Effects of Different Calcium Supplementation Patterns on Production Performance, Tibia Quality, and Serum Biochemical Indices in Laying Hens (Postprint)

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Abstract

This experiment aimed to investigate the effects of different calcium increment patterns on production performance, tibia quality, and serum-related biochemical indices in laying hens, in order to determine the dietary calcium increment pattern and supplementation level during the production process. A total of 480 Hy-Line Gray commercial laying hens aged 18 weeks were randomly divided into 4 groups, with 4 replicates per group and 30 hens per replicate. The time points for calcium increment were at 18 weeks of age and when the laying rate reached 5%, 50%, and 90%. The calcium supplementation levels at each time point were as follows: Group I: 2.00%, 2.20%, 2.40%, and 3.75%; Group II: 2.00%, 2.50%, 3.00%, and 3.75%; Group III: 2.00%, 3.00%, 3.75%, and 3.75%; Group IV: 2.00%, 3.75%, 3.75%, and 3.75%. The experimental period lasted 9 weeks. The results showed that: 1) The laying rate of Group III was significantly higher than that of Groups I and IV ($P<0.05$), while there was no significant difference in average daily egg weight among groups ($P>0.05$); 2) Tibial strength, tibial weight, and tibial calcium content in Group III were significantly higher than those in Groups I and II ($P<0.05$), with no significant difference from Group IV ($P>0.05$); 3) When the laying rate reached 50%, serum calcium content in Groups I and II was significantly higher than that in Groups III and IV ($P<0.05$); when the laying rate reached 90%, serum calcium content in Group IV was significantly higher than that in Groups I and II ($P<0.05$), with no significant difference between Groups III and IV ($P>0.05$). The results suggest that when the laying rate of hens reaches 5%, 50%, and 90%, dietary calcium levels of 3.00%, 3.75%, and 3.75%, respectively, are beneficial for improving laying rate, body condition, and stabilizing bone quality, without causing waste of calcium sources.

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Full Text

Different Calcium Supplementation Modes Affect Production Performance, Tibia Quality and Serum Biochemical Indices of Laying Hens

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Abstract

This study was conducted to investigate the effects of different calcium supplementation modes on production performance, tibia quality, and serum biochemical indices of laying hens, aiming to determine the optimal calcium supplementation strategy and level during egg production. Four hundred and eighty 18-week-old Hy-Line Gray commercial layers were randomly divided into 4 groups with 4 replicates each and 30 hens per replicate. Calcium supplementation was implemented at 18 weeks of age and when laying rates reached 5%, 50%, and 90%. The calcium levels for each group were: Group I: 2.00%, 2.20%, 2.40%, and 3.75%; Group II: 2.00%, 2.50%, 3.00%, and 3.75%; Group III: 2.00%, 3.00%, 3.75%, and 3.75%; Group IV: 2.00%, 3.75%, 3.75%, and 3.75%. The experiment lasted for 9 weeks. The results showed: 1) The laying rate of Group III was significantly higher than that of Groups I and IV ($P<0.05$), with no significant differences in average daily egg weight among all groups ($P>0.05$). 2) Tibia strength, tibia weight, and tibia calcium content in Group III were significantly higher than those in Groups I and II ($P<0.05$), but not significantly different from Group IV ($P>0.05$). 3) When the laying rate reached 50%, serum calcium content in Groups I and II was significantly higher than in Groups III and IV ($P<0.05$); when the laying rate reached 90%, serum calcium content in Group IV was significantly higher than in Groups I and II ($P<0.05$), with no significant difference between Groups III and IV ($P>0.05$). These findings indicate that dietary calcium levels of 3.00%, 3.75%, and 3.75% at laying rates of 5%, 50%, and 90%, respectively, can improve laying rate, enhance body condition, stabilize bone quality, and avoid calcium waste.

Keywords: calcium; laying hens; production performance; tibia quality; serum biochemical indices

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China' s current "Feeding Standard of Chickens" [1] divides the nutritional requirements of laying hens into two stages: from first lay to >85% laying rate and <85% laying rate, while NRC (1994) [2] provides requirements from 18 weeks to laying period. However, the nutrient requirements differ substantially from onset of lay to peak production. In practice, various calcium supplementation strategies are employed: some switch to high-calcium diets at 5% laying rate, others at 50%, and some at 90%. Dietary calcium directly affects production performance; insufficient calcium reduces eggshell deposition, increasing soft-shelled and broken eggs, and impairs bone development. Laying hens mobilize bone calcium, causing mineral decomposition and osteoporosis [3]. Conversely, excessive calcium causes severe diarrhea and even gout [4], resulting in significant losses. Therefore, investigating different calcium supplementation modes during lay is crucial for meeting production needs. This study examines how different calcium supplementation modes affect production performance, tibia quality, and serum biochemical indices to determine optimal strategies for pullets.

1. Materials and Methods

1.1 Experimental Period and Location The trial was conducted from July to October 2014 at the Specimen Garden Farm of the College of Animal Science and Technology, Agricultural University of Hebei. The experimental period was 10 weeks, including a 1-week pre-trial period and a 9-week formal trial period.

1.2 Experimental Animals and Treatments Four hundred and eighty 18-week-old Hy-Line Gray layers were randomly allocated into 4 groups (I, II, III, IV) with 4 replicates each and 30 hens per replicate. Different calcium levels were added to the basal diet (corn-soybean meal type) for each group as shown in . All other nutrients were formulated according to NRC (1994) [2] poultry standards, with consistent levels of dietary energy, crude protein, crude fat, crude fiber, and available phosphorus. Diet composition and nutrient levels are presented in .

Hens were housed in three-tier full-step cages with free access to feed and water. Daily observations included mental state, appetite, and fecal condition, with routine immunization procedures followed. The chicken house used artificial lighting and natural ventilation to maintain a good environment.

1.3 Measurement Indicators 1.3.1 Production Performance

At 16:00 daily, egg number and weight were recorded per replicate. Weekly feed intake and mortality were recorded to calculate laying rate, average daily egg mass, average daily feed intake, feed-to-egg ratio, and deformed egg rate.

1.3.2 Body Measurements

When the laying rate reached 90%, three hens were randomly selected from each replicate to measure body slanting length, keel length, pubic space, hip width, tibia length, and tibia girth using vernier calipers and flexible rulers.

1.3.3 Bone Quality

At the end of the trial, three hens per replicate were slaughtered according to anatomical requirements. Both legs were separated and completely defleshed. The right tibia was used to determine tibia strength (breaking force) using a CMT5504 digital universal testing machine with parameters set at 40 mm span and 10 mm/min displacement speed, recording the force at fracture (expressed in N). The left tibia was used to determine tibia weight, tibia index, and calcium/phosphorus content. After ether defatting for 24 h and drying at 105°C to constant weight, tibia weight was measured and tibia index was calculated relative to body weight. The tibia was then ashed in a muffle furnace at 600°C for 18 h to determine crude ash content, followed by hydrochloric acid dissolution for calcium and phosphorus measurement. Tibia index (%) = (tibia weight (g) / body weight (g)) × 100.

1.3.4 Serum Biochemical Indices

When laying rates reached 50% and 90%, three hens per replicate were randomly selected for 10 mL wing vein blood collection. After centrifugation at 3,000 r/min for 10 min, serum was collected and stored at -20°C. Serum calcium was measured by methyl thymol blue colorimetry, phosphorus by phosphomolybdic acid method, and parathyroid hormone (PTH), calcitonin (CT), and osteocalcin (BGP) by enzyme-linked immunosorbent assay (ELISA).

1.4 Data Processing Data were analyzed using SPSS 17.0 software for one-way ANOVA. All data are expressed as mean ± standard deviation. $P<0.05$ was considered statistically significant, and Duncan' s multiple comparison test was used when significant differences were detected.

2. Results

2.1 Effects of Different Calcium Supplementation Modes on Production Performance As shown in , the laying rate of Group III was 6.34% higher than Group I ($P<0.05$), 1.75% higher than Group II ($P>0.05$), and 3.51% higher than Group IV ($P<0.05$). No significant differences were observed in average daily egg weight among groups ($P>0.05$). Average daily feed intake of Group I was significantly higher than other groups ($P<0.05$), with no significant differences among Groups II, III, and IV ($P>0.05$). The soft-shelled and deformed egg rate of Group I was significantly higher than other groups ($P<0.05$), with no significant differences among Groups II, III, and IV ($P>0.05$).

2.2 Effects of Different Calcium Supplementation Modes on Body Size Indices As shown in , Group III had the largest pubic space, which was 21.88% larger than Group I ($P<0.05$), 1.30% larger than Group II ($P>0.05$), and 1.56% larger than Group IV ($P>0.05$). Hip width of Groups III and IV was significantly higher than Groups I and II ($P<0.05$). No significant effects were observed on body slanting length, keel length, tibia length, or tibia girth ($P>0.05$).

2.3 Effects of Different Calcium Supplementation Modes on Tibia Quality As shown in , different calcium supplementation modes significantly affected tibia strength, tibia weight, and tibia calcium content ($P<0.05$). Tibia strength of Groups III and IV was significantly higher than Groups I and II ($P<0.05$). Tibia weight of Group III was significantly higher than Groups I and

II (P<0.05). Tibia calcium content of Group III was 7.31% higher than Group I (P<0.05), 3.04% higher than Group II (P<0.05), and 1.98% higher than Group IV (P>0.05). No significant differences were observed in tibia index or tibia phosphorus content among groups (P>0.05).

2.4 Effects of Different Calcium Supplementation Modes on Serum Biochemical Indices As shown in , at 50% laying rate, serum calcium content decreased sequentially from Groups I to IV, with Groups I and II significantly higher than Groups III and IV (P<0.05). At 90% laying rate, serum calcium content increased sequentially from Groups I to IV, with Group IV significantly higher than Groups I and II (P<0.05), but not significantly different from Group III (P>0.05). At 50% laying rate, serum phosphorus content decreased sequentially from Groups I to IV, with Group I significantly higher than Groups III and IV (P<0.05). At 90% laying rate, no significant differences were observed in serum phosphorus among groups (P>0.05). Throughout the laying period, serum PTH content in Groups III and IV was significantly lower than Group I (P<0.05), while serum CT content was significantly higher than Groups I and II (P<0.05). No significant differences in serum BGP were observed at 50% laying rate (P>0.05), but at 90% laying rate, Groups III and IV were significantly lower than Group I (P<0.05).

3. Discussion

3.1 Effects on Production Performance Calcium regulation in laying hens is a complex and rigorous process constrained by average daily feed intake and hormone secretion, with calcium-regulating hormones acting through the intestine, bone, and kidney [5]. For chicks and pullets, most calcium is used for bone formation, while in laying hens, most dietary calcium is used for eggshell formation and excreted with eggs [6]. Compared with other livestock, laying hens maintain high calcium metabolism, requiring large daily calcium absorption to meet eggshell calcification needs [7-8]. This study found that different calcium supplementation modes significantly affected laying rate. When dietary calcium levels were 3.00%, 3.75%, and 3.75% at laying rates of 5%, 50%, and 90%, respectively, laying rate increased by 6.34%, 1.75%, and 3.51% compared with other modes, indicating that appropriate calcium levels and supplementation modes significantly improve laying rate, consistent with Zhang et al. [9] on New Yangzhou chickens. However, average daily feed intake decreased with increasing dietary calcium, likely related to calcium' s regulatory effect on feed intake. Although energy is the primary factor controlling feed intake, calcium also significantly affects feed intake, enabling hens to regulate calcium consumption [10]. Mongin et al. [11] similarly found that hens significantly increased feed intake on low-calcium diets to obtain adequate calcium. Dietary calcium level is also crucial for eggshell quality; Gao [12] found that calcium content below 3.5% in peak-laying hen diets significantly increased thin-shelled egg rate, consistent with our finding that high calcium supplementation significantly reduced deformed egg rate.

3.2 Effects on Body Size Indices Body measurements are important indicators of animal growth and development, influenced by breed, age, sex, nutrition, and environment [13]. This study showed that pubic space initially increased then decreased with increasing dietary calcium, with the largest space observed when calcium levels were 3.00%, 3.75%, and 3.75% at 5%, 50%, and 90% laying rates, respectively, significantly higher than other groups. However, hip width significantly decreased with increasing calcium levels. Dietary calcium had no significant effects on body slanting length, keel length, tibia length, or tibia girth, consistent with Zhu et al. [14]. Research suggests that dietary nutrition primarily affects hen body size before 7 weeks, with minimal impact on body measurements and secondary sexual characteristics during lay [15], and different local chicken breeds show significant differences in body size traits [16].

3.3 Effects on Tibia Quality Calcium is the most abundant mineral element in laying hens, with both deficiency and excess causing diseases, the most basic being skeletal disorders [16]. Calcium deficiency in young animals reduces bone hardness and causes leg bone curvature, while in adults it causes osteomalacia. Currently, caged layer bone metabolic disorders mainly result from insufficient dietary calcium. This study demonstrated that calcium supplementation levels and modes significantly affected tibia strength, weight, and calcium content, with tibia strength increasing with calcium level, consistent with Frost et al. [17]. Tibia calcium and phosphorus content showed an initial increase then decrease with increasing dietary calcium [18], with calcium levels of 3.00%, 3.75%, and 3.75% at 5%, 50%, and 90% laying rates being beneficial for bone growth and mineral deposition.

3.4 Effects on Serum Biochemical Indices Different calcium supplementation modes significantly affected bone metabolism hormones. PTH, produced by the parathyroid gland, inhibits osteoclast-to-osteoblast transformation and activates bone lining cells to form pre-osteoclasts and new osteoclasts [19]. CT inhibits bone resorption, reduces calcium release from bone, and decreases calcium transfer from bone fluid by lowering osteocyte calcium concentration, while also inhibiting osteoclast activity and promoting conversion to osteoblasts. As two important hormones regulating calcium metabolism, PTH and CT interact to maintain blood calcium stability. This study found that serum PTH decreased significantly while CT increased with higher dietary calcium, indicating that higher calcium supplementation promotes osteoclast-to-osteoblast conversion, reduces bone calcium mobilization, and effectively protects bone tissue. Comparison of serum calcium and phosphorus differences suggests that higher calcium supplementation helps reduce blood calcium and phosphorus, possibly related to PTH and CT regulation.

BGP is a non-collagen protein produced by osteoblasts that is important for evaluating bone formation and turnover [20]. In this study, serum BGP decreased at 90% laying rate with 3.75% dietary calcium, indicating that higher calcium levels can reduce bone metabolic activity and decrease bone calcium metabolic disease incidence [9].

4. Conclusion

Under the conditions of this experiment, different calcium supplementation modes significantly affected production performance and endocrine function of laying hens. Dietary calcium levels of 3.00%, 3.75%, and 3.75% at laying rates of 5%, 50%, and 90%, respectively, improved laying rate, enhanced body condition, stabilized bone quality, and avoided calcium waste.

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