Effects of Alternate Feeding of High- and Low-Calcium Diets on Production Performance, Egg Quality, and Serum Indices in Laying Hens During the Late Laying Period: Postprint

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Abstract

This experiment aimed to investigate the effects of alternating high- and low-calcium diets on production performance, egg quality, and serum indices of laying hens during the late laying period. Sixty-four Dawu Fen-1 breeding hens at 68 weeks of age were selected and randomly divided into 2 groups with 32 hens per group, individually housed in single cages of an intelligent individual cage system. The control group was fed 43.3 g and 86.7 g of medium-calcium diet (calcium content 3.66%) at 08:00 and 14:00, respectively, while the treatment group was fed 43.3 g of low-calcium diet (calcium content 2.00%) at 08:00 and 86.7 g of high-calcium diet (calcium content 4.49%) at 14:00. The experimental period lasted for 5 weeks, including a 1-week pre-trial period and a 4-week formal trial period. The results showed that there were no significant differences in average daily feed intake, average egg weight, laying rate, or feed-to-egg ratio between the treatment group and the control group across all weeks ($P>0.05$); however, the laying rate of the treatment group was numerically higher than that of the control group in each week. The eggshell thickness of the treatment group was significantly higher than that of the control group in week 2 ($P<0.05$), while there were no significant differences in albumen height or Haugh unit between the two groups across all weeks ($P>0.05$). In the control group, serum calcium content at 18:00 and 22:00 was significantly higher than that at 10:00 and 14:00 ($P<0.05$), and higher than that at 06:00 and 02:00 on the following day ($P>0.05$). In the treatment group, serum calcium content at 22:00 was extremely significantly higher than that at 06:00, 10:00, 18:00, and 02:00 on the following day ($P<0.01$), and significantly higher than that at 14:00 and 18:00 ($P<0.05$). From 06:00 to 22:00, serum calcium content in the treatment group showed a continuous increasing trend. At 22:00, both serum calcium and

calcitonin contents in the treatment group were significantly higher than those in the control group (P<0.05). It can be concluded that alternating feeding of high- and low-calcium diets (feeding high-calcium diet in the afternoon) can improve the laying rate and calcium absorption and deposition in laying hens during the late laying period, and can improve eggshell quality to a certain extent.

Full Text

Effects of Alternating High and Low Calcium Diets on Performance, Egg Quality, and Serum Indices of Laying Hens During Late Production Period

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Abstract: This experiment investigated the effects of alternating high and low calcium diets on production performance, egg quality, and serum indices of laying hens during the late production period. Sixty-four 68-week-old Dawufen No. 1 breeder hens were randomly divided into two groups of 32 birds each and housed individually in intelligent cages. The control group received 43.3 g and 86.7 g of a medium-calcium diet (3.66% Ca) at 08:00 and 14:00, respectively. The experimental group received 43.3 g of a low-calcium diet (2.00% Ca) at 08:00 and 86.7 g of a high-calcium diet (4.49% Ca) at 14:00. The trial lasted five weeks, including a one-week pre-trial period and a four-week formal experimental period. Results showed no significant differences between groups in average daily feed intake, average egg weight, laying rate, or feed-to-egg ratio ($P > 0.05$), though laying rates were numerically higher in the experimental group each week. Eggshell thickness in the experimental group was significantly greater than the control group in week 2 ($P < 0.05$), while albumen height and Haugh unit did not differ significantly between groups ($P > 0.05$). In the control group, serum calcium levels at 18:00 and 22:00 were significantly higher than at 10:00 and 14:00 ($P < 0.05$), and higher than at 06:00 and 02:00 the following day ($P > 0.05$). In the experimental group, serum calcium at 22:00 was extremely significantly higher than at 06:00, 10:00, 18:00, and 02:00 the following day ($P < 0.01$), and significantly higher than at 14:00 and 18:00 ($P < 0.05$). Serum calcium in the experimental group showed a continuous increasing trend from 06:00 to 22:00. At 22:00, both serum calcium and calcitonin levels in the experimental group were significantly higher than in the control group ($P < 0.05$). These findings indicate that alternating high and low calcium diets (feeding high-calcium diet in the afternoon) can improve laying rate, enhance calcium absorption and deposition, and improve eggshell quality in aging laying

hens.

Keywords: calcium content; laying hens; performance; egg quality; serum calcium

Introduction

The most pressing challenge facing China' s egg industry is how to extend the laying cycle of caged hens. Several companies worldwide have initiated breeding programs targeting 100-week-old layers to improve economic efficiency, and Chinese researchers have pursued similar objectives. ISA' s "100-500 Program," proposed in 2011, advocates extending the traditional culling age from 72 weeks to 80 or even 100 weeks, thereby maximizing facility utilization and reducing rearing costs. Consequently, research on "improving laying rates during 80-100 weeks" and "enhancing hatchability and egg quality in late production" has intensified. Various methods have been employed to improve performance and shell quality in aging hens, with dietary manipulation being the most practical and economical approach. Proper calcium supplementation strategies can prevent losses from deficiency or excess while optimizing production and shell quality. The optimal timing for calcium supplementation is from afternoon until lights-out, as calcium consumed in the morning primarily deposits in bone, whereas calcium absorbed in the afternoon or evening directly contributes to shell formation. Since 14:00-16:00 represents the peak feeding period, supplementation during this window capitalizes on heightened appetite and maximizes calcium utilization. Previous research by Wang Yulin et al. found that 3.5% dietary calcium optimized performance and egg quality in late-stage Hy-Line Brown hens. Therefore, this study designed a control diet with 3.66% calcium, while the experimental group received equivalent total daily calcium but split between low-calcium (morning) and high-calcium (afternoon) diets. This approach tested whether afternoon high-calcium feeding could improve shell quality and calcium utilization, providing a nutritional strategy to enhance shell quality, reduce breakage and abnormal eggs, and improve performance in aging hens.

1. Materials and Methods

1.1 Experimental Design Sixty-four 68-week-old Dawufen breeder hens (purchased from Hebei Dawu Group) were randomly allocated to two groups of 32 birds each and housed individually in intelligent cages with 16-hour lighting (06:00-22:00). Birds received timed, quantitative feeding: the control group (CG) consumed 43.3 g and 86.7 g of medium-calcium diet (3.66% Ca) at 08:00 and 14:00, respectively. The experimental group (EG) consumed 43.3 g of low-calcium diet (2.00% Ca) at 08:00 and 86.7 g of high-calcium diet (4.49% Ca) at 14:00. Residual feed was removed and weighed at each feeding to record

consumption. Total daily feed intake and calcium intake were equivalent between groups. The trial lasted five weeks (one-week pre-trial, four-week formal period).

1.2 Experimental Diets Diets were purchased from Hebei Dawu Group and formulated according to Chinese agricultural industry standards (NY/T 33-2004). Diet composition and nutrient levels are presented in .

1.3 Management The trial was conducted in individual intelligent cages developed by Hebei Agricultural University (two-tier stacked system). The system featured automated feeding, residual feed weighing, data recording, free access to water and feed, automatic egg collection, and manure removal. Birds were fed at 08:00 (morning) and 14:00 (afternoon) daily: CG received medium-calcium diet at both times, while EG received low-calcium diet in the morning and high-calcium diet in the afternoon. Standard disinfection and vaccination protocols were followed.

1.4 Sample Collection and Measurements 1.4.1 Sample Collection
Individual records were maintained daily for morning/afternoon feed allocation and residual amounts, egg weight, and egg number. Weekly calculations determined average daily feed intake, laying rate, and feed-to-egg ratio. Eight eggs per group were randomly selected weekly for quality analysis. Blood samples were collected from eight randomly selected hens per group at 08:00 weekly and at 06:00, 10:00, 14:00, 18:00, 22:00, and 02:00 the following day on the final experimental day. Samples were centrifuged at 3,000 rpm for 15 minutes, and serum was stored at -20°C for analysis.

1.4.2 Performance Metrics
Average daily feed intake (g) = feed offered - residual feed
Laying rate (%) = 100 × total eggs / total hen-days
Feed-to-egg ratio = total feed intake / total egg weight

1.4.3 Egg Quality Metrics
Eggshell thickness: Measured using an eggshell thickness gauge (ESTG-01, Israel) at three points (blunt end, sharp end, equator) after applying petroleum jelly; values were averaged.
Eggshell strength: Determined using an eggshell force gauge (EFR-01, Israel) with compression speed of 10 mm/min; the force at first crack was recorded.
Relative eggshell weight: Calculated as (eggshell weight / egg weight) × 100 after weighing on an electronic balance.
Egg shape index: Measured using an egg shape index gauge (NFN385, Israel).
Egg weight, albumen height, yolk color, Haugh unit: Egg weight was measured electronically, then an egg quality analyzer (EA-01, Israel) determined albumen height, yolk color, and Haugh unit.

1.4.4 Serum Index Determination
Serum calcium was measured by methylthymol blue (MTB) colorimetry, and

serum phosphorus by molybdic acid method using kits from Nanjing Jiancheng Bioengineering Institute. Serum calcitonin (CT) was determined by ELISA using kits from Shanghai Yili Biotechnology.

1.4.5 Calcium Metabolism Rate
Daily calcium intake was calculated weekly to determine calcium metabolism rate:
Calcium metabolism rate (%) = 100 × (daily calcium intake - fecal calcium) / daily calcium intake [5].

1.5 Statistical Analysis Data were initially processed using Excel 2007. SPSS 17.0 was used for t-tests between control and experimental groups within the same week, and one-way ANOVA for the same index across different weeks. Results are expressed as mean ± standard deviation. $P < 0.05$ indicated significant difference, $P < 0.01$ indicated extremely significant difference, and $P > 0.05$ indicated no significant difference.

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2. Results

2.1 Effects on Production Performance As shown in , no significant differences were observed between groups in average daily feed intake, average egg weight, laying rate, or feed-to-egg ratio across all weeks ($P > 0.05$). However, laying rates were numerically higher in the experimental group each week. Neither group showed significant temporal changes in these performance metrics ($P > 0.05$).

2.2 Effects on Egg Quality In week 1, eggshell thickness was significantly lower in the experimental group than the control group ($P < 0.05$). By week 2, this pattern reversed, with experimental group shells significantly thicker ($P < 0.05$). No significant differences were observed in weeks 3 or 4 ($P > 0.05$). Eggshell strength and relative eggshell weight did not differ significantly between groups in any week ($P > 0.05$), and neither metric changed significantly over time in either group ($P > 0.05$).

Egg shape index, albumen height, yolk color, Haugh unit, and egg weight showed no significant differences between groups in any week ($P > 0.05$) (). Neither group exhibited significant temporal changes in these indices ($P > 0.05$), though egg shape index tended to increase during weeks 1-3 in both groups.

2.3 Effects on Serum Indices In the control group, serum calcium at 18:00 and 22:00 was significantly higher than at 10:00 and 14:00 ($P < 0.05$), and higher than at 06:00 and 02:00 the following day ($P > 0.05$). In the experimental group, serum calcium at 22:00 was extremely significantly higher than at 06:00, 10:00, 18:00, and 02:00 the following day ($P < 0.01$), and significantly higher than at 14:00 and 18:00 ($P < 0.05$). Serum calcium in the experimental group

increased continuously from 06:00 to 22:00. Serum calcitonin in the control group was significantly higher at 06:00, 10:00, and 14:00 than at 18:00 and 02:00 the following day ($P < 0.05$). In the experimental group, calcitonin at 06:00 was significantly higher than at 18:00 and 02:00 the following day ($P < 0.05$), while levels at 22:00 were also elevated but not significantly ($P > 0.05$). At 22:00, both serum calcium and calcitonin were significantly higher in the experimental group than the control group ($P < 0.05$). Serum phosphorus showed no significant differences between groups or across time points ($P > 0.05$) ().

Weekly serum calcium levels were numerically higher in the experimental group ($P > 0.05$), while serum phosphorus was numerically lower ($P > 0.05$). Serum calcitonin was also numerically lower in the experimental group across all weeks ($P > 0.05$) ().

2.4 Effects on Calcium Metabolism Rate No significant differences were observed between groups in daily calcium intake, fecal calcium, or calcium metabolism rate across weeks ($P > 0.05$) (). However, calcium metabolism rates were numerically higher in the experimental group each week.

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3. Discussion

3.1 Effects on Production Performance Dietary calcium is crucial for normal physiological function, bone development, and optimal production performance in laying hens. This study found that feeding high-calcium diet in the afternoon increased average daily feed intake and decreased feed-to-egg ratio in the experimental group compared to controls, consistent with Sauveur et al. [6] who reported that dietary calcium level significantly affects feed intake, allowing hens to regulate consumption to maintain calcium intake. Although laying rates did not differ significantly between groups, the experimental group maintained more stable and higher average laying rates across the four-week period. Zhang Shiyuan et al. [7] demonstrated that appropriate calcium supplementation methods can effectively improve laying rates, while maintaining high dietary calcium levels supports performance [8,9]. The improved laying rate in the experimental group indicates that alternating high and low calcium diets enhances production performance in aging hens.

3.2 Effects on Egg Quality Eggshell quality is a critical indicator of egg quality [10]. Calcium is the primary component of eggshells, and its absorption and utilization directly determine shell formation and quality [11]. Shell strength, shell weight, and shell proportion are important indicators for evaluating dietary calcium and phosphorus adequacy [12]. Xue Jian et al. [13] reported that 60-75% of eggshell calcium originates from dietary sources, with the remainder from bone mobilization. Therefore, dietary calcium directly affects shell and bone quality. This study revealed that while control group shells were thicker

in week 1, experimental group shells became significantly thicker by week 2. Relative eggshell weight was higher in the experimental group during weeks 2-4, indicating that afternoon high-calcium feeding enhances calcium deposition and improves shell quality. Since shell formation occurs primarily at night, calcium supplemented during this period is absorbed directly into the shell gland, aligning with active intestinal absorption and improving calcium utilization. The optimal calcium supplementation period is 16:00-18:00, when two-thirds of the daily requirement can be fed to significantly improve shell quality.

3.3 Effects on Serum Indices Hormones play a vital role in maintaining blood calcium and phosphorus homeostasis. Serum calcium and phosphorus are regulated by parathyroid hormone, calcitonin, and vitamin D, which interact to modulate bone metabolism and shell formation [14]. Calcitonin is a key hormone that inhibits osteoclast activity, preventing bone calcium release into blood and thereby reducing blood calcium levels [15]. This study demonstrated that afternoon high-calcium feeding increased serum calcium levels, with a clear increasing trend from 14:00 to 22:00. Since shell formation occurs primarily at night, calcium absorption increases during this period. Wang Hongzhe [16] found that granular limestone delays calcium digestion, prolonging intestinal retention and improving absorption, which aligns with our findings. Serum calcium was significantly higher in the experimental group at 14:00 and 22:00 on the final day, coinciding with calcium deposition for shell formation. Elevated serum calcitonin at these times likely reflects increased bone calcium mobilization to maintain calcium balance and prevent osteoporosis when large amounts of calcium are consumed. Therefore, nocturnal calcium deposition is critical for aging hens. This study confirms that afternoon high-calcium feeding increases serum calcium levels, which is essential for egg production [17].

3.4 Effects on Calcium Absorption and Metabolism Eggshell calcium formation is closely related to calcium metabolism and deposition. Hens obtain calcium primarily through diet, which is absorbed in the small intestine, partially deposited in bone, and partially utilized for shell formation in the shell gland. Under normal conditions, calcium metabolism maintains dynamic equilibrium between absorption and deposition. Shell formation requires 60-75% of calcium from dietary sources, with the remainder from bone mobilization [13]. Chronically low dietary calcium forces bone calcium mobilization, causing osteoporosis and compromising laying rate and shell quality. Li Zhixue et al. [18] found that calcium metabolism becomes severely disrupted in aging hens, representing a key factor affecting performance. Although both groups consumed equivalent daily calcium, the experimental group showed numerically higher calcium metabolism rates, indicating improved calcium absorption and deposition and consequently better shell quality.

4. Conclusion

Alternating high and low calcium diets (feeding high-calcium diet in the afternoon) improved laying rate and nocturnal serum calcium levels, increased calcium absorption and deposition, and enhanced eggshell quality in aging laying hens.

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