line Brown	65–73	A sodium salt of butyrate	0.05, 0.10, 0.20%							↑				[37]
Lohman layers	26–38	70% propionic acid, 5% citric acid, 25% modified ligonosulphonic acid	1.5, 3.0, 4.5 kg/ton					↑		↑				[66]
Lohman strains of layers	26–38	60% formic acid, 20% propionic acid, and 20% lignosulphonic acid	1.5, 3.0, 4.5 kg/ton	↑										[70]
Hy-Line brown	25–35	17% fumaric acid, 13% citric acid, 10% malic acid, 1.2% capric and caprylic acid, a carrier	0.05, 0.10, 0.20%				↑			↑				[42]
Hyline-White	59–75	Boron acid (17.5% boron) / ascorbic acid / combination of boric acid and ascorbic acid	200 mg/kg / 120 mg/kg / 200 mg/kg ascorbic acid + 120 mg/kg boric acid						↑		↑			[55]
Hybrid Lohman Brown Lite	17–38	Humic acid	0.5%		↑		↑							[58]
Hy-Line W-36 laying hens	34–44	Citric, butyric, fumaric acid supplementation in 2 different NPP levels (60 or 100% of Hy-Line W-36 recommended values)	5 g/kg	↑					↑					[43]
Dekalb White	61–76	30% protected sodium butyrate	0, 105, 210, 300 g/ton	↓					↑	↑	↑			[98]
Lohman pink-shell laying hens	45–61	Benzoic acid	1,000, 2,000 mg/kg			↑	↑							[48]
Isa Brown hens	70–78	Calcium propionate, calcium butyrate (The calcium content in calcium propionate and calcium butyrate was 21.48% and 18.69%, respectively)	0.5%						↑	↑	↑			[41]
Hy-Line Brown layers	43–51	*B. subtilis* + 25% Formic acid, 10% fumaric acid, and 10% sorbic acid	0.15%							↑				[97]

OAs, organic acids; NPP, non-phytate phosphorus; HU, Haugh units; SCFAs, short-chain fatty acids; MCFAs, medium-chain fatty acids.

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calcium and protein deposition facilitated by better nutrient absorption. A mixture containing formic, lactic, malic, citric, and tartaric acids supplemented to 32-week-old W-36 hens improved albumen quality and eggshell parameters [25]. Another OAs blend (15% formic acid, 14% acetic acid, 7% propionic acid, and others) in 73-week-old Shaver 579 hens improved eggshell thickness and albumen percentage [26]. Formic acid supplementation in drinking water for 68-week-old Hy-Line W-98 Leghorns under heat stress improved shell thickness and HU, supporting shell quality and albumen freshness even under high temperatures [23]. Gul et al. [52] also reported improved yolk color in Lohmann Brown hens with formic acid mixtures. These studies suggest formic acid and blends enhance mineral utilization, albumen quality, and overall egg freshness.

Acetic acid

Acetic acid supplementation in drinking water for 30-week-old Brown Leghorn hens under heat stress conditions improved albumen height, albumen percentage, and HU [29]. Since heat stress negatively affects protein metabolism [30], acetic acid likely helps maintain protein quality through better nutrient digestibility. Similarly, bamboo vinegar, rich in acetic acid, improved shell thickness and yolk color in 25-week-old ISA Brown hens [31]. Dietary bamboo vinegar inhibits pathogenic bacteria [32], while enhanced mineral utilization supports skeletal metabolism [33,34] and protein digestion [53], thereby contributing to better albumen and shell quality. Collectively, acetic acid helps maintain albumen and shell quality, especially under heat stress, by stabilizing gut microbiota and supporting protein metabolism.

Propionic acid and salts

A mixture of calcium-formate, calcium-propionate, calcium-lactate, and citric acid in the diet of 75-week-old Hy-Line Browns reduced the incidence of soft and broken shells [4]. Another combination of fumaric acid, calcium formate, calcium propionate, and potassium sorbate improved shell thickness and yolk color in 27-week-old Hy-Line Browns [36]. Propionic acid in such blends enhances nutrient solubility, resulting in improved shell integrity and yolk pigmentation.

Butyric acid and salts

Sodium butyrate supplementation in 65-week-old Hy-Line Brown hens improved eggshell strength and reduced the percentage of broken eggs [37]. This improvement was associated with improved mineral utilization and gut health [38,39]. A protected sodium butyrate diet improved shell thickness, eggshell percentage, and shell strength in Dekalb White hens, while also reducing yolk index values, indicating better protein incorporation into the eggshell membrane [54]. Encapsulated butyric acid and protected sodium butyrate in Lohmann Brown hens enhanced shell thickness and eggshell weight as a proportion of EW [55]. Calcium propionate and calcium butyrate supplementation in ISA Brown hens improved eggshell percentage, thickness, and strength [41]. These findings suggest that butyrate-based acidifiers enhance intestinal function and mineral absorption, resulting in stronger shells and improved shell quality.

Citric and fumaric acids

Citric acid, by lowering gastrointestinal pH, enhances mineral solubility and phytase activity [27,56]. Co-supplementation of phytase with citric acid improved shell quality in Brown Nick layers on a low-phosphorus diet [57]. Fumaric acid inclusion in Hy-Line Browns improved shell thickness and yolk color [36]. Citric, butyric, and fumaric acids tested in Hy-Line W-36 hens improved shell thickness and yolk index under different phosphorus levels, suggesting better mineral utilization even under nutritional challenge [43]. Microencapsulated blends containing fumaric and citric acids in Hy-Line Browns improved HU and shell strength [42]. Together, citric and fumaric acids enhance calcium utilization and improve shell quality and albumen quality.

Humic, benzoic, and caprylic acids

Humic acid supplementation in Gimmizah hens improved eggshell percentage and shell thickness, which was supported by higher plasma calcium levels [47]. Similarly, humic acid increased albumen index and HU in Lohmann Brown Lite hens [58]. Benzoic acid supplementation in Lohmann Pink-Shell hens improved albumen height and HU, suggesting better protein quality in the egg white [48]. However, responses to benzoic acid vary due to age, breed, and dietary factors, and the exact mechanisms underlying these variations remain unclear. MCFAs blends containing caprylic acid improved shell quality in Bovans Brown hens, enhancing shell-breaking strength, shell percentage, and shell density at different laying phases [59]. These effects were linked to better gut health and mineral availability. Overall, humic, benzoic, and caprylic acids improve both shell quality and albumen freshness by supporting calcium metabolism and protein utilization.

EFFECTS OF ORGANIC ACIDS ON BLOOD PARAMETERS

Blood parameters reflect the physiological and metabolic status of laying hens, including nutrient utilization, immune function, and stress responses. OAs support immune regulation and mitigate stress-induced physiological changes, thereby contributing to improved overall health and productivity. Previous studies that investigated the effects of OAs on blood parameters are summarized in Table 3.

Blood biochemistry and mineral metabolism

Supplementation with formic acid and its salts, including butyric, propionic, and lactic acids, has been shown to enhance blood protein profiles and mineral metabolism in laying hens [5]. In 53-week-old White Lohmann LSL hens, Soltan [5] observed that dietary inclusion of these acids for 16 weeks significantly increased serum total protein and albumin concentrations, likely due to improved gut health that facilitated the absorption of nitrogen and calcium. Similarly, sodium formate administered to hens exposed to heat stress increased plasma calcium and phosphorus concentrations while simultaneously reducing total lipid and cholesterol levels, suggesting improved mineral availability and modulation of microbial activity in the gastrointestinal tract [60,61]. These findings align with the observation that OAs blends, particularly those containing fumaric and citric acids, improved serum calcium concentrations in Hy-Line Browns by lowering intestinal pH and enhancing nutrient solubility [42]. Moreover, citric and fumaric acids have been linked to enhanced phytase activity, thereby improving the release and utilization of bound minerals [27,56,57].

Immune and stress responses

OAs also play an essential role in regulating immune function and mitigating stress in laying hens. Formic acid supplementation in drinking water for 68-week-old Hy-Line W-98 hens under heat stress increased hemagglutination inhibition titers against Newcastle Disease, indicating enhanced systemic immune responses through the suppression of pathogenic gut microbiota [23]. Propionic acid-based blends have demonstrated similar immunomodulatory effects, increasing plasma anti-*Salmonella* IgA levels following vaccination while modulating IgG titers depending on the infection challenge [27]. Humic acid, when fed to Gimmizah hens, improved hematological parameters, including red and white blood cell counts, hemoglobin levels, plasma calcium levels, and total protein levels [47]. Furthermore, humic acid supplementation in high-density housing conditions significantly

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Table 3. Effects of organic acids on blood parameter in laying hens

Breed	Age (weeks)	Composition of OAs	Dosage	Blood parameters	References
Brown laying hens	40–48	Humic acid (160 mg polymeric polyhydroxy acids (humic, fulvic, ulmic, humatomelanic acids)), 663.3 mg SiO_2 and other minerals) / OAs mixture (15% propionic acid, 24% formic acid, 3% ammonium hydroxide)	0.15% humate / 0.20% OAs mixture	↑ Lymphocyte, ↓ Heterophil, ↓ Heterophil to lymphocyte ratio	[18]
White Lohmann LSL	53–70	Formic acid and salt of butyric, propionic, and lactic acids	0.5, 1.0, 1.5 kg/ton (260, 520, 780 ppm, respectively)	↑ Total protein, ↑ Albumin	[5]
Gimmizah strain	20–44	Humic acid (85% polymeric polyhydroxy acid, 10% phophorus, 2% magnesium, 2% sulfur, 1% trace minerals)	100, 200 mg/kg	↑ Calcium, ↑ Total protein, ↑ Red blood cell, ↑ White blood cell, ↑ Hemoglobin	[47]
ISA Brown	36–41	Phenyllactic acid	0.1, 0.2, 0.3%	↑ Total protein, ↑ Albumin, ↑ Lymphocyte, ↑ Red blood cell, ↑ White blood cell	[94]
Laying hens (heat stress conditions)	53–61	Sodium formate	0.1, 0.2, 0.3%	↑ Calcium, ↑ Phosphorus, ↑ Lactate dehydrogenase, ↓ Total lipid, ↓ Cholesterol, ↓ Alkaline phosphatase	[60]
Hy-Line W-98 commercial White Leghorn (heat stress conditions)	68–76	Formic acid	0.5, 1.0, 1.5 mL/liter of drinking water	↑ Haemagglutination inhibition titers (Newcastle Disease)	[23]
Lohman layers	26–38	70% propionic acid, 5% citric acid, 25% modified ligonosulphonic acid	1.5, 3.0, 4.5 kg/ton	↑ Calcium, ↑ phosphorus, ↑ glucose, ↑ Alkaline phosphatase	[66]
Hy-Line brown	25–35	17% fumaric acid, 13% citric acid, 10% malic acid, 1.2% capric and caprylic acid, a carrier	0.05, 0.10, 0.20%	↑ Calcium	[42]
Hyline-White	59–75	Boron acid (17.5% boron) / ascorbic acid / combination of boric acid and ascorbic acid	200 mg/kg / 120 mg/kg / 200 mg/kg ascorbic acid + 120 mg/kg boric acid	↓ Cholesterol	[64]
Hy-Line W-36 laying hens	34–44	Citric, butyric, fumanric acid supplementation in 2 different NPP levels (60 or 100% of Hy-Line W-36 recommended values)	5 g/kg of diet	↓ Cholesterol, ↑ HDL, ↓ Alkaline phosphatase	[43]
Hy-Line W-36 commercial layers	32–42	A combination of formic, lactic, malic, citric, tartaric, and orthophosphoric acids containing 38% OAs and 62% silicates as carriers	0.005%	↓ LDL	[96]
Layers	0–28	Formic, propionic, and acetic acids in ammonium salt form, and cinnamaldehyde	1 g/kg	↑ Anti-*Salmonella* IgA, ↓ Anti-*Salmonella* IgG	[27]
Lohmann Brown hens	24–36	Caprylic acid	500, 1000, 2000 mg/kg	↑ Total protein, ↑ Albumin, ↑ Globulin, ↑ Glucose, ↑ IgG, ↑ T3, ↑ T4, ↓ Heterophils to lymphocytes ratio, ↓ Cholesterol	[51]
Isa Brown hens	70–78	Calcium propionate, calcium butyrate (The calcium content in calcium propionate and calcium butyrate was 21.48% and 18.69%, respectively)	0.5%	↑ Calcium, ↑ Phosphorus	[41]

OAs, organic acids; NPP, non-phytate phosphorus; LDL, low-density lipoprotein; HDL, high-density lipoprotein; IgA, immune globulin A; IgG, immune globulin G; T3, triiodothyronine; T4, thyroxine.

reduced heterophil counts and heterophil-to-lymphocyte ratios, while increasing lymphocyte counts, indicating reduced stress responses that may be associated with enhanced interleukin-2 signaling and reduced stress hormone secretion [62–64]. These results collectively suggest that OAs modulate both innate and adaptive immunity while mitigating environmental and social stressors.

Lipid and energy metabolism

Beyond their effects on minerals and immunity, OAs influence lipid and energy metabolism. Sodium formate and citric acid supplementation reduced total cholesterol and low-density lipoprotein (LDL) levels while increasing high-density lipoprotein (HDL) concentrations in Hy-Line W-36 hens, suggesting improved lipid profiles [43,60]. Benzoic acid exhibited similar lipid-lowering effects in Hy-Line White layers without altering yolk cholesterol levels, indicating systemic lipid regulation without affecting egg composition [48,65]. Moreover, acidification of the diet with fumaric, citric, and propionic acids was suggested to stimulate glycogenesis by increasing glucose-6-phosphate influx into glycogen synthesis pathways, possibly through the inhibition of glycolysis via elevated citrate levels [66,67]. Medium-chain fatty acids, such as caprylic acid, when combined with OAs blends, further enhance gut health, indirectly supporting serum mineral balance and overall metabolic stability [42,59]. Together, these findings highlight the role of OAs in optimizing lipid metabolism, reducing energy losses, and improving the overall metabolic resilience of laying hens.

EFFECTS OF ORGANIC ACIDS ON INTESTINAL MORPHOLOGY

Intestinal morphology directly affects nutrient absorption efficiency, as villus height (VH), villus width (VW), and crypt depth (CD) determine the functional surface area for digestion and nutrient transfer [68]. Increased VH and VW enhance absorption, whereas excessive CD may indicate villus atrophy and reduced nutrient utilization [69]. Therefore, evaluating villus and crypt structures is crucial for assessing the impact of OAs supplementation on gut functionality. Previous studies that investigated the effects of OAs on intestinal morphology are summarized in Table 4.

Villus and crypt morphology

Dietary OAs significantly influenced villus and crypt development in laying hens, primarily by lowering intestinal pH and modulating microbial colonization. Supplementation with mixtures containing formic, propionic, and citric acids in Lohmann Brown hens increased VH over a 10-week period [52], while increasing inclusion levels linearly enhanced VW and CD in Lohman layers [66,70]. These morphological improvements are associated with reduced intestinal colonization by both pathogenic and non-pathogenic bacteria, thereby lowering local inflammation and promoting nutrient absorption. Longer villi increase the absorptive surface area, improving

Table 4. Effects of organic acids on intestinal morphology in laying hens

Breed	Age (weeks)	Composition of OAs	Dosage	Intestinal Morphology	References
Lohmann Brown Commercial hens	45–55	60% formic acid, 20% propionic acid, 20% lignosulphonic acid / 70% propionic acid, 5% citric acid, 25% lignosulphonic acid	2.5 kg per ton	↑ VH	[52]
Lohman layers	26–38	70% propionic acid, 5% citric acid, 25% modified ligonosulphonic acid	1.5, 3.0, 4.5 kg/ton	↑ VW, ↑ CD, ↑ tunica mucosal width	[66]
Lohman strains of layers	26–38	60% formic acid, 20% propionic acid, and 20% lignosulphonic acid	1.5, 3.0, 4.5 kg/ton	↑ VH, ↑ VW, ↑ tunica mucosal width	[70]
Hyline-White	59–75	Boron acid (17.5% boron) / ascorbic acid / combination of boric acid and ascorbic acid	200 mg/kg / 120 mg/kg / 200 mg/kg ascorbic acid + 120 mg/kg boric acid	↑ VH, ↑ CD, ↑ thickness of the glandular layer	[55]
ISA Brown	25–41	Silicic acid powder containing bamboo vinegar liquid	2, 4, 6 g/kg	↑ VH in jejunum, ↑ villus area in duodenum and jejunum	[31]
Lohman pink-shell laying hens	45–61	Benzoic acid	1000, 2000 mg/kg	↑ VH in duodenum and ileum, ↑ CD in duodenum, jejunum, and ileum, ↓ VH:CD ratio	[48]
Layers	0–28	Formic, propionic, and acetic acids in ammonium salt form, and cinnamaldehyde	1 g/kg	↑ VH in the jejunum	[27]

OAs, organic acids; VH, villus height; CD, crypt depth; VW, villus width.

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digestive efficiency and nutrient uptake [71,72]. Similarly, supplementation of day-old layer chicks with an OAs blend of formic, propionic, and acetic acids plus cinnamaldehyde extended jejunal VH following *Salmonella Enteritidis* vaccination [27]. However, benzoic acid supplementation exhibited segment-specific effects, with duodenal VH and CD increasing at higher doses, while altering VH:CD ratios differently in the jejunum and ileum [48]. This variability may result from differences in benzoic acid formulation, dosage, or experimental conditions, and excessive inclusion levels could induce mild toxicity, impairing gut architecture.

Epithelial and mucosal development

Beyond villus morphology, OAs support epithelial cell proliferation and the development of the mucosal layer. Encapsulated butyric acid and protected sodium butyrate improved glandular layer thickness, VH, and CD in the ileum of Lohmann Brown hens over a 24-week period, with encapsulated butyric acid showing superior effects [55]. Butyrate serves as a direct energy source for epithelial cells, promoting mucosal repair and proliferation [73]. Supplementation with silicic acid powder containing bamboo vinegar in ISA Brown hens enhanced jejunum VH and increased villus areas in the duodenum and jejunum at optimal inclusion levels, likely by suppressing pathogenic bacteria and reducing villus atrophy [31]. These findings collectively indicate that OAs and their derivatives modulate gut morphology by maintaining a balanced microbial ecosystem, reducing intestinal inflammation, and providing metabolic substrates that enhance epithelial growth, ultimately improving nutrient absorption and intestinal health.

EFFECTS OF ORGANIC ACIDS ON FECAL AND INTESTINAL MICROBIOTA IN LAYING HENS

The gut microbiota is essential for nutrient digestion, immune regulation, and protection against pathogens [74]. Optimal microbial diversity enhances colonization resistance and improves gut health [75]. Previous studies on the effects of OAs on fecal and intestinal microbiota are summarized in Table 5.

Modulation of gut microbiota composition

OAs supplementation significantly alters the composition and diversity of intestinal microbiota in laying hens by lowering gut pH and creating an environment favorable for beneficial bacteria. Sodium butyrate supplementation in Hy-Line Brown hens increased total anaerobic bacteria and *Lactobacillus* spp., which are essential for maintaining gut homeostasis through epithelial stimulation, immune enhancement, and nutrient metabolism [37,76]. Similarly, microencapsulated blends containing fumaric, citric, and malic acids along with MCFAs promoted *Lactobacillus* populations and reduced *Escherichia coli* counts in Hy-Line Browns [42]. *In vitro* analyses also showed that sodium butyrate shifted microbial populations toward carbohydrate fermenters such as *Bacteroides* and *Faecalibacterium*, while reducing populations of nitrogenous fermentation bacteria, including *Desulfovibrio*, *Helicobacter*, and *Campylobacter*, ultimately mitigating ammonia production [77]. Additionally, butyric, citric, and fumaric acids enhanced the abundance of beneficial *Lactobacillus* while simultaneously suppressing *Salmonella* in the ileum of Hy-Line W-36 hens [43]. These effects are attributed to the ability of OAs to penetrate bacterial cell membranes and disrupt intracellular metabolism, favoring probiotic populations over pathogenic species.

Pathogen control and microbial balance

Beyond promoting beneficial microbiota, OAs directly inhibit pathogens and restore microbial

Table 5. Effects of organic acids on fecal and intestinal microbiota in laying hens

Breed	Age (weeks)	Composition of OAs	Dosage	Fecal microbiota composition	Intestinal microbiota	References
Hy-Line Brown	65–73	A sodium salt of butyrate	0.05, 0.10, 0.20%	↑ Total anaerobic bacteria, ↑ *Lactobacillus* spp		[37]
Hy-Line brown	25–35	17% fumaric acid, 13% citric acid, 10% malic acid, 1.2% capric and caprylic acid, a carrier	0.05, 0.10, 0.20%	↑ *Lactobacillus* spp, ↓ *Escherichia coli*		[42]
Hyline Grey laying hens	NS (28 days of feeding trial)	Sodium butyrate	10, 15, and 20 mg		↑ *Clostridia* (class), ↑ *Clostridiales* (order), ↑ *Ruminococcaceae* (family), ↑ *Lachnospiraceae* (famliy), ↑ *Bacterodies caecicola* (species)	[77]
Hy-Line W-36 laying hens	34–44	Citric, butyric, fumaric acid supplementation in 2 different NPP levels (60 or 100% of Hy-Line W-36 recommended values)	5 g/kg		↑ *Lactobacillus* spp, ↓ *Escherichia coli*, ↓ *Salmonella* in ileum	[43]
Layers	0–28	Formic, propionic, and acetic acids in ammonium salt form, and cinnamaldehyde	1 g/kg		↑ Lactic acid bacteria in ceca	[27]
Lohman pink-shell laying hens	45–61	Benzoic acid	1000, 2000 mg/kg		↑ *Bacteroides*, ↑ *Faecalibacterium*, ↓ *Desulfovibrio*, ↓ *Helicobacter*, ↓ *Campylobacter*	[48]
Hy-Line Brown layers	43–51	*B. subtilis* + 25% Formic acid, 10% fumaric acid, and 10% sorbic acid	0.15%		↓ *Salmonella Enteritidis*, ↑ *Bifidobacterium in ceca*	[97]

OAs, organic acids; NS, not stated; NPP, non-phytate phosphorus.

balance. Acidifier supplementation containing formic, propionic, and acetic acids, combined with cinnamaldehyde, significantly reduced cecal *Salmonella Enteritidis* in vaccinated layer chicks and simultaneously increased *Bifidobacterium* populations at multiple post-challenge time points, supporting the competitive exclusion of pathogens [27,78–80]. Fumaric acid is particularly effective in suppressing coliforms and *Salmonella* while sparing beneficial *Lactobacillus* due to its slow dissociation rate and the inherent acid tolerance of *Lactobacillus* strains [81–84]. On the other hand, benzoic acid supplementation in Lohmann Pink-Shell hens exhibited dose-dependent effects; moderate inclusion enhanced the *Firmicutes*/*Bacteroidetes* ratio and enriched beneficial bacterial families such as *Clostridiales* and *Lachnospiraceae*, whereas higher doses reduced microbial richness and diversity and selectively enriched *Bacteroides caecicola* [48,85,86]. These findings underscore the importance of achieving optimal inclusion rates to maintain eubiosis and prevent potential dysbiosis. Overall, OAs act as selective antimicrobial agents, reducing pathogenic loads while supporting the growth of probiotic communities, thereby improving gut health and nutrient utilization in laying hens.

EFFECTS OF ORGANIC ACIDS ON BONE HEALTH IN LAYING HENS

Bone health is crucial in laying hens, as impaired calcium metabolism compromises both eggshell quality and skeletal integrity. OAs support bone mineralization by enhancing calcium absorption and utilization, helping reduce bone resorption that accelerates during the laying cycle. Previous studies on the effects of OAs on bone health are summarized in Table 6.

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Table 6. Effects of OAs on bone health in laying hens

Breed	Age (weeks)	Composition of OAs	Dosage	Bone health	References
Hyline-White	59–75	Boron acid (17.5% boron) / ascorbic acid / combination of boric acid and ascorbic acid	200 mg/kg / 120 mg/kg / 200 mg/kg ascorbic acid + 120 mg/kg boric acid	↑ Bone length	[55]
Hyline-White	59–75	Boron acid (17.5% boron) / ascorbic acid / combination of boric acid and ascorbic acid	200 mg/kg / 120 mg/kg / 200 mg/kg ascorbic acid + 120 mg/kg boric acid	↑ Tibia calcium	[65]
Hy-Line W-36 laying hens	34–44	Citric, butyric, fumaric acid supplementation in 2 different NPP levels (60 or 100% of Hy-Line W-36 recommended values)	5 g/kg	↑ Tibia ash, ↑ tibia phosphorus	[43]
Isa Brown hens	70–78	Calcium propionate, calcium butyrate (The calcium content in calcium propionate and calcium butyrate was 21.48% and 18.69%, respectively)	0.5%	↑ Tibia calcium, ↑ tibia phosphorus	[41]

OAs, organic acids; NPP, non-phytate phosphorus.

Calcium absorption and bone formation

Supplementation with specific OAs improves calcium absorption and supports bone development in laying hens. Encapsulated butyric acid at 500 g/ton in Lohmann Brown hens increased tibia length compared with both the basal diet and higher inclusion of protected sodium butyrate, indicating reduced mobilization of bone calcium and enhanced mineral retention in the skeleton [55]. Similarly, boric acid supplementation, either alone or in combination with ascorbic acid, elevated tibia calcium content in Hy-Line White hens, highlighting boron's role in stimulating the formation and maturation of the bone matrix required for optimal mineralization [65,87,88]. These results suggest that specific OAs and mineral-associated compounds can positively modulate bone composition by improving gastrointestinal calcium uptake and overall mineral utilization.

Phosphorus utilization

Citric, fumaric, and butyric acids also enhance phosphorus utilization by promoting phytate hydrolysis. In Hy-Line W-36 hens fed non-NPP-deficient diets, fumaric acid supplementation increased tibia ash content. In contrast, citric acid increased tibia phosphorus levels, demonstrating a significant interaction between OAs and NPP levels [43]. These effects are attributed to the acidification of the gastrointestinal tract, which creates a favorable environment for endogenous phytase activity and improves the release of phytate-bound phosphorus. Such mechanisms highlight the indirect benefits of OAs on bone mineralization, which is achieved through improved nutrient availability.

Bone resorption and structural changes

While some OAs enhance bone health, others may influence bone turnover differently depending on diet composition and age. Calcium butyrate supplementation in older Isa Brown hens decreased tibia calcium and phosphorus content, although tibia bone index and bending strength remained unaffected [41]. This reduction may be linked to increased medullary bone resorption during eggshell formation, a process that can be exacerbated by metabolic acids stimulating osteoclast activity through extracellular acidification, which lowers intracellular pH and destabilizes calcium [89–91]. These findings suggest that while OAs can enhance bone mineral metabolism under optimal conditions, specific organic acids and physiological demands, such as prolonged egg production, may alter their effects to promote increased bone turnover.

CONCLUSION

The inclusion of OAs in laying hen diets has been shown to positively influence egg production, eggshell quality, and overall nutrient utilization by improving intestinal health and mineral metabolism. By modulating gastrointestinal pH, OAs create a more favorable environment for enzymatic activity and microbial homeostasis, ultimately enhancing digestive efficiency. Furthermore, their role in promoting calcium and phosphorus absorption contributes to improved eggshell integrity and skeletal strength, which are critical factors in sustaining long-term laying performance. However, variations in the efficacy of different OAs, influenced by their specific chemical properties, dietary interactions, and environmental conditions, underscore the need for precise formulation strategies. Future research should aim to elucidate the long-term physiological effects of OAs supplementation while optimizing inclusion levels to maximize production efficiency without compromising metabolic balance.

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