Effects of Dietary Vitamin D3 Supplementation at Different Calcium Levels on Production Performance, Egg Quality, Tibia Quality, and Plasma Calcium and Phosphorus Metabolism in Late-Phase Laying Hens: Postprint

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Abstract

This experiment was conducted to investigate the effects of dietary vitamin D3 supplementation at different calcium levels on production performance, egg quality, tibia quality, and plasma calcium and phosphorus metabolism in laying hens during the late laying period. A 3×3 factorial design was employed, consisting of three calcium levels (3.0%, 3.5%, and 4.0%) and three vitamin D3 supplementation levels (0, 2 500, and 5 000 IU/kg). A total of 810 Lohmann pink-shell laying hens aged 60 weeks were selected and randomly allocated into 9 groups, with 6 replicates per group and 15 hens per replicate. A corn-soybean meal diet was used in the experiment, with a 1-week preliminary period and a 6-week formal experimental period. The results showed: 1) Compared with the group without vitamin D3 supplementation, the vitamin D3-supplemented groups exhibited extremely significantly increased average daily feed intake and laying rate ($P<0.01$), and extremely significantly decreased feed-to-egg ratio, broken egg rate, and soft-shell egg rate ($P<0.01$). With the continuous increase of dietary calcium level and vitamin D3 supplementation level, the average egg weight increased and the dirty egg rate decreased, but the differences were not significant ($P>0.05$). 2) Compared with the group without vitamin D3 supplementation, the vitamin D3-supplemented groups showed extremely significantly increased eggshell strength, eggshell weight, and eggshell percentage ($P<0.01$), significantly increased eggshell thickness ($P<0.05$), and significantly decreased yolk weight ($P<0.05$). With the increase of dietary calcium level, eggshell strength showed an increasing trend, but it did not reach a significant level ($P>0.05$). 3) Dietary vitamin D3 supplementation at different calcium levels had an extremely significant effect on tibia strength in laying

hens (P<0.01); compared with the group without vitamin D3 supplementation, the vitamin D3-supplemented groups exhibited extremely significantly increased tibia strength (P<0.01); with the increase of dietary calcium level, tibia calcium content showed an increasing trend, but the difference was not significant (P>0.05); when the dietary calcium level was 4.0%, tibia strength was significantly higher than when the dietary calcium levels were 3.0% and 3.5% (P<0.05). 4) Compared with the group without vitamin D3 supplementation, the vitamin D3-supplemented groups showed extremely significantly decreased plasma alkaline phosphatase activity (P<0.01) and extremely significantly increased plasma calcium and phosphorus contents (P<0.01); with the increase of dietary calcium level, plasma calcium and phosphorus contents did not change significantly (P>0.05); compared with the 3.0% calcium group, the 4.0% calcium group showed significantly decreased plasma alkaline phosphatase (P<0.05). The results showed that appropriately increasing dietary calcium level during the late laying period was beneficial to production performance of laying hens, but vitamin D3 deficiency would reduce production performance and even cause production cessation; dietary supplementation with 2 500 IU/kg vitamin D3 could both meet the vitamin D3 requirements of laying hens during the late laying period and avoid resource waste, thereby reducing costs.

Full Text

Title and Authors

Effects of Different Calcium Level Diets Supplemented with Vitamin D3 on Performance, Egg Quality, Tibia Quality and Plasma Calcium and Phosphorus Metabolism of Laying Hens in Late Laying Period

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Abstract

This experiment was conducted to investigate the effects of different calcium level diets supplemented with vitamin D3 on performance, egg quality, tibia quality, and plasma calcium and phosphorus metabolism in laying hens during the late laying period. A 3×3 factorial design was employed with three dietary calcium levels (3.0%, 3.5%, and 4.0%) and three vitamin D3 supplemental levels (0, 2,500, and 5,000 IU/kg), forming nine experimental groups. Eight hundred ten healthy 60-week-old Lohmann laying hens were randomly allocated to nine groups with six replicates per group and 15 hens per replicate. A corn-soybean meal basal diet was formulated according to NRC (1994) and Chinese Feeding Standard of Chickens (NY/T 33-2004). The pre-trial pe-

riod lasted one week, followed by a six-week formal experimental period. The results demonstrated that compared with the group without vitamin D3 supplementation, the vitamin D3-supplemented groups showed extremely significant increases in average daily feed intake and laying rate ($P<0.01$), and extremely significant decreases in feed-to-egg ratio, broken egg rate, and soft-shell egg rate ($P<0.01$). As dietary calcium and vitamin D3 levels increased, average egg weight tended to increase while dirty egg rate tended to decrease, though these differences were not significant ($P>0.05$). Vitamin D3 supplementation also produced extremely significant improvements in eggshell strength, eggshell weight, and eggshell proportion ($P<0.01$), significant increases in eggshell thickness ($P<0.05$), and significant reductions in yolk weight ($P<0.05$). With increasing dietary calcium level, eggshell strength showed an upward trend but did not reach significance ($P>0.05$). Different calcium level diets with vitamin D3 supplementation had extremely significant effects on tibia strength ($P<0.01$). The vitamin D3-supplemented groups showed extremely significant elevations in tibia strength compared with the non-supplemented group ($P<0.01$). As dietary calcium level increased, tibia calcium content tended to increase but not significantly ($P>0.05$). The 4.0% calcium group exhibited significantly higher tibia strength than the 3.0% and 3.5% calcium groups ($P<0.05$). Plasma alkaline phosphatase (ALP) activity was extremely significantly decreased in vitamin D3-supplemented groups compared with the non-supplemented group ($P<0.01$), while plasma calcium and phosphorus contents were extremely significantly increased ($P<0.01$). With increasing dietary calcium level, no significant changes were observed in plasma calcium and phosphorus contents ($P>0.05$), but the 4.0% calcium group showed significantly lower plasma ALP activity compared with the 3.0% calcium group ($P<0.05$). In conclusion, appropriately increasing dietary calcium level benefits the performance of laying hens in the late laying period, whereas vitamin D3 deficiency reduces performance and may even cause production cessation. Under the conditions of this experiment, supplementation with 2,500 IU/kg vitamin D3 can meet the vitamin D3 requirements of laying hens in the late laying period without causing resource waste, thus reducing costs.

Keywords: vitamin D3; calcium level; laying hens; performance; egg quality; tibia quality; plasma calcium and phosphorus

Introduction

Numerous non-nutritional and nutritional factors influence the performance of laying hens, among which calcium and vitamin D3 play crucial roles. Calcium serves as an essential raw material for eggshell formation, while vitamin D3 acts as a necessary cofactor to promote calcium absorption. Previous research on dietary calcium levels in laying hens has generally established the optimal range as 3%–4%; however, studies combining calcium and vitamin D3 are scarce. Therefore, this experiment investigated the effects of different dietary calcium and vitamin D3 levels on production performance, egg quality, tibia quality,

and plasma calcium-phosphorus metabolism in laying hens, providing an experimental basis for the rational use of calcium and vitamin D3 in production practice.

Materials and Methods

Experimental Design

A 3×3 factorial design was adopted, consisting of three dietary calcium levels (3.0%, 3.5%, and 4.0%) and three vitamin D3 supplemental levels (0, 2,500, and 5,000 IU/kg), forming nine treatment groups.

Experimental Animals and Basal Diet

Eight hundred ten healthy 60-week-old Lohmann laying hens were randomly divided into nine groups with six replicates per group and 15 hens per replicate. The basal diet was formulated based on NRC (1994) [1] and Chinese Feeding Standard of Chickens (NY/T 33-2004) [2], combined with practical production conditions. The composition and nutrient levels are presented in Table 1 , and the diet was provided in mash form.

Management Practices

The experiment was conducted at the experimental farm of the Institute of Animal Nutrition, Sichuan Agricultural University. All hens were housed in three-tier step cages with three hens per cage. Five consecutive cages constituted one replicate, and the six replicates per group were evenly distributed throughout the hen house, with hens in each replicate uniformly distributed across upper, middle, and lower tiers. The experiment lasted seven weeks, including a one-week pre-trial period and a six-week formal trial period. At the beginning of the pre-trial period, the egg production of each replicate was recorded and adjusted appropriately to ensure no significant differences in laying rate among groups during the pre-trial period ($P>0.05$). Hens were allowed free access to feed and water, with feeding twice daily at 09:00 and 14:00. Natural ventilation was employed with a 16-hour lighting regimen. Daily observations included hen mental state, abnormal behaviors, appetite, and fecal conditions. House sanitation was maintained regularly, and routine immunization procedures were followed.

Performance Measurements

Daily records were maintained for each replicate, including mortality, feed intake, egg number, broken eggs, dirty eggs, soft-shell eggs, and egg weight. Weekly calculations were performed for average daily feed intake (ADFI), average egg weight, feed-to-egg ratio, laying rate, dirty egg rate, broken egg rate, and soft-shell egg rate.

Egg Quality Assessment

During the sixth experimental week, three eggs per replicate (n=18) with weights close to the average were selected for quality analysis. Protein height, eggshell strength, yolk color, and Haugh unit were measured using a Robotmation EMT-5200 egg quality analyzer purchased by Sichuan Agricultural University. Eggshell thickness was measured with a micrometer, and eggshell weight and yolk weight were accurately weighed.

Tibia Quality Evaluation

The left tibia was collected intact and tibia strength was determined using the three-point bending method with a TAXT-Plus texture analyzer (Stable Micro Systems Corp, UK), expressed in kgf. The right tibia was dissected, defatted with ether for 8 hours, dried to constant weight at 105°C, ashed at 550°C, and analyzed for calcium and phosphorus content.

Plasma Calcium and Phosphorus Metabolism

At the end of the sixth experimental week, one healthy laying hen per replicate was randomly selected, weighed, and blood was collected from the wing vein. Plasma was separated by centrifugation at 3,000 r/min for 10 minutes after clotting and stored at -20°C for subsequent analysis. Plasma calcium and phosphorus contents and alkaline phosphatase (ALP) activity were determined at the Institute of Animal Nutrition, Sichuan Agricultural University.

Statistical Analysis

Experimental data were analyzed using the General Linear Model (GLM) procedure in SPSS 21.0 for two-way ANOVA. The model included main effects of dietary calcium level, vitamin D3 supplemental level, and their interaction. Duncan' s multiple range test was used for post-hoc comparisons. Results are expressed as means and standard errors. Statistical significance was declared at $P<0.05$ and extreme significance at $P<0.01$.

Results

Effects on Laying Hen Performance

As shown in Table 2 , different calcium level diets supplemented with vitamin D3 had no significant effects on average egg weight or dirty egg rate ($P>0.05$), but exerted extremely significant effects on average daily feed intake, feed-to-egg ratio, laying rate, broken egg rate, and soft-shell egg rate ($P<0.01$). With increasing dietary calcium and vitamin D3 levels, average egg weight tended to increase while dirty egg rate tended to decrease, though these differences were not significant ($P>0.05$). Compared with the non-supplemented group, vitamin D3 supplementation resulted in extremely significant increases in average daily

feed intake and laying rate (P<0.01) and extremely significant decreases in feed-to-egg ratio, broken egg rate, and soft-shell egg rate (P<0.01). No significant interaction effects were observed between dietary calcium level and vitamin D3 supplemental level on average daily feed intake, average egg weight, feed-to-egg ratio, laying rate, dirty egg rate, broken egg rate, or soft-shell egg rate (P>0.05).

Effects on Egg Quality

As presented in Table 3 , no significant interaction effects were detected between dietary calcium level and vitamin D3 supplemental level on eggshell strength, yolk color, eggshell weight, albumen height, yolk weight, eggshell thickness, Haugh unit, or eggshell proportion (P>0.05). Different calcium level diets supplemented with vitamin D3 had no significant effects on eggshell strength, yolk color, albumen height, eggshell thickness, or Haugh unit (P>0.05), but significantly affected eggshell weight, yolk weight, and eggshell proportion (P<0.05). Regarding vitamin D3 supplemental level, compared with the non-supplemented group, vitamin D3 supplementation produced extremely significant increases in eggshell strength, eggshell weight, and eggshell proportion (P<0.01), a significant increase in eggshell thickness (P<0.05), and a significant reduction in yolk weight (P<0.05). Regarding dietary calcium level, eggshell strength tended to increase with increasing calcium level but did not reach significance (P>0.05).

Effects on Tibia Quality

As shown in Table 4 , different calcium level diets supplemented with vitamin D3 had extremely significant effects on tibia strength (P<0.01) but no significant effects on tibia calcium or phosphorus content (P>0.05). Regarding vitamin D3 supplemental level, compared with the non-supplemented group, vitamin D3 supplementation resulted in extremely significant increases in tibia strength (P<0.01), and the 5,000 IU/kg vitamin D3 group showed significantly higher tibia calcium content than the non-supplemented group (P<0.05). Regarding dietary calcium level, the 4.0% calcium group exhibited significantly higher tibia strength than the 3.0% and 3.5% calcium groups (P<0.05). Tibia calcium content tended to increase with increasing dietary calcium level but did not reach significance (P>0.05). No significant interaction effects were observed between dietary calcium level and vitamin D3 supplemental level on tibia calcium content or tibia strength (P>0.05), though a significant interaction effect was detected on tibia phosphorus content (P<0.05).

Effects on Plasma Calcium and Phosphorus Metabolism

As presented in Table 5 , no significant interaction effects were found between dietary calcium level and vitamin D3 supplemental level on plasma ALP activity, calcium content, or phosphorus content (P>0.05). Regarding vitamin D3 supplemental level, compared with the non-supplemented group, vitamin D3 supplementation resulted in extremely significant reductions in plasma ALP activity (P<0.01), with the lowest activity observed in the 5,000 IU/kg vitamin

D3 group. Vitamin D3 supplementation also produced extremely significant increases in plasma calcium and phosphorus contents ($P<0.01$), with the highest values observed in the 5,000 IU/kg vitamin D3 group. Regarding dietary calcium level, the 4.0% calcium group showed significantly lower plasma ALP activity compared with the 3.0% calcium group ($P<0.05$). No significant changes in plasma calcium and phosphorus contents were observed with increasing dietary calcium level ($P>0.05$).

Discussion

Effects on Production Performance

Calcium regulation in laying hens is a complex and tightly controlled process influenced by feed intake and various hormones, all acting through the intestine, bone, and kidney [3]. Numerous studies have demonstrated that dietary calcium content directly affects laying hen performance, with calcium deficiency reducing calcium deposition in eggshells and increasing soft-shell and broken eggs [4]. The current results showed that different calcium level diets supplemented with vitamin D3 had extremely significant effects on average daily feed intake, feed-to-egg ratio, laying rate, broken egg rate, and soft-shell egg rate. As dietary calcium level increased, average egg weight tended to increase while dirty egg rate tended to decrease, though these differences were not significant. These findings align with previous reports. Ma et al. [5] reported that low-calcium diets reduced performance in Hy-Line Brown laying hens. The present study found that reducing dietary calcium from 3.5% to 3.0% did not significantly affect performance, which contradicts some previous results. This discrepancy may be attributed to the relatively small reduction in calcium, differences in calcium intake, varying feeding durations, and different laying stages, which may not have reached the threshold for affecting performance [6]. However, excessive dietary calcium can cause severe diarrhea and even gout in laying hens [7], thereby reducing performance. Previous studies have reported that many metabolic diseases, including visceral gout, polyuria, urolithiasis, and poor performance, are associated with excessive dietary calcium [3,8]. Conversely, appropriate vitamin D3 levels significantly impact performance by promoting calcium-binding protein formation in intestinal mucosal cells, thereby enhancing active calcium absorption and reducing soft-shell and broken egg proportions [9]. Yang et al. [10] suggested that the optimal dietary vitamin D3 level for laying hens should be controlled between 900–2,700 IU/kg. In this study, hens in the non-supplemented group exhibited lethargy and reduced feed intake during the fourth week of the formal trial, and showed signs of ceasing production by the sixth week. In contrast, vitamin D3 supplementation produced extremely significant improvements in average daily feed intake and laying rate, and extremely significant reductions in feed-to-egg ratio, broken egg rate, and soft-shell egg rate, consistent with previous research.

Effects on Egg Quality

Dietary calcium nutrition is directly related to calcium involvement in eggshell formation and eggshell quality. Gao [11] found that low-calcium diets reduced egg quality, depending on the degree of calcium reduction. The current results indicated that different calcium level diets supplemented with vitamin D3 had no significant effects on eggshell strength, yolk color, albumen height, eggshell thickness, or Haugh unit, but significantly affected eggshell weight, yolk weight, and eggshell proportion. When dietary calcium level decreased from 3.5% to 3.0%, no significant effects on egg quality were observed, which contradicts some previous findings. This inconsistency may be due to insufficient calcium reduction or a relatively short feeding period. As calcium level increased, eggshell strength tended to increase, and maximum values for eggshell weight, eggshell thickness, and eggshell proportion were achieved at the 4.0% calcium level. Gao [12] reported that 1,500 IU/kg vitamin D3 was appropriate for laying hens, with excessive or insufficient levels affecting calcium absorption and utilization. Navickis et al. [9] found that appropriate vitamin D3 increased eggshell calcium content and thickness. The present study demonstrated that vitamin D3 supplementation produced extremely significant increases in eggshell strength, eggshell weight, and eggshell proportion, significant increases in eggshell thickness, and significant reductions in yolk weight, consistent with previous research. Frost et al. [13] reported that high-dose vitamin D3 and 1,25-dihydroxyvitamin D3 had no significant effects on egg quality in 36-week-old Hy-Line Brown hens. The current study found no significant differences between the 2,500 IU/kg and 5,000 IU/kg vitamin D3 groups in egg quality, suggesting that the higher dose of 2,500 IU/kg may have already met the maximum requirement.

Effects on Tibia Quality

Numerous studies have reported that bone is the primary calcium storage site in animals, particularly important in laying hens. As the most abundant mineral element in laying hen production, long-term calcium deficiency or excess can cause various diseases [14]. Kong [15] found that long-term feeding of low-calcium diets reduced calcium utilization in laying hens, forcing them to mobilize tissue calcium to meet eggshell requirements, resulting in substantial bone calcium loss, lighter and more brittle bones, and subsequent fractures, reduced feed intake, and decreased performance. Roland et al. [16] confirmed that low-calcium diets adversely affected calcium deposition in laying hens. When dietary calcium level was 2.5%, tibia bone mineral density was significantly reduced [17]. High dietary calcium levels can cause calcium-phosphorus imbalance and reduce tibial performance [18]. The current results showed that different calcium level diets supplemented with vitamin D3 had extremely significant effects on tibia strength but no significant effects on tibia calcium and phosphorus content. The 4.0% calcium group exhibited significantly higher tibia strength than the 3.0% and 3.5% calcium groups. Tibia calcium content tended to increase with increasing dietary calcium level but did not reach significance, possibly

due to insufficient calcium variation or a relatively short experimental period. Additionally, Driver et al. [19] found that long-term supplementation of 2,000 IU/kg vitamin D3 in broiler breeders effectively reduced leg problems. The present study demonstrated that the 5,000 IU/kg vitamin D3 group showed significantly higher tibia calcium content than the non-supplemented group, and vitamin D3 supplementation produced extremely significant increases in tibia strength. However, the 5,000 IU/kg vitamin D3 group showed a tendency for reduced tibia strength compared with the 2,500 IU/kg group. Previous research reported that tibia strength increased with vitamin D3 level within a certain range [20], which aligns with the current findings. However, no significant differences in tibia strength or calcium content were observed between the 2,500 and 5,000 IU/kg vitamin D3 groups, possibly because excessive dietary vitamin D3 reduced absorption efficiency, stabilizing calcium metabolism and deposition. This is consistent with Yang et al. [10], who reported that appropriate vitamin D3 levels improved calcium absorption and deposition, thereby enhancing tibia quality.

Effects on Plasma Calcium and Phosphorus Metabolism

Ding et al. [21] reported that low-calcium diets increased serum ALP activity and reduced plasma calcium and phosphorus contents, affecting osteogenesis and bone metabolism, thereby impairing normal skeletal development. Guo et al. [22] noted that high-calcium diets caused gout and hypercalcemia in laying hens, with long-term elevation of serum calcium leading to calcium overload, increased calcium-dependent protease activation, and elevated uric acid content. The current study found that the 4.0% calcium group showed significantly lower plasma ALP activity than the 3.0% calcium group. Although plasma calcium and phosphorus contents did not change significantly with increasing dietary calcium level, their highest values were observed at the 4.0% calcium level. These results suggest that dietary calcium content can be appropriately increased during the late laying period to meet calcium requirements for egg production and compensate for body calcium loss. However, excessive calcium may cause calcium-phosphorus imbalance, leading to metabolic diseases and affecting performance, consistent with previous research. Regarding vitamin D3 supplementation, vitamin D3-supplemented groups showed extremely significant reductions in plasma ALP activity compared with the non-supplemented group, with the lowest activity observed at 5,000 IU/kg vitamin D3. Plasma calcium and phosphorus contents were extremely significantly increased in vitamin D3-supplemented groups, with the highest values also observed at 5,000 IU/kg vitamin D3. Guo et al. [23] found that dietary vitamin D3 levels from 500–5,000 IU/kg produced varying degrees of increase in serum calcium-binding protein concentration in broilers, with the 5,000 IU/kg group showing relatively high values. Zhang [24] reported that appropriately increasing vitamin D3 levels improved broiler performance when calcium requirements were met. The current study reached similar conclusions, demonstrating that appropriately increasing vitamin D3 levels can promote performance in laying hens during the

late production period.

Conclusion

1. Vitamin D3 deficiency adversely affects laying hen performance and may even cause production cessation in the late laying period.
2. Under the conditions of this experiment, dietary calcium level can be appropriately increased for laying hens in the late laying period. A dietary calcium level of 4.0% did not affect performance but significantly improved tibia strength.
3. In this experiment, no significant differences were observed between supplementation with 2,500 IU/kg and 5,000 IU/kg vitamin D3 on laying hen performance, egg quality, tibia quality, or plasma calcium-phosphorus metabolism. Dietary supplementation with 2,500 IU/kg vitamin D3 can meet the vitamin D3 requirements of laying hens in the late laying period without causing resource waste, thereby reducing production costs.

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Note: Figure translations are in progress. See original paper for figures.