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Research Progress on the Regulation of Eggshell Quality by Trace Elements and Feed Additives: Postprint

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Abstract

Eggshell breakage is a significant issue in the poultry industry, influenced by age, genetics, environment, nutrition, and the health status of laying hens. In recent years, research on nutritional regulation of eggshell quality has primarily focused on trace elements and feed additives. This article briefly reviews research progress on the effects of trace elements and feed additives on eggshell quality, aiming to provide novel measures for regulating eggshell quality in production practice. Dietary supplementation with specific levels and forms of manganese, microecological preparations, organic acids, and traditional Chinese medicine extracts can all improve eggshell quality.

Full Text

Research Progress of Microelement and Feed Additives on Eggshell Quality Regulation

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Abstract: Eggshell breakage represents a significant challenge in the poultry industry, influenced by factors including hen age, genetics, environment, nutrition, and health status. Recent nutritional research on eggshell quality regulation has primarily focused on trace elements and feed additives. This paper reviews research progress on the effects of trace elements and feed additives on eggshell quality, aiming to provide novel strategies for eggshell quality control in production practice. Dietary supplementation with appropriate levels and forms

of manganese, probiotics, organic acids, and herbal extracts can all improve eggshell quality.

Key words: eggshell quality; trace element; probiotics; organic acids; essential oils and plant extracts

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Poor eggshell quality constitutes a major issue in layer production, affecting egg output and economic returns while reducing hatchability and embryonic survival rates [1]. The eggshell protects embryos from harmful environmental factors, regulates gas and moisture exchange, and provides calcium for embryonic development [2]. Improving eggshell quality and reducing breakage prevents pathogen invasion, making eggshell quality a critical concern.

Eggshell breakage severely impacts the healthy development of the layer industry. Statistics indicate that broken eggs account for 6%–10% of total production, causing economic losses for the poultry industry and egg processing sector while raising consumer concerns about egg quality [3]. Among poor-quality eggs, broken eggs represent 80%–90% [4]. With advancing age, vitamin D3 metabolism becomes disrupted in laying birds [5], and breakage rates in late lay can exceed 20% of total production [6]. Consequently, the layer industry urgently needs effective measures to improve eggshell quality.

Numerous factors influence eggshell mineralization and quality, including age, genetics, environment, nutrition, and hen health status. Early nutritional research on eggshell quality regulation primarily focused on minerals such as calcium and phosphorus and vitamin D3. Recent studies demonstrate that trace elements like manganese at appropriate sources and levels, or functional feed additives, can promote enzyme activity or affect gastrointestinal metabolism, thereby enhancing eggshell mineralization and improving quality. Zhang et al. [7–9] previously reviewed the effects of nutrients and glycosaminoglycans on eggshell quality, briefly describing manganese and zinc influences. Based on recent research, this paper comprehensively reviews the regulatory effects of trace elements and feed additives on eggshell quality.

Trace Elements and Eggshell Quality

During eggshell and bone mineralization, enzymes related to trace elements (such as carbonic anhydrase and glucuronyltransferase) play crucial roles. Research indicates that trace elements (zinc, manganese, copper) serve as cofactors for certain enzymes, regulating eggshell mechanical properties by influencing calcium carbonate formation and eggshell crystal structure [4].

Zinc Few studies have investigated zinc alone regarding eggshell quality. Adding 60 mg/kg zinc to a basal diet (containing 32.6 mg/kg zinc) significantly

improved eggshell strength in aged hens (69–82 weeks); however, since the diet simultaneously included 60 mg/kg manganese and 10 mg/kg copper, whether zinc alone caused the improvement remains unclear [5]. Dietary zinc/manganese supplementation at 30/0, 65/30, and 100/60 mg/kg increased shell thickness in early-lay hens [10], while 200/160 mg/kg significantly improved eggshell strength without affecting thickness [11]. Our research group previously demonstrated that zinc significantly increased shell thickness, shell weight percentage, and eggshell index, with zinc amino acid complexes showing superior effects compared to zinc sulfate, though eggshell strength was not significantly affected [12–13]. Zinc acts as an activator of carbonic anhydrase; dietary zinc promotes calcium carbonate deposition by increasing carbonic anhydrase activity in plasma and the shell gland, thereby enhancing eggshell weight and thickness. Zinc may also affect eggshell elasticity and toughness, potentially through its influence on matrix proteins or eggshell crystal texture [14].

Zinc metabolism must maintain homeostasis in the body. Zinc deficiency inhibits layer growth, causes incomplete bone mineralization [15], reduces carbonic anhydrase activity, and decreases eggshell quality [13]. The general dietary zinc recommendation is 70–135 mg/kg [16]; higher levels (170 or 200 mg/kg) show no significant improvement [14,17], while excessive levels (137–655 mg/kg) impair layer performance and reduce eggshell weight [18].

Manganese Compared to zinc, more research has investigated manganese's effects on eggshell quality with significant results. Dietary supplementation with 80 mg/kg manganese (as manganese oxide) increased shell thickness [19]. In molted layers, shell thickness and eggshell index increased linearly with manganese dosage (40–200 mg/kg) [20]. Manganese at 100 mg/kg improved eggshell ultrastructure and quality by enhancing glycosaminoglycan and uronic acid synthesis in the shell gland, significantly increasing eggshell strength, thickness, and toughness [21]. However, other studies reported that dietary manganese (25, 50, and 70 mg/kg) did not affect eggshell quality [22]. Most research indicates that dietary manganese improves eggshell mechanical properties, though its effects on shell weight and thickness remain inconsistent.

Manganese serves as an activator for galactose- β -1,3-glucuronosyl transferase I (GlcAT-I), which transfers a glucuronic acid residue from UDP-glucuronic acid to glycosaminoglycan chains, catalyzing proteoglycan synthesis [23]. Dietary manganese at 116 mg/kg increased GlcAT-I mRNA and protein expression in the shell gland, elevated glycosaminoglycan and uronic acid content in the eggshell membrane, improved eggshell ultrastructure, and enhanced eggshell strength. Comparative proteomics analysis further revealed that dietary manganese upregulated seven proteins potentially related to eggshell formation, including two galactosyltransferases and glucuronosyltransferases [24]. Thus, manganese likely regulates eggshell quality by modulating glycosaminoglycan synthesis, affecting glycosaminoglycan formation and eggshell ultrastructure.

Manganese deficiency thins the eggshell, alters ultrastructure (particularly the mammillary layer), and reduces hexuronic acid and hexosamine content in the organic matrix [25]. Insufficient dietary manganese decreases GlcAT-I mRNA and protein expression in the shell gland and reduces glycosaminoglycan and uronic acid content in the eggshell membrane, impairing ultrastructure and weakening eggshell strength [21]. The appropriate dietary manganese level is 80–120 mg/kg, with supplementation generally not exceeding 200 mg/kg. Levels beyond the optimal range provide no further eggshell quality improvement and do not significantly affect performance [26].

Copper The eggshell membrane consists of numerous collagen fibers, and lysyl oxidase plays a significant role in maintaining membrane integrity, with copper participating in lysyl oxidase formation [27]. Copper deficiency can alter lysine-dependent fiber cross-linking, deform the matrix membrane, and cause irregular egg size and shell breakage [28–29]. Since copper requirements in layers are low, deficiency rarely occurs in practice, while high copper negatively affects production and health. Our previous research showed that 125 mg/kg copper (as copper sulfate) produced the thinnest shells and poorest strength, while 6–30 mg/kg copper was more appropriate [29]. Adding organic copper to low-phosphorus diets containing phytase did not affect eggshell quality [30], and replacing copper sulfate with copper lysine did not improve shell quality (thickness, shell weight percentage, eggshell index) in breeding hens [31].

Comparative Studies of Organic vs. Inorganic Trace Element Sources

Recent research indicates that trace element level and form (inorganic or organic) affect eggshell quality (Table 1), though results remain inconsistent. Replacing inorganic zinc and manganese (oxides) with organic forms (amino acid salts) alleviated age-related eggshell quality decline, improving eggshell strength in 62–70-week-old layers without affecting shell weight percentage or thickness [32]. Dietary supplementation with zinc, manganese, and copper amino acid salts or sulfates (40, 40, and 7 mg/kg respectively) increased shell thickness by 3.8% in the amino acid salt group [33]. Simultaneous addition of organic and inorganic zinc, manganese, and copper significantly increased eggshell weight and thickness [34]. Dietary zinc, manganese, and copper supplementation improved eggshell strength and thickness without differences between sources [35]. Adding manganese (120 mg/kg) to a basal diet (46.4 mg/kg manganese) significantly improved eggshell quality without source differences [36]. Our research showed that organic manganese relative bioavailability values compared to inorganic manganese were 159.1% (eggshell strength), 107.8% (eggshell elasticity), and 189.2% (eggshell thickness) at dietary levels of 25, 50, 100, and 200 mg/kg [26]. Additionally, research on other elements has increased. Chromium propionate supplementation (600 g/kg chromium) in late lay improved shell thickness [37]. Sodium selenite and selenium yeast increased eggshell weight and eggshell index, with selenium yeast also improving strength [38], while organic selenium (selenomethionine) replacing sodium selenite did not significantly improve shell

quality [39]. Boron significantly improved eggshell strength and thickness in aged layers without source differences [40]. Magnesium (2.3, 3.0, 3.5, and 4.2 g/kg) increased eggshell strength and thickness in late-lay hens [41]. Despite extensive research on inorganic and organic trace elements regulating eggshell quality, inconsistent results persist, primarily due to insufficient mechanistic investigation.

In summary, trace elements zinc, manganese, and copper play important roles in eggshell formation, yet minimal research has examined their interactions or antagonistic relationships affecting eggshell quality regulation. Comparative studies investigating synergistic or antagonistic effects among trace elements could provide theoretical support for better utilizing these elements to solve eggshell quality problems while reducing waste.

Probiotics and Feed Additives

Research shows that probiotics, organic acids, and other additives can improve mineral element utilization (e.g., calcium) and eggshell quality (Table 2). Dietary supplementation with 1% fructan significantly improved eggshell strength and shell weight percentage while increasing bone ash, calcium, and phosphorus levels [43]. Adding inulin or fructooligosaccharides to late-lay hen diets increased shell weight percentage, eggshell density, and strength [44]. Inulin supplementation in diets containing high levels of corn dried distillers grains with solubles (DDGS) for 50-week-old layers significantly improved shell weight percentage, thickness, and density [45]. Adding prebiotics (3% or 4%) to diets containing lactic acid bacteria significantly improved eggshell quality, primarily because probiotics and lactose promoted short-chain fatty acid production in the intestine [46]. However, other studies reported that dietary inulin had no significant effect on eggshell weight, thickness, or strength [47], and did not affect eggshell ash, calcium content, or shell weight percentage [48]. Yeast culture-containing diets with prebiotics also showed no eggshell quality improvement [49].

Dietary probiotic supplementation significantly improved eggshell quality [50-52]. Adding *Bacillus subtilis* (2.3×10^8 CFU/g) to aged hen diets (64 weeks) significantly increased laying rate, egg weight, and shell thickness while reducing breakage rate [53]. *Bacillus subtilis* (0.10%) and inulin (0.10%) added separately or together to diets improved performance, eggshell quality, and calcium storage by increasing microbial colonization in villus absorption sites [54]. Probiotic and prebiotic improvements in eggshell quality relate to enhanced mineral absorption and utilization, increasing mineral solubility. This occurs primarily through increased substrate synthesis promoting short-chain fatty acid formation, intestinal mucosal changes and cell colonization increasing small intestinal absorption area, phytase degradation by probiotics, and increased calcium-binding protein expression, thereby promoting healthy intestinal growth [55]. However, some studies reported no significant eggshell quality improvement from dietary probiotics [45,56].

Short-chain fatty acid (SCFA) supplementation (0.05%) in late-lay hen diets improved eggshell strength and reduced soft and broken egg rates [57]; SCFA (0.078%) increased shell thickness in late-lay hens (70 weeks) and reduced breakage rate without affecting shell weight, as organic acids promoted calcium absorption and increased blood calcium, thereby improving eggshell quality [58]. Medium-chain fatty acid (MCFA) supplementation in layer diets (46–70 weeks) improved shell weight percentage, density, and strength by increasing small intestinal villus height and reducing upper gut pH, thereby enhancing calcium and phosphorus utilization and eggshell quality [43]. However, formic and propionic acids showed no improvement in shell thickness or strength for Lohmann layers [59].

Essential Oils and Plant Extracts

Dietary essential oil blends (oregano, bay leaf, sage, myrtle leaf, fennel seed, and citrus peel) reduced soft and broken egg rates by 15.5% in late-lay hens (54–74 weeks) [60]. Essential oil complexes (sage, thyme, and mint extracts at 0.015% or 0.030%) improved eggshell hardness and weight [61]. Plant extracts (red clover and allicin at 0.10%) increased eggshell strength [62], while black cumin seed extracts (1%, 2%, and 3%) significantly improved shell thickness and strength [63]. Chinese herbal extracts increased shell thickness by 10.0%, strength by 15.2%, and reduced breakage rate by 2.5% [64]. Chinese herbal complexes increased eggshell strength by 19.3% in late-lay hens, with increased tibia strength, suggesting the mechanism may involve reduced bone loss and enhanced mineral absorption, though the active components require further investigation [65]. Some studies reported no eggshell quality improvement from dietary essential oils or herbal extracts [45,66].

Most research indicates that dietary probiotics, organic acids, and plant extracts can improve eggshell quality in late-lay hens, primarily by regulating healthy intestinal metabolism, promoting mineral absorption and utilization, and enhancing eggshell mineralization. Particularly in late lay, metabolic processes become more disordered compared to younger birds, predisposing them to absorption and metabolic issues. However, the mechanisms by which these feed additives regulate mineral absorption remain unclear. Molecular biology techniques such as metabolomics could identify gut microbiota associated with eggshell quality regulation for better mechanistic explanation. Furthermore, combining trace elements with probiotics, organic acids, or extracts to co-regulate eggshell quality represents a new direction for nutritional regulation, providing a basis for improved additive utilization.

Table 1 Effects of dietary microelements on eggshell quality of laying hens

Table 2 Effects of feed additives on eggshell quality of laying hens

Dietary supplementation with appropriate forms and levels of manganese, probiotics, organic acids, and herbal extracts can improve eggshell quality, with more pronounced effects in aged layers, making them applicable in production. Incon-

sistent results from trace element and additive supplementation relate to hen age, physiological status, additive form and composition, with the fundamental reason being unclear regulatory mechanisms.

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