

## 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 68-week-old Dawu Fen No. 1 breeder hens were selected and randomly allocated into 2 groups with 32 hens per group, housed individually in individual intelligent cages. The control group was fed medium-calcium diets (calcium content 3.66%) at 08:00 and 14:00 with amounts of 43.3 g and 86.7 g, respectively. 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 preliminary period and a 4-week formal experimental 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 in week 2 of the treatment group was significantly higher than that of the control group ( $P < 0.05$ ), 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 the next 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 the next 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 Alternation Feeding of Low and High Calcium Diets on Performance, Egg Quality and Serum Indexes of Laying Hens at Late Period of Laying

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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 in laying hens during the late laying 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 5-week trial consisted of a 1-week pre-test period followed by a 4-week formal test 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 concentrations were significantly higher in the experimental group 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 to some extent in late-period laying hens.

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

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In recent years, the most critical challenge facing the layer industry has been how to extend the laying cycle of caged hens [1]. Several companies worldwide have initiated breeding programs targeting 100-week-old layers to improve economic efficiency, and Chinese poultry breeding researchers have also conducted related studies. ISA Corporation proposed the “100-500 Program” in 2011, which extended the traditional culling age from 72 weeks to 80 or even 100 weeks, thereby improving facility utilization and reducing rearing costs [2]. Consequently, research on “improving laying rates during 80-100 weeks” and “enhancing hatchability and egg quality in late laying period” has gained prominence. With scientific and technological advances, numerous methods have been employed to improve production performance and eggshell quality in aging layers. Among these, modifying feeding strategies is the most practical and economical approach. Proper calcium supplementation can prevent losses from deficiency or excess, thereby enhancing performance and eggshell quality. The optimal timing for calcium supplementation is from afternoon to before lights-out, as calcium consumed in the morning primarily deposits in bones, while calcium absorbed in the afternoon or evening can be directly utilized for eggshell formation. Moreover, 14:00-16:00 represents the peak feeding period, allowing maximum exploitation of hens’ strong appetite and high feed intake for optimal calcium supplementation [3]. Wang Yulin et al. [4] found that a dietary calcium level of 3.5% optimized performance and egg quality in late-period Hy-Line Brown layers. Therefore, this experiment designed a control diet with 3.66% calcium, while the experimental group received the same daily calcium intake but split between low-calcium diet (morning) and high-calcium diet (afternoon). This study aimed to determine whether feeding high-calcium diet in the afternoon could improve eggshell quality and calcium utilization in late-period layers, providing scientific evidence for optimal feeding strategies to enhance eggshell quality, reduce breakage and abnormal eggs, and improve performance in aging hens from a nutritional perspective.

### 1.1 Experimental Design

Sixty-four 68-week-old Dawufen breeder hens (purchased from Hebei Dawu Group) were randomly allocated into two groups of 32 birds each and housed individually in intelligent cages under 16-hour lighting (06:00-22:00). Feed was provided at fixed times and quantities daily. 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 recorded at each feeding to monitor consumption. Both groups received identical daily feed intake and calcium intake. The trial lasted 5 weeks, including a 1-week pre-test period and a 4-week formal test

period.

## 1.2 Experimental Diets

Experimental 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 Table 1 .

## 1.3 Management

The trial was conducted in individual intelligent cages developed by Hebei Agricultural University, featuring two-tier stacking with single-bird housing, automatic feeding, residual feed weighing before delivery, intelligent data recording and output, ad libitum water and feed intake, automatic egg collection, and automatic manure removal. The 5-week trial included a 1-week pre-test period and a 4-week formal test period. Feed was provided at fixed times (morning: 08:00; afternoon: 14:00). The control group received medium-calcium diet at both feedings, while the experimental group received low-calcium diet in the morning and high-calcium diet in the afternoon. The house was regularly disinfected, and routine immunization protocols were followed.

## 1.4 Sample Collection and Measurements

**1.4.1 Sample Collection** Daily records were maintained for each hen, including morning feed provision and residual amounts, afternoon feed provision and residual amounts, egg weight, and egg number. Weekly calculations were performed for average daily feed intake, laying rate, and feed-to-egg ratio. Each week, eight eggs per group were randomly selected for egg quality measurement. Blood samples were collected weekly from eight randomly selected hens per group at 08:00, with additional collections at 06:00, 10:00, 14:00, 18:00, 22:00, and 02:00 the following day on the final day of the trial. Blood samples were centrifuged at 3,000 r/min for 15 minutes to separate serum, which was stored at -20°C for analysis.

**1.4.2 Performance Indices** Average daily feed intake (g) = feed provision - residual feed

Laying rate (%) =  $100 \times \text{total eggs} / \text{total hen-days}$

Feed-to-egg ratio = total feed intake / total egg weight

**1.4.3 Egg Quality Indices** **Eggshell thickness:** Measured using an eggshell thickness gauge (ESTG-01, Israel). Eggs were coated with petroleum jelly and placed on the test probe for direct reading. Measurements were taken at three points (blunt end, sharp end, and middle) and averaged.

**Eggshell strength:** Measured using an eggshell strength analyzer (EFR-01, Israel). Eggs were placed blunt-end up on the analyzer, and the probe descended

at 10 mm/min. The force at the first visible crack was recorded as eggshell strength.

**Relative eggshell weight:** Calculated after weighing eggshell and whole egg using an electronic balance.

Relative eggshell weight (%) =  $100 \times \text{eggshell weight} / \text{egg weight}$

**Egg shape index:** Measured using an egg shape index gauge (NFN385, Israel).

**Egg weight, albumen height, yolk color, Haugh unit:** Egg weight was measured using an electronic balance, followed by determination of albumen height, yolk color, and Haugh unit using an egg quality analyzer (EA-01, Israel).

**1.4.4 Serum Indices Determination** Serum calcium was determined by methylthymol blue (MTB) colorimetric method, and serum phosphorus by molybdcic acid method. Kits for both assays were purchased from Nanjing Jiancheng Bioengineering Institute. Serum calcitonin (CT) was measured by enzyme-linked immunosorbent assay (ELISA) using kits from Shanghai Yili Biotechnology Co., Ltd.

**1.4.5 Calcium Metabolism Rate Determination** Daily calcium intake was calculated weekly, and calcium metabolism rate was determined on a weekly basis.

Calcium metabolism rate (%) =  $100 \times (\text{daily calcium intake} - \text{fecal calcium}) / \text{daily calcium intake}$  [5].

## 1.5 Statistical Analysis

Data were initially organized using Excel 2007, followed by t-tests for comparisons between control and experimental groups at the same week using SPSS 17.0. One-way ANOVA was performed for the same index across different weeks. Results are expressed as mean  $\pm$  standard deviation.  $P < 0.05$  indicated significant difference,  $P < 0.01$  indicated extremely significant difference, and  $P > 0.05$  indicated no significant difference.

## 2.1 Effects of Alternating High and Low Calcium Diets on Performance of Late-Period Laying Hens

As shown in Table 2, the experimental group showed no significant differences from the control group 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 exhibited significant changes in these performance metrics over the feeding period ( $P > 0.05$ ).

## 2.2 Effects of Alternating High and Low Calcium Diets on Egg Quality of Late-Period Laying Hens

Table 3 shows that in week 1, eggshell thickness was significantly lower in the experimental group than the control group ( $P < 0.05$ ). By week 2, however, eggshell thickness was significantly higher in the experimental group ( $P < 0.05$ ). No significant differences were observed in weeks 3 and 4 ( $P > 0.05$ ). Eggshell strength and relative eggshell weight did not differ significantly between groups across all weeks ( $P > 0.05$ ). Neither group showed significant temporal changes in eggshell thickness, strength, or relative weight ( $P > 0.05$ ).

Table 4 indicates that egg shape index, albumen height, yolk color, Haugh unit, and egg weight did not differ significantly between groups across all weeks ( $P > 0.05$ ). Neither group showed significant temporal changes in these parameters ( $P > 0.05$ ), though both groups exhibited an increasing trend in egg shape index from weeks 1 to 3.

## 2.3 Effects of Alternating High and Low Calcium Diets on Serum Indices of Late-Period Laying Hens

Table 5 reveals that 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, 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. In the control group, serum calcitonin at 06:00, 10:00, and 14:00 was significantly higher than at 18:00 and 02:00 the following day ( $P < 0.05$ ). In the experimental group, serum 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 higher but not significantly ( $P > 0.05$ ). At 22:00, both serum calcium and calcitonin were significantly higher in the experimental group than in the control group ( $P < 0.05$ ). No significant differences were observed in serum phosphorus between groups or across time points within groups ( $P > 0.05$ ).

Table 6 shows that serum calcium levels were numerically higher in the experimental group across all weeks ( $P > 0.05$ ), while serum phosphorus was numerically lower ( $P > 0.05$ ). Serum calcitonin was also numerically lower in the experimental group ( $P > 0.05$ ).

## 2.4 Effects of Alternating High and Low Calcium Diets on Calcium Metabolism Rate of Late-Period Laying Hens

Table 7 demonstrates no significant differences between groups in daily calcium intake, fecal calcium, or calcium metabolism rate across all weeks ( $P > 0.05$ ).

However, calcium metabolism rates were numerically slightly higher in the experimental group.

### 3.1 Effects on Performance of Late-Period Laying Hens

Dietary calcium content plays a crucial role in normal physiological functions, bone development, and optimal performance in laying hens. This experiment found that feeding high-calcium diet in the afternoon resulted in higher average daily feed intake but lower feed-to-egg ratio in the experimental group, consistent with Sauveur et al. [6] who reported that dietary calcium level significantly affects feed intake, allowing hens to regulate calcium consumption through feed intake. Although laying rates did not differ significantly between groups, the experimental group maintained more stable and higher average laying rates across the 4-week period. Zhang Shiyuan et al. [7] found that appropriate calcium supplementation methods can effectively improve laying rates, and maintaining high dietary calcium levels benefits performance [8-9]. The improved laying rate in the experimental group suggests that alternating high and low calcium diets enhances performance in late-period laying hens.

### 3.2 Effects on Egg Quality of Late-Period Laying Hens

Eggshell quality is a critical indicator of egg quality [10]. Calcium is the main component of eggshells, and its absorption and utilization directly determine eggshell formation and quality [11]. Eggshell strength, weight, and 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 resorption. Therefore, dietary calcium level directly affects eggshell and bone quality. This experiment showed that eggshell thickness was significantly lower in the experimental group in week 1 but significantly higher in week 2, indicating improvement. Relative eggshell weight was higher in the experimental group during weeks 2-4, suggesting that afternoon high-calcium feeding enhances calcium deposition and eggshell quality. Since eggshell formation occurs primarily at night, calcium supplemented during this period is directly transported to the shell gland after intestinal absorption, coinciding with active intestinal absorption and improving calcium utilization. The optimal calcium supplementation time is afternoon (16:00-18:00), when two-thirds of the daily requirement can be fed to significantly improve eggshell quality.

### 3.3 Effects on Serum Indices of Late-Period Laying Hens

Hormones play a vital role in maintaining stable serum calcium and phosphorus levels. Serum calcium and phosphorus are primarily regulated by parathyroid hormone, calcitonin, and vitamin D, which interact to modulate bone metabolism and eggshell formation, maintaining dynamic balance [14]. Calcitonin is an important hormone regulating calcium and phosphorus metabolism

by inhibiting osteoclast activity and preventing bone calcium release into blood, thereby reducing blood calcium levels [15]. This experiment demonstrated that feeding high-calcium diet in the afternoon increased serum calcium levels, with a clear increasing trend from 14:00 to 22:00. Since eggshell formation occurs mainly at night, calcium absorption is enhanced during this period. Wang Hongzhe [16] found that granular limestone prolongs calcium digestion time and intestinal retention, improving calcium absorption, which aligns with our findings. Serum calcium levels in the experimental group were significantly higher than the control group at 14:00 and 22:00 on the final day, coinciding with calcium deposition for eggshell formation. Serum calcitonin was also highest at these times, likely because massive calcium consumption triggers bone calcium mobilization, increasing blood calcium and stimulating calcitonin secretion to maintain calcium balance and prevent osteoporosis. Therefore, nocturnal calcium deposition is crucial for late-period layers. These results indicate 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 calcium deposition is critically important in production. Dietary intake is the primary calcium source for layers. After intestinal absorption, calcium enters the bloodstream, with some depositing in bones and the remainder forming eggshells in the shell gland. Under normal conditions, calcium metabolism maintains dynamic balance between absorption and deposition. Sixty to seventy-five percent of eggshell calcium must be supplied by diet, with the remainder mobilized from bone [13]. Chronically low dietary calcium forces bone calcium mobilization when dietary calcium cannot meet eggshell requirements, causing osteoporosis and impairing performance and eggshell quality. Li Zhixue et al. [18] found that calcium metabolism becomes severely disrupted in late laying period, significantly affecting performance. Although both groups received identical daily calcium intake, the experimental group showed numerically higher calcium metabolism rates, indicating better calcium absorption and improved eggshell quality through enhanced calcium deposition.

## 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 to some extent in late-period laying hens.

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