DOI: http://dx.doi.org/10.18782/2320-7051.6509

ISSN: 2320 – 7051 *Int. J. Pure App. Biosci.* **6** (5): 480-487 (2018)



Review Article

Dietary Factors Improving Egg Shell Quality in Layer Chicken: A Review

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Received: 24.05.2018 | Revised: 30.06.2018 | Accepted: 7.07.2018

ABSTRACT

Poor egg shell quality is a significant problem in the poultry industry. Incidence of inferior shell quality still remains as one of the major causes of economic losses to egg producers throughout the world. Egg shell quality is also an important concern for consumers, as strong resistance to breaking and lack of shell defects are essential for protection against the penetration of pathogenic bacteria in to eggs. Many factors affect egg shell mineralization and quality, including genetic, environmental and nutritional factors, as well as the health status of hens. Most studies on dietary effects on egg shell and bone quality in laying hens have focused on macro minerals and Vitamin D_3 . However the results of some recent experiments have demonstrated that certain dietary levels and sources of certain micro elements, mainly Zinc and Manganese as well as diet supplementation with certain feed additives influencing the metabolic indices of the gastro intestinal tract can beneficially affect the egg shell mineralization process and egg shell quality. Thus Eggshell quality may be beneficially affected in certain conditions by optimal level and form of micro elements, as well as pre- and probiotics, organic acids, and herb extracts. It should be also stressed that the observed improvement of eggshell quality is often of low magnitude in young layers. So their use can be more economically justified in aged hens.

Key words: Egg shell quality, Pre and Probiotics, Organic acids, Herb extracts.

INTRODUCTION

Poor egg shell quality is a significant problem in the poultry industry, negatively affecting the economic results of egg production, as well as decreasing the hatchability of eggs and increasing embryonic mortality. Protection of the embryo from the harmful influence of outside environmental factors, regulation of gas and water exchange and serving as a Ca supply for embryonic development are the main functions of the egg shell. Incidence of inferior shell quality still remains as one of the major causes of economic losses to egg producers throughout the world. The estimated total cracked eggs or eggs lost prior to reaching their final destination from the point of lay might be 5 to 10 per cent in India. The breakup of this may be 1.0 to 3.0 per cent in collection, 2.0 to 3.0 per cent in packing and 2.0 to 4.0 per cent during transportation and trade channels.

Cite this article: Harikrishnan, S. and Mohan, R., Dietary Factors Improving Egg Shell Quality in Layer Chicken: A Review, *Int. J. Pure App. Biosci.* **6(5)**: 480-487 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6509

The approximate loss incurred by the layer industry in India due to poor shell quality was estimated to be around Rs. 3000 million per annum¹⁵. The highest rate (1.07 per cent) of egg breakage was noticed among the age group of 61-80 weeks and the lowest rate (0.48 per cent) of egg breakage was noticed among the age group of 20-40 weeks. The incidences of eggshell breakages in commercial layer farms were increasing as age advances. It may be due to insufficient mobilization of calcium from bones for eggshell formation $et al.^2$. Since, the proportion of cracked eggs increases with the age of the bird, it is especially important to reduce egg breakage during later part of the production period.

Egg shell quality is also an important concern for consumers, as strong resistance to breaking and lack of shell defects are essential for protection against the penetration of pathogenic bacteria in to eggs. This is an especially crucial problem in the European Union, where scale of egg production in cage systems is being gradually reduced. It was well demonstrated by several researchers that total microbial load of egg shells is lower for cage system eggs compared with free-range.Many factors affect egg shell mineralization and quality, including genetic, environmental and nutritional factors, as well as the health status of hens. Most studies on dietary effects on egg shell and bone quality in laying hens have focused on macro minerals and Vitamin D₃. However the results of some recent experiments have demonstrated that certain dietary levels and sources of certain micro

elements, mainly Zinc and Manganese as well as diet supplementation with certain feed additives influencing the metabolic indices of the gastro intestinal tract can beneficially affect the egg shell mineralization process and egg shell quality.

Egg: The fertile egg is highly complexed reproductive cell and is a tiny center of life, where initial development of embryo takes place. Most of the commercial eggs are infertile. The yolk is surrounded by albumen, having high water content, elasticity and shock absorbing capacity. This entire mass is surrounded by two membranes and an external covering called egg shell. The shell provides a proper shape to the egg and is meant for conserving the valuable nutrients within the egg. Hen egg contains approximately 76% water, 12% protein, 10% lipids and rest vitamins, minerals and carbohydrates. Egg is a major source of human dietary protein with high biological value and excellent protein efficiency ratio.

Egg Shell: The outer cover of the egg, the shell comprises 10-11% of total egg weight. On an average the eggshell weighs 5-6g, with remarkable mechanical properties of breaking strength and is 300-350 micrometer thick. This structure plays a crucial role in protecting the contents of the egg from the microbial and physical environment and in controlling the exchange of water and gases. The calcium content of the eggshell is approximately 1.7-2.5g. An average eggshell contains:

Calcium carbonate	:94-97%
Phosphorus	:0.3%
Magnesium	:0.2%
Sodium, Potassium, Manganese, Iron and Copper	:traces
Organic matter	:<2%
Pore Cuticle	
Palisade L Cleft Mammilla Outer Men	ayer ry Laye mbrane

Structure of the egg shell

The small amount of organic matter mostly consists of matrix proteins (mixture of proteins and polysaccharides rich in sulphated molecules) and shell pigment. The matrix proteins are critically important in determining the egg shell structure and serves as foundation for the deposition of calcium carbonate. There are about 8000 microscopic pores on the shell. The outer surface of the shell itself consists of a mucous coating (cuticle) which is deposited on the egg just prior to the lay. This proteinous covering helps to protect the interior content of the egg from bacterial penetration through the egg shell.

Shell Quality: The aesthetic quality of the egg shell relates to the quality factors which one can observe; such as soundness of the shell, shape of the shell and colour of the shell. However, for commercial layer and breeder operations, shell quality means increased shell thickness and shell breaking strength to reduce number of cracked eggs, an increased number of saleable/ hatching eggs and a higher number of viable day old chicks.

Diet formulation: Shell breaking strength was greater for the sorghum diet than wheat or barley based diet and less for maize-soya diet. High levels of calcium and phytate in the diet of laying hen reduce the availability of trace minerals, especially manganese and zinc. Addition of non starch polysaccharides and phytase enzymes to the feed tends to improve eggshell quality.

No deleterious effects on egg and eggshell quality were observed when levels of chloride and magnesium were up to three times higher than recommended levels. Excess dietary chlorine, however, decreases blood bicarbonate concentration, which plays a pivotal role in eggshell calcification. Low dietary cationic-anionic balance, presence of non starch polysaccharides, mycotoxins and contaminants results in poor shell quality.

Results of recent studies on the effect of nutrition on egg shell quality

There is a complex relationship between calcium, phosphorus, vitamin D_3 and the hormonal system of the layer in calcium metabolism during lay. Calcium and

phosphorus balance is critical for proper egg production and eggshell quality. Layer ration should be formulated with correct amount of calcium and phosphorus (usually 3.5 - 4.0% calcium, 0.35-0.40% phosphorus).

Calcium: Both excess and deficiency of calcium will negatively affect the shell quality. An egg contains almost 2 grams calcium; hence an average of 4 grams of calcium intake per day is required by a layer to maintain good shell quality since only 50 - 60% of dietary calcium is actually used in shell formation. Calcium requirement of a laying hen is 4 - 6 times that of a non-laying hen. The egg enters the shell-gland region of the oviduct - the uterus, 19 hours prior to oviposition, and the shell does not store calcium ions to attach on protein matrix. During the last 15 hours of shell formation, calcium movement across the shell gland reaches a rate of 100-150 mg/hr. This process draws calcium from two sources: diet and bone. Normal blood calcium level is about 20 - 30 mg/dl with a normal layer ration of 3.56% calcium or higher, while layers on a 2% calcium diet, 30- 40% of the calcium is derived from bone. It is therefore important to have pullets, prior to lay, on a high level of calcium to store it on body. Intestinal absorption of calcium in the diet is about 40% when the shell gland is inactive, but reaches 72% when active. This time closely coincides with late afternoon or the dark hours for the layer. Having higher calcium levels in the gut during this time is important to ensure calcium is being taken from the diet and not bone. Large particle sizes of calcium sources allow calcium to be metered throughout this time. In growers, most importantly, high calcium levels during the growth period will interfere with the proper development of the parathyroid gland by increasing gut pH, which will decrease absorption. The damage to the parathyroid would be permanent and would affect the bird's laying cycle afterwards.

Laying hens fed diets with high Ca concentrations (4.4%) experienced decreased egg shell quality in comparison with a control group (3.7% Ca). Increase in Ca dietary level above 3.6-3.9% usually has no positive

influence on egg shell quality¹⁰. Many studies have reported the beneficial effect of replacing fine limestone with coarse limestone or oyster shell, both of which has higher retention times in the gizzard, is dissolved more slowly and thus supplies the hen's organism more evenly with Ca along with maintaining an adequate Ca blood concentration also during the night, has been observed. Cufadar et al.7 investigated the effects of Ca dietary levels (3.0, 3.6 or 4.2 % Ca) and limestone particle sizes (<2mm, 2-5mm, >5mm) in moulted laying hens (76 weeks of age). The obtained results showed that medium or large particle sizes of limestone had a positive effect on egg shell breaking strength when the diet was low in Ca, but this effect was not found in hens fed with a normal or high Ca dietary content.

Phosphorus: The phosphorus content of the eggshell is small i.e. 20mg, compared with 120mg in the egg contents. There is also uneven distribution of the phosphorus in the inner and outer layers of the shell. Phosphate ions have an inhibitory effect on the CaCO₃ and bring the shell formation to an end. High levels of phosphorus in the blood will inhibit the mobilization of calcium from bone. The absorption of calcium and phosphorus are interrelated and can be influenced by:

- a. Source and form of calcium and phosphorus: Calcium source and particle size plays a role in calcium level in the gut when needed. Phosphorus must be in a form that is available and usable by the layer.
- **b.** *Intestinal pH:* Phosphorus absorption is optimal at pH 5.5-6.0. When the pH is higher than 6.5, absorption of phosphorus markedly decreases. Excess free fatty acids in the diet can cause the pH to decrease and therefore, interfere with calcium and phosphorus absorption.
- *c. Calcium and phosphorus ratio:* High calcium or phosphorus levels in the intestine reduce the absorption of both. High calcium increase the pH in the gut and phosphorus absorption is decreased along with zinc and manganese absorption.

High plasma phosphorus decreases calcium absorption from the gut and calcium mobilization from the bone. Phosphorus is an integral part of the acid-base balance in the body. The proper ratio of calcium to phosphorus (Ca: P ratio) for growing birds is 1.5-2.0: 1.0

Dietary levels of 0.30-0.35% available P enables optimal egg shell quality, whereas too high level of available P, above 0.40-0.45% can interfere with intestinal Ca absorption, resulting in a reduction in egg shell quality Waldroup *et al.*²⁴.

Vitamin D₃: Vitamin D_3 and its metabolite (25-OH-D₃) are vital for absorption and mobilization of calcium during shell synthesis. Vitamin D_3 is the major control element in stimulating calcium absorption from the intestine and the effect is facilitated by the synthesis of calcium-binding protein (CBP). The importance of adequate vitamin D_3 intake by the hen is obvious and it is essential for proper calcium and phosphorus utilization. However, excess vitamin D₃ and its metabolites have not shown to benefit eggshell quality.

Microelements

Studies on nutritional effects on eggshell and bone quality in laying hens have focused on the effects of dietary Ca, P, and vitamin D3. However, it is known that some enzymes, e.g. anhydrase, carbonic related to certain microelements are important in the mineralization process, and in recent years there have been an increasing number of studies with laying hens on the relationship between micro minerals and eggshell quality.

Trace elements such as Zn, Mn and Cu, as cofactors of certain enzymes, could affect the mechanical properties of eggshells through exerting an influence on calcite crystal formation and modifying the crystallographic structure of egg shells. A dietary concentration of 28 mg/kg Zn was sufficient for good eggshell quality Stahl *et al.*²⁰. Mabe *et al.*¹⁴ reported the positive effect of eggshell resistance in older hens (69 to 82 weeks of age) by feeding a diet with 60mg/kg Zn. But in younger hens they observed no such effect.

ISSN: 2320 - 7051

Eggshells from hens fed a diet deficient in Mn are thinner and show alterations in shell ultra structure in the mamillary layer. Sazzad et al.¹⁶ found that the content of Mn in a basal, cornsoybean diet (25 mg/kg) is sufficient for optimal laying performance in hens; however, eggshell thickness increased with increasing dietary supplementation of Mn until 105 (25 mg/kg from the basal diet and 80 mg/kg mg/kg from added MnO). Results of a recent study with Hy-Line Grey layers by Xiao et al.²³ indicated that dietary Mn supplementation (100 mg/kg) positively affects eggshell quality, i.e. breaking strength, thickness, and fracture toughness, by increasing the synthesis of glycosaminoglycan and uronic acid in eggshell glands, as well as improving eggshell ultra structure. In general experimental data show that dietary Mn can improve the mechanical properties of eggshell, while the effects on eggshell weight or thickness is rather inconsistent. Discussing the effects of dietary levels of Zn and Mn, it should be also mentioned that maximal concentrations of these trace elements in poultry diets were reduced by European legislation to 150 mg/kg.

Several recent experiments have demonstrated that not only the level of microelements in a layer's diet but also their form (inorganic vs. organic complexes) may affect eggshell quality. Swiatkiewicz and Koreleski²² found that substituting Zn and Mn oxides with amino acid complexes of these microelements alleviates the negative effect of hen age on eggshell quality, in the late phase of the laying cycle. Gheisari et al.⁹ supplemented corn-soybean diet with Zn, Mn, and Cu amino acid complexes or sulphates (40, 40, and 7 mg/kg, respectively) and reported that eggshell thickness was higher by in hens fed diet with organic 3.8% microelements. Favero et al.8 evaluated organic amino acid complexes of Zn, Mn, and Cu added to Cobb 500 breeder hen diets containing inorganic sulphates of these microelements. The results indicated that supplementation simultaneous diet with inorganic and organic forms of Zn, Mn, and

Cu significantly increased eggshell quality, i.e. eggshell weight and thickness. It should be underlined that the effect of organic minerals corresponded to an improvement of eggshell weight by about 2%. In a recent experiment Stefanello et al.,²¹, diet supplementation with increasing levels of Zn, Mn, and Cu had a positive influence on eggshell quality, however no significant differences between inorganic and organic sources (proteinates) were observed. Ma reported that supplementation of late-phase layers' diets with chromium propionate (600 μ g/kg) improved eggshell quality, measured as eggshell thickness. Results of a study with dual-purpose breeding hens (Attia et al., 2010) demonstrated that the substitution of an organic selenium source (selenomethionine) for an inorganic (sodium selenite) did not affect such eggshell quality traits as shell percentage and thickness or shell weight.

FEED ADDITIVES

Pre- and probiotics

Feed additives, mainly pre- and probiotics, as well as organic acids, may improve eggshell quality and this effect can be attributed mainly to increasing the availability of Ca and other minerals. Chen and Chen⁶ reported that diet supplementation with 1% prebiotic fructans significantly increased eggshell percentage and breaking strength, as well as levels of crude ash, Ca and P in tibia bones. Positive effects of dietary inulin or oligofructose on certain eggshell quality indices were found in aged hens by Swiatkiewicz et al.²³. The same authors reported that dietary inulin positively affected eggshell quality (shell percentage, thickness and density) in older hens (50 weeks of age). Cesari et al.⁵ evaluated the influence of prebiotic (skim milk powder containing 54% lactose) added to a diet containing Lactobacillus acidophilus on the performance and egg quality of Hy-Line layers. The results proved the positive influence of prebiotics (3 or 4%) on eggshell quality, which could be due to the increased production of short-chain fatty acids in the intestine of hens fed with the combination of probiotic bacteria and lactose.

The beneficial effects of the use of dietary probiotics in the nutrition of laying hens on egg shell characteristics have been found in some experiments. Abdelgader et $al.^1$ evaluated the influence of dietary inclusion of Bacillus subtilis (2.3×108cfu/g of probiotic) on the performance and eggshell quality of aged laying hens (64 weeks of age). They found a positive effect of probiotics on egg production and eggshell quality, i.e. eggshell weight and thickness, as well reduced number of unmarketable eggs. The efficacy of the dietary inclusion of Bacillus subtilis and inulin, individually or in combination were tested. The results showed a beneficial effect of diet supplementation with probiotic (0.10%), inulin (0.10%), or symbiotic on egg performance, eggshell quality, and calcium retention in aged hens. The improvements in performance and eggshell quality were directly related to the colonisation of beneficial micro flora along with an increase in the villi-crypt absorptive area.

Organic acids

The diet supplemented with short-chain fatty acids (SCFA, 0.05% in the diet) improved egg shell breaking strength, as well as reducing the number of dirty, cracked and misshapen eggs in White Bovans hens et al.¹⁷ Soltan¹⁹ reported that dietary SCFA (a mixture of formic acid and salts of butyric, propionic and lactic acids, 0.078% in the diet) increased eggshell thickness from hens at 70 weeks of age and reduced the number of broken eggs, with no effect on eggshell weight. The author stressed that the improvement in eggshell quality was related to an increase in Ca concentration in serum, which could be attributed to the beneficial effect of organic acids on Ca absorption.

Swiatkiewicz *et al.*²³ observed a positive influence of dietary medium-chain fatty acids (MCFA), i.e., capric and caproic acid and, to a lesser extent, SCFA, on eggshell quality (eggshell percentage, density and breaking strength) in older hens (46-70 weeks of age). The authors attributed this effect to increased Ca and P availability, brought about by a decrease in pH in the upper part of the

intestinal tract and the stimulating effect of organic acids on villus height.

Essential oils and plant extracts

The results of a study with older laying hens (54 to 74 weeks of age) demonstrated that

diet supplementation with a mixture of essential oils (oregano, laurel leaf, sage leaf, myrtle leaf, fennel seeds, and citrus peel oil) reduced the number of cracked or broken eggs by 15.5% et al.⁴. Similarly, Kaya et al.¹¹ reported a beneficial effect of a dietary essential oil combination (sage, thyme, and mint extracts, 0.015 or0.030%) on shell stiffness shell weight. As well, and Lokaewmanee et al.¹² found, in an experiment with Boris Brown layers, that dietary plant extracts (red clover and garlic, 0.10%) improved eggshell quality measured as eggshell breaking strength, and that these positive effects could be probably attributed to observed improvements in small-intestine histological parameters.

Sharma et al.18 observed increased eggshell thickness (by 10.0%) and breaking strength (by 15.2%), as well as decreased number of eggs with shell defects (by 2.5 percentage points) in hens fed diet supplemented with herbal products. Correspondingly, Zhou et al.²⁶ reported improved eggshell breaking strength (by 19.3%) and tibia bone quality in aged hens fed a diet supplement with a mixture of traditional Chinese herbs, indicating that the mechanism of this positive effect of herbs was possibly associated with minimizing structural bone loss and stimulating bone mineral absorption in osteoporotic layers, however the detailed composition of used herbs mixture was not shown.

CONCLUSION

The layer diet supplemented with microelements and feed additives is not consistent and depends on factors like age and physiological stage of the hens, as well as the chemical form and composition of used additives. Eggshell quality may be beneficially affected in certain conditions by optimal level and form of micro elements, as well as pre-and probiotics, organic acids, and herb

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