Effects of Different Dietary Metabolizable Energy and Crude Protein Levels on Production Performance, Egg Quality, and Serum Biochemical Indices of Xinyang Green-Shell Laying Hens during the Late Laying Period (Postprint)

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Abstract

This experiment aimed to investigate the effects of different dietary metabolizable energy (ME) and crude protein (CP) levels on production performance, egg quality, and serum biochemical indices of Xinyang green-shell laying hens during the late laying period (44-56 weeks of age), and to determine the optimal dietary ME to CP ratio for this period. A total of 768 43-week-old Xinyang green-shell laying hens with similar body weight were randomly allocated to 6 groups, with 4 replicates per group and 32 hens per replicate. A 2$×$3 factorial design was employed (ME levels: 10.87 and 11.08 MJ/kg; CP levels: 15.00%, 15.50%, and 16.00%), resulting in 6 experimental diets. The trial consisted of a 1-week pretest period and a 12-week test period. The results showed: 1) Dietary ME and CP levels significantly affected laying rate and feed-to-egg ratio ($P<0.05$), with a highly significant interaction between ME and CP for laying rate ($P<0.01$). The high ME low CP group (ME at 11.08 MJ/kg, CP at 15.00%) exhibited the highest laying rate and the lowest feed-to-egg ratio. 2) A higher dietary CP level (16.00%) significantly increased Haugh unit ($P<0.05$); a higher dietary ME level (11.08 MJ/kg) significantly increased yolk color ($P<0.05$); no significant interaction between dietary ME and CP levels was observed for egg quality ($P>0.05$). 3) The high ME low CP group had the lowest serum urea nitrogen content; a higher dietary ME level (11.08 MJ/kg) significantly reduced serum total cholesterol content ($P<0.05$). In conclusion, under the conditions of this experiment, dietary ME and CP levels of 11.08 MJ/kg and 15.00%, respectively, were more conducive to optimizing production performance and improving egg quality in green-shell laying hens during the late laying period.

Full Text

Effects of Different Dietary Metabolizable Energy and Crude Protein Levels on Production Performance, Egg Quality, and Serum Biochemical Indices of Xinyang Green-Shell Laying Hens during the Late Stage of Egg Production

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Abstract: This study investigated the effects of different dietary metabolizable energy (ME) and crude protein (CP) levels on production performance, egg quality, and serum biochemical indices of Xinyang green-shell laying hens during the late laying period (44–56 weeks of age) to determine the optimal dietary ME and CP combination. A total of 768 healthy Xinyang green-shell laying hens at 43 weeks of age were randomly allocated to six groups with four replicates of 32 hens each. A 2$×$3 factorial design was employed with two ME levels (10.87 and 11.08 MJ/kg) and three CP levels (15.00%, 15.50%, and 16.00%), resulting in six experimental diets. The pre-trial period lasted one week, followed by a 12-week formal trial period. The results showed that: (1) Dietary ME and CP levels significantly affected laying rate and feed-to-egg ratio ($P<0.05$), with a highly significant interaction between ME and CP for laying rate ($P<0.01$). The high-energy low-protein group (11.08 MJ/kg ME, 15.00% CP) achieved the highest laying rate and lowest feed-to-egg ratio. (2) The higher CP level (16.00%) significantly increased Haugh unit ($P<0.05$), while the higher ME level (11.08 MJ/kg) significantly improved yolk color ($P<0.05$). No significant interaction between ME and CP was observed for egg quality parameters ($P>0.05$). (3) The high-energy low-protein group exhibited the lowest serum urea nitrogen content. The higher ME level (11.08 MJ/kg) significantly reduced serum total cholesterol content ($P<0.05$). In conclusion, under the conditions of this experiment, a dietary ME level of 11.08 MJ/kg combined with a CP level of 15.00% is optimal for maximizing production performance and improving egg quality in Xinyang green-shell laying hens during the late laying period.

Keywords: green-shell laying hens; late laying period; metabolizable energy; crude protein; optimal requirement

Introduction

Feed represents the primary cost in poultry production, and feed costs are largely determined by dietary metabolizable energy and crude protein levels, as well as the supplementation of essential amino acids [1]. Dietary energy and protein levels significantly influence the production performance and body composition of laying hens [2], making the determination of appropriate ME and CP levels crucial for meeting production requirements and ensuring efficient feed resource utilization. The Xinyang green-shell laying hen is an excellent domestic poultry breed independently developed in China through hybridization of Dongxiang green-shell chickens with White Leghorn or Hy-Line White chickens after compatibility testing, conducted by Shanghai Poultry Breeding Co., Ltd., China Agricultural University, and the National Poultry Engineering Technology Research Center. However, recent research on green-shell chickens has primarily focused on the effects of essential amino acids such as methionine, lysine, and tryptophan on production performance [3-4], with most studies limited to the rearing period [5]. To date, no feeding standards exist for the laying period of this breed. Therefore, this study aimed to investigate the effects of dietary ME and CP levels and their interaction on production performance, egg quality, and serum biochemical indices of Xinyang green-shell laying hens during the late laying period, thereby establishing optimal dietary ME and CP combinations to provide a theoretical basis for formulating feeding standards and guiding production practices.

1.1 Experimental Materials

The experimental animals were Xinyang green-shell laying hens during the late laying period (44-56 weeks of age). The trial was conducted at the Huaqing Breeding Base of Shanghai Poultry Breeding Co., Ltd. from May 21 to August 29, 2015.

1.2 Experimental Design

A total of 768 healthy 43-week-old green-shell laying hens with similar body weights were randomly divided into six groups, each comprising four replicates of 32 hens. After a one-week pre-trial period, during which laying rates were monitored and groups were adjusted to ensure no significant differences in initial laying rates ($P>0.05$), the formal 12-week trial commenced. Based on NRC (1994) guidelines for Leghorn-type layers and China' s "Feeding Standard of Chickens" (NY/T 33—2004) for quality egg-type chickens, a 2$×$3 factorial design was employed with two ME levels (10.87 and 11.08 MJ/kg) and three CP levels (15.00%, 15.50%, and 16.00%), resulting in six experimental diets. The composition and nutrient levels of the experimental diets are presented in Table 1 .

1.3 Management Practices

Hens were housed in three-tier step cages with semi-mechanical ventilation and artificial lighting. Feed was provided twice daily at 08:00 and 14:00. Feed allowance was adjusted based on residual feed to maintain ad libitum intake, stimulate appetite, and minimize waste. Birds had free access to water throughout the pre-trial period (one week) and the formal trial period (12 weeks).

1.4 Measurement Indices

1.4.1 Production Performance Daily records were maintained for mortality, feed intake, egg number, and egg weight within each replicate to calculate average daily feed intake (ADFI), laying rate, average egg weight, and feed-to-egg ratio. At the beginning of the trial, eight hens with similar body weights were selected and marked in each replicate; these same hens were weighed again at the end of the trial to calculate average body weight gain per replicate.

1.4.2 Egg Quality Eggs were collected every six weeks (two collections total). For each replicate, six eggs were randomly selected (24 eggs per group, 144 eggs total) for quality assessment using a Nippon Denshi DET 6000 egg quality analyzer. Measured parameters included albumen height, yolk color, Haugh unit, eggshell thickness, and eggshell strength.

1.4.3 Serum Biochemical Indices At the end of the feeding trial, four hens were randomly selected from each replicate (16 per group), fasted for 12 hours with free access to water, and then blood samples were collected from the wing vein. Serum was prepared by centrifugation at 3,500 r/min for 15 minutes, immediately aliquoted, temporarily stored in ice boxes, and then preserved at -80°C. Serum total protein (TP), albumin (ALB), urea nitrogen (UN), triglycerides (TG), and total cholesterol (T-CHO) were measured using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.5 Statistical Analysis

All data are expressed as means $\pm$ standard deviation. Data were analyzed using the univariate procedure in SPSS 20.0 general linear model, and Duncan' s multiple range test was used for post-hoc comparisons. Significance was declared at $P<0.05$.

Results

2.1 Effects of Dietary ME and CP Levels on Production Performance

As shown in Table 2 , dietary ME and CP levels significantly affected laying rate and feed-to-egg ratio ($P<0.05$), with a highly significant interaction between ME and CP for laying rate ($P<0.01$). The high-energy low-protein group (HELP) achieved the highest laying rate, which was 10.62% ($P<0.05$), 11.80% ($P<0.05$), 7.06% ($P>0.05$), 7.48% ($P<0.05$), and 11.00% ($P<0.05$) higher than the low-energy low-protein (LELP), low-energy medium-protein (LEMP), low-energy high-protein (LEHP), high-energy medium-protein (HEMP), and high-energy high-protein (HEHP) groups, respectively. The HELP group also had the lowest feed-to-egg ratio, which was 7.57% (P>0.05), 9.23% (P<0.05), 7.85% (P>0.05), 7.29% (P>0.05), and 7.01% (P>0.05) lower than the LELP, LEMP, LEHP, HEMP, and HEHP groups, respectively.

At the ME level of 10.87 MJ/kg, the high-protein group exhibited the highest laying rate and ADFI, which were 3.32% (P>0.05) and 4.42% (P>0.05) higher than the low-protein group, and 2.53% (P>0.05) and 0.82% (P>0.05) higher than the medium-protein group, respectively. At the ME level of 11.08 MJ/kg, the low-protein group achieved the highest laying rate, which was 7.48% (P<0.05) and 11.00% (P<0.05) higher than the medium- and high-protein groups, respectively.

When CP level was considered as the fixed factor, the low-protein group showed a 4.80% (P<0.05) and 3.94% (P>0.05) lower feed-to-egg ratio compared with the medium- and high-protein groups, respectively. When ME level was considered as the fixed factor, no significant effects on laying rate, average egg weight, ADFI, or feed-to-egg ratio were observed (P>0.05), although the high-energy group showed a 3.60% higher laying rate, 0.50% lower average egg weight, and 3.61% lower feed-to-egg ratio compared with the low-energy group.

2.2 Effects of Dietary ME and CP Levels on Egg Quality

As shown in Table 3 , dietary ME and CP levels significantly affected Haugh unit, yolk color, and eggshell strength (P<0.05). The low-energy high-protein group exhibited the highest Haugh unit, which was 7.25% higher than the low-energy low-protein group (P<0.05). The high-energy high-protein group also showed a 6.80% higher Haugh unit compared with the low-energy low-protein group (P<0.05). The high-energy high-protein group had the highest yolk color, which was 11.86% higher than the low-energy high-protein group (P<0.05). The low-energy medium-protein group demonstrated the greatest eggshell strength, which was 20.59% higher than the high-energy high-protein group (P<0.05).

At the ME level of 10.87 MJ/kg, Haugh unit increased with increasing dietary CP level, with the high-protein group showing a 7.25% higher Haugh unit than the low-protein group (P<0.05). At the ME level of 11.08 MJ/kg, the high-protein group exhibited higher albumen height, Haugh unit, yolk color, and eggshell thickness than the low- and medium-protein groups, though these differences were not significant (P>0.05).

When CP level was considered as the fixed factor, increasing dietary CP significantly improved Haugh unit (P<0.05), with the high-protein group showing 4.19% (P<0.05) and 4.72% (P<0.05) higher Haugh units than the medium- and low-protein groups, respectively. When ME level was considered as the fixed factor, increasing dietary ME significantly improved yolk color, with the high-energy group showing 4.58% higher yolk color than the low-energy group (P<0.05). The high-energy high-protein group had the lowest eggshell strength, which was 17.07% lower than the low-energy medium-protein group (P<0.05). No significant interaction between ME and CP was observed for egg quality parameters (P>0.05).

2.3 Effects of Dietary ME and CP Levels on Serum Biochemical Indices

As shown in Table 4 , dietary ME and CP levels significantly affected serum UN, ALB, and T-CHO contents (P<0.05). A significant interaction between ME and CP was observed for serum UN content (P<0.05). The HELP group exhibited the lowest serum UN content, which was 22.02% (P<0.05) and 21.08% (P<0.05) lower than the LEMP and HEHP groups, respectively. The HELP group also showed a 31.11% lower serum T-CHO content compared with the LELP group (P<0.05).

When CP level was considered as the fixed factor, the medium-protein group exhibited 10.30% (P<0.05) and 8.35% (P<0.05) lower serum ALB contents than the low- and high-protein groups, respectively. When ME level was considered as the fixed factor, increasing dietary ME significantly reduced serum T-CHO content, with the high-energy group showing a 20.12% lower serum T-CHO content than the low-energy group (P<0.05).

Discussion

3.1 Effects of Dietary Crude Protein Level on Production Performance

Laying rate and average egg weight are key indicators of production performance, influenced by breed, age, dietary nutrition, and environmental factors. Geng et al. [6] reported that during the early laying period at 20 weeks of age, the 15.50% CP group showed significantly higher laying rates than the 16.00% CP group. As the laying period progressed, the 16.00% CP group exhibited decreased laying rates and increased feed-to-egg ratios compared with the 15.00% CP group during the late laying stage, which aligns with our findings. The present study demonstrated that in green-shell laying hens after 45 weeks of age, increasing dietary CP level tended to decrease laying rate, with the 15.00% CP group achieving the highest laying rate. Silva et al. [7] investigated the effects of different dietary CP levels on performance of 48-week-old Hisex White layers and found that average egg weight increased linearly with dietary CP level, consistent with our results. Although increasing dietary CP level did not significantly affect average egg weight in this study, an increasing trend was observed, which may be attributed to differences in animal species and dietary composition. Westerterp-Plantenga [8] suggested that increasing dietary CP level increases heat increment, thereby enhancing satiety and reducing metabolizable energy utilization efficiency. Our results indicate that dietary CP level significantly affected feed-to-egg ratio during the late laying stage, with the ratio decreasing as CP level increased, suggesting that 15.00% CP is beneficial for optimizing feed efficiency in late-stage green-shell laying hens.

3.2 Effects of Dietary Metabolizable Energy Level on Production Performance

Poultry possess the innate ability to regulate feed intake according to their energy requirements. The effect of ME on laying hen performance primarily manifests through altered feed intake, which subsequently affects nutrient intake [9]. This study found that dietary ME levels of 10.87 and 11.08 MJ/kg did not significantly affect feed intake, laying rate, average egg weight, or feed-to-egg ratio in late-stage green-shell laying hens. This may be attributed to the relatively small difference in ME levels, as well as variations in animal species and dietary composition.

3.3 Combined Effects of Dietary ME and CP on Production Performance

The balance between dietary ME and CP levels plays a crucial role at all growth stages. The nutritional relationship between ME and CP is not independent; rather, an inherent connection exists between them [10]. Optimal performance and feed conversion efficiency are achieved when dietary ME and CP maintain an appropriate ratio. Conversely, an imbalanced ME:CP ratio can impair nutrient utilization efficiency and cause metabolic disorders due to overall nutritional imbalance [11]. This study demonstrated a highly significant interaction between dietary ME and CP levels for laying rate in late-stage green-shell laying hens, consistent with the findings of Sell et al. [12]. Under our experimental conditions, the combination of 11.08 MJ/kg ME and 15.00% CP yielded the highest laying rate.

3.4 Effects of Dietary ME and CP Levels on Egg Quality

Egg quality and composition change with hen age and production level as laying rate declines and egg weight increases with advancing age [13]. Albumen height and Haugh unit are important indicators of egg white quality. While albumen height is primarily influenced by environmental conditions during egg formation and storage, with minimal impact from nutrition [14], Haugh unit is commonly used to evaluate egg physical quality. A higher Haugh unit indicates greater albumen viscosity and superior protein quality. Chen et al. [15] reported that in Luxi game fowl after 41 weeks of age, the higher CP level group (17.00%) showed significantly higher Haugh units than the lower CP group (15.50%). Our results demonstrate that increasing dietary CP level significantly improved Haugh units in green-shell eggs, possibly because absorbed protein beyond maintenance requirements is deposited primarily in the thick albumen. Yang [16] reported that yolk color deepened significantly with increasing dietary ME level, which aligns with our findings. The present study showed that dietary ME level significantly affected yolk color, with the high-energy high-protein group (11.08 MJ/kg ME, 16.00% CP) producing the best yolk color. Bi [17] found that eggshell weight is related to egg shape index and dietary calcium content, calcium source utilization, and timing of calcium supplementation. The significantly reduced eggshell strength observed in the high-energy high-protein group may be attributed to lower feed intake and insufficient calcium intake.

3.5 Effects of Dietary ME and CP Levels on Serum Biochemical Indices

Malmiof [18] suggested that serum UN accurately reflects protein metabolism and dietary amino acid balance, with lower UN indicating well-balanced dietary amino acids and efficient protein metabolism. Our results show that increasing dietary ME level reduced serum UN content, consistent with the findings of Yuan et al. [19] from our research group. The HELP group (11.08 MJ/kg ME, 15.00% CP) significantly reduced serum UN content, likely because this ME-CP combination promoted protein metabolism more effectively. Serum T-CHO content reflects fat deposition, while serum TG content indicates lipogenesis intensity. Cao et al. [20] demonstrated a relationship between lipid metabolism and fat deposition, with higher fat deposition corresponding to elevated serum TG and T-CHO levels. This study indicates that 11.08 MJ/kg ME effectively reduced serum TG and T-CHO contents, possibly because the ME-CP composition met the nutritional requirements of late-stage green-shell laying hens, preventing excessive fat accumulation in subcutaneous tissue and liver. Protein deposition depends on the balance between protein synthesis and catabolism. Serum TP and ALB contents reflect protein synthesis capacity, and ALB also functions as a nutrient carrier and maintains plasma osmotic pressure [21]. The HELP group significantly increased serum ALB content, suggesting that this ME-CP combination enhanced protein anabolism in late-stage green-shell laying hens.

Conclusions

Under the conditions of this experiment:

1. A dietary ME level of 11.08 MJ/kg combined with a CP level of 15.00% optimized production performance in late-stage green-shell laying hens, achieving the highest laying rate, lowest feed-to-egg ratio, lowest serum UN and T-CHO contents, and highest serum ALB content.
2. A dietary ME level of 11.08 MJ/kg effectively improved yolk color in late-stage green-shell laying hens.
3. A dietary CP level of 16.00% effectively improved Haugh unit in late-stage green-shell laying hens.

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