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Review Article
Effects of Vitamin D and Calcium for the Prevention of Osteoporosis at Various Stages of Life of Laying Hens-Review

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Abstract

Laying hens have been selected for high egg production in a prolonged period. This has negatively affected the health of laying hens, specifically in weakening their bones to the point of developing osteoporosis. Nutrients such as vitamin D and calcium have been studied in order to find a prevention for osteoporosis. The objective of this review was to examine the effects of these nutrients on bone turnover in laying hens in order to find a prevention for osteoporosis through nutritional strategies. Several studies have demonstrated that particulate forms of calcium enhance the absorption of calcium, and thus incorporation of calcium into the bone. Vitamin D has been shown to play a decisive role in blood calcium concentration, and osteoclast activity to break down bone; however, vitamin D continues to play a critical role in laying hen reproduction and is thus necessary. Further research should be done on the ideal concentration of particulate calcium sources and vitamin D in laying hens in order to maintain healthy bone strength and metabolism during the egg laying period.

Key words: Laying hen, osteoporosis, vitamin D, calcium, egg production

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INTRODUCTION

Osteoporosis results when bone resorption occurs at a greater rate than bone formation, thus leaving a fragile bone matrix susceptible to fractures[1]. The decrease in bone density and increase in fractures in laying hens is mainly due to osteoporosis[2]. When laying hens are being disemboweled, 98% of carcasses were found to have broken bones[3]. Fragments of bone puncturing the hen's organs resulting from bone fractures leads to enormous economic loss in the poultry industry[4]. In order to prevent economic loss and ensure the welfare of laying hens, it is important to study the nutritional factors such as vitamin D and calcium that may help prevent bone fractures. Calcium and cholecalciferol (vitamin D) have been shown to prolong the onset of osteoporosis in humans[1], but their effects and mechanisms need to be comprehensively investigated in laying hens.

Although genetically selecting hens for bone strength that have stronger bones produces less fractures[5], it is important to study the nutritional factors that can help prevent osteoporosis due to the fact that nutrients such as vitamin D and calcium are key players in bone formation pathways. 7-Dehydrocholesterol found in the skin is converted into cholecalciferol by ultraviolet rays[6]. Cholecalciferol is converted to 25-hydroxycholecalciferol in the liver, which is converted to 1,25-dihydroxycholecalciferol by the kidney[7]. 1,25-Dihydroxycholecalciferol promotes the absorption of calcium ions by the intestinal epithelial cells[8]. Vitamin D facilitates the absorption of dietary calcium in the small intestine[8]. With the addition of calcitonin, the ionized calcium is able to be stored within the bone[9]. During embryonic development, calcium phosphate is deposited in the osteoblasts and then crystallized in the collagen matrix[10]. When calcium is required for biological processes, such as eggshell formation, osteoclasts degrade bone to release calcium ions into the bloodstream to be transported to the oviduct[10].

Laying hens have cancellous, cortical, and medullary bones that are used in associated with bone turnover for eggshell formation[11]. Specifically, the medullary bone serves as a calcium reservoir ready to release calcium at the onset of eggshell formation[11]. Cortical bone formation is caused by a decrease in osteoclast activity in the inner endosteal surface and an increase in osteoblast activity in the periosteal layer[12,13]. Cortical bone formation continues until the onset of sexual maturity[12]. At the onset of sexual maturity, the increase in free estrogen causes structural bone to become medullary bone, and continued formation of the medullary bone at the expense of the structural bone results in osteoporosis[13].

The objective of this review is to describe critical roles of vitamin D and calcium for the prevention of osteoporosis in laying hens. An in-depth review of the role of vitamin D and calcium in the life cycle of laying hens will enable thorough understanding of the metabolisms of these nutrients in order to minimize the development of osteoporosis in laying hens.

EGGSHELL FORMATION

Once the F1 follicle is sufficiently mature, it is released from the ovary into the avian oviduct where the follicle becomes packaged with albumen and calcium that will form the eggshell[14]. Creation of the eggshell begins in the isthmus of the avian oviduct[14]. Calcium carbonate crystals are stacked vertically to form a crystalloid structure[15]. Pores naturally form between the vertical stacks of calcium carbonate to allow gas exchange of oxygen and carbon dioxide for respiration of the embryo[15]. Shells that are too rich in the calcium crystal structure cause the embryo to experience great difficulties when it is ready to hatch; however, a thin calcium crystal structure can also be detrimental for the embryo in that it is more vulnerable to parasites and diseases[15]. Therefore, finding the appropriate calcium carbonate crystal thickness is crucial for the survival of the embryo[15].

Studies have shown that eggshell formation and calcium concentrations in the diet of laying hens are interconnected[16,17]. Feeding laying hens low-calcium diets results in poor eggshell quality and poor bone homeostasis which increases the risk of osteoporosis. Likewise, Jiang *et al.*[16] fed a low-calcium diet (2.62%) to laying hens which resulted in poor bone quality and weaker eggshells when compared to hens fed a normal calcium diet (3.7%). This finding demonstrates how crucial calcium is to the well-being of the chicken throughout its lifespan. In addition, commercial chickens were traditionally fed ground limestone as their source of calcium[17]. However, the strength of the eggshells is greater in hens that are fed particulate forms of calcium as opposed to ground limestone[17].

In addition, vitamin D has been linked to viability of the chicken embryo[18-20]. Vitamin D3, specifically 1,25-dihydroxyvitamin D3, has shown to stimulate the formation of cartilage in chick embryos[18]. Providing laying hens with a vitamin D3 deficient diet produced eggs; yet, none of the eggs hatched[19]. Further analysis revealed decreased bone resorption and mineralization in the deceased embryos[19]. However, hatchability increased in the vitamin D3 deficient embryos when injected with calcitrol, 24,25-dihydroxyvitamin D3, or 25-hydroxyvitamin[19] D3. These results demonstrate the

vitality of vitamin D in the survival of chicks. Likewise, 1α, 25-Dihydroxyvitamin D3 works with transforming growth factor beta 3 to stimulate bone growth in chicken embryos[20]. Since the skeleton is a major organ that is necessary for survival in vertebral species such as chickens, association of vitamin D with the development of the skeleton in the preliminary stages of life demonstrates its cruciality in bone formation.

MATURATION

Preventing osteoporosis begins with building strong bones before puberty in order to diminish the occurrence of fractures as bone turnover becomes uneven, and nutrition plays a critical role in the prevention of osteoporosis in the future[1].

It has been reported that the medullary bone of laying hens fed with calcium and vitamin D3 deficient diets was almost completely resorbed[21]. Since the medullary bone gets resorbed for calcium to be used in biological pathways, calcium and vitamin D3 play a critical role in the development of the medullary bone[22].

Calcification of the osteoid increases in chicks fed a diet rich in vitamin D, whereas calcification of osteoid decreases in chicks with vitamin D deficient diets[23]. Dickson and Kodicek[23] predicted that calcification is related to the concentration of cholecalciferol. This prediction is supported by Wasserman[24] who provided evidence that calcium is absorbed in the small intestine using vitamin D-dependent pathways. Feeding particulate forms of calcium instead of ground limestone, is beneficial not only in the embryonic stages of life, but also as the chick matures[24]. Particulate form of calcium resulted in higher duodenal and gizzard soluble calcium toward the end of eggshell calcification in laying hens[25]. Although only a small percentage of calcium is absorbed by the duodenum, the small intestine plays a crucial role in absorbing 90% of calcium[24]. The amount of calcium absorbed by the intestine is critical to maintaining balanced bone turnover, and thus is important to overall bone health and prevention of osteoporosis[9].

Dacke *et al.*[22] suggest that free calcium ions contribute to the quick, local control of the osteoclast activity in the medullary bone. Osteoclast activity declines with an increase in free calcium ions, and osteoblast activity increases in order to store the calcium ions within the bone[22]. In addition, Mechanic *et al.*[26] have demonstrated that vitamin D induces bone collagen maturation which is important for bone strength and health. Therefore, vitamin D is not only important for regulating bone turnover, but also for providing bone stabilization and flexibility in the skeletal system.

EGG-LAYING PERIOD

Once the laying hen reaches sexual maturity and starts laying eggs, bone health declines due to the frequent bone turnover for egg shell formation[27]. Calcium-rich diets are more beneficial to eggshell quality during the egg-laying period than before the egg-laying period begins[27]. Calcium is most incorporated into the eggshell by the laying hen in the evening, correlating with the fact that the eggs were laid in the morning[28]. This is because from the time a follicle is released to laying the egg takes one day, and the time the egg is laid is heavily regulated by the circadian rhythm of the bird[29]. Therefore, it is advantageous for hens to optimally build up their calcium storage content to produce a strong eggshell and reduce the effects of fragile bones. A study performed by Comar and Diggers[30] concluded that between 60 and 75% of calcium that is deposited to form the egg shell comes from dietary/ingested sources, and the rest of the calcium comes from the body reserves[31].

Candlish[32] revealed that during the egg-laying cycle, the amount of cortical and medullary bone did not vary. However, further analysis revealed that the strength of the humeral bones of the laying hens directly correlated with the amount of medullary bone present during the laying period[33]. These seemingly opposite observations are due to the fact that there are few variabilities in the number of osteoclasts present within the daily egg laying cycle[32,33]. Nonetheless, the morphology of the osteoclasts significantly changes due to the calcium requirement for eggshell formation[11]. Similar to the results shown by Guinotte and Nys[17], feeding laying hens a particulate form of calcium can reduce the consequences of osteoporosis[13], as it can in embryonic and maturation stages of the hen's life cycle. Another study found that feeding hens with prominent levels of large particle calcium, independent of vitamin D3 concentration, ameliorated the quality of the eggshell and did not negatively affect the performance of first cycle of the laying period[34].

In respect to vitamin D, supplementing hens with cholecalciferol at concentrations of up to 102, 200 IU kg^{-1} of diet did not reduce the egg quality or the laying hen's performance[35]. However, increasing the dosage of vitamin D3 to 5000 IU kg^{-1} in the feed increased the albumen content of the egg[36]. Likewise, when concentration of vitamin D3 decreased in the feed, hens responded by reducing the thickness of the shells they produced[37]. When fed an extreme-vitamin D3 deficient diet, laying hens did not produce follicular activity[21].

CONCLUSION

Since calcium plays a critical role in bone turnover, finding the optimal source and concentration to put into the feed is important for decreasing the risk of osteoporosis in laying hens.

Furthermore, Vitamin D regulates bone turnover due to its activity in blood calcium concentration. Vitamin D increases blood calcium levels with the help of the parathyroid hormone. Therefore, vitamin D is crucial in regulating bone turnover and follicle development and must be monitored in order to prevent osteoporosis and maintain efficient egg production. Vitamin D also regulates the maturation of collagen due to its role as a transcription regulator in the bone. As a transcription regulator, vitamin D reduces the synthesis of collagen. Since collagen is a prominent protein in bones, maintaining proper collagen production is important for establishing overall bone health. Further research should be done to find the ideal concentration of particulate calcium and vitamin D that should be fed to laying hens that would result in optimal calcium absorption and collagen synthesis, minimal osteoclast activity for the maintenance of healthy bone turnover, and maximal follicular development.

SIGNIFICANCE STATEMENT

This review shows the effect of dietary calcium and vitamin D on bone development of laying hens and shell formation at various stages of their life.. This study will help the researchers to uncover the critical relationship of osteoporosis with dietary calcium particle size and level, and the important function of vitamin D on calcium homeostasis and bone metabolism throughout the unique life span of laying hens. Thus, optimizing the combination of optimal particle size and level of calcium with ideal form and concentration of vitamin D base on the different life stage of layer would improve skeletal integrity and eggshell quality and furthermore prevent layer osteoporosis.

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