Effects of Different Supplementary Feeding Levels on Production Performance, Egg Quality, Visceral Organ Development, and Blood Indices of Free-Range Luhua Laying Hens under Locust Forest Postprint

Authors: Xiong Mangli, Wu Xujin, Zhu Xiaofu, Zhang Wenjuan, Zhang Zhaoshun

Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment was conducted to determine the appropriate supplementation level of diet for free-range Luhua laying hens under Chinese scholar trees by investigating the effects of different supplementation levels on production performance, egg quality, visceral organ development, and blood parameters. A total of 200 healthy 45-week-old Luhua laying hens with similar laying performance were randomly allocated to 5 groups with 4 replicates per group and 10 hens per replicate. The five groups of hens were free-ranged under Chinese scholar trees, with supplementation levels of 60%, 70%, 80%, 90%, and 100% of ad libitum intake of the experimental diet, respectively, where 100% supplementation corresponded to 90 g of experimental diet per day. The preliminary period lasted 7 days, and the formal experimental period lasted 70 days. The results showed: 1) The laying rate and average egg weight of the 60% supplementation group were significantly lower than those of the other groups ($P<0.05$). 2) There were no significant differences in egg shape index, eggshell thickness, eggshell strength, and eggshell ratio among groups ($P>0.05$). The Haugh units of the 80%, 90%, and 100% supplementation groups were significantly higher than those of the 60% and 70% supplementation groups ($P<0.05$), the yolk ratio of the 70% supplementation group was significantly higher than that of the 90% and 100% supplementation groups ($P<0.05$), and the yolk color of the 60%, 70%, and 80% supplementation groups was significantly higher than that of the 90% and 100% supplementation groups ($P<0.05$). 3) The liver index and heart index of the 60% and 70% supplementation groups were significantly lower than those of the other groups ($P<0.05$), the gizzard index and cecal index of the 60% and 70% supplementation groups were significantly higher than

those of the other groups (P<0.05), the proventriculus index of the 80% supplementation group was significantly higher than that of the 70%, 90%, and 100% supplementation groups (P<0.05), the oviduct weight of the 80% supplementation group was significantly higher than that of the 60% supplementation group (P<0.05), and the total follicle number of the 80% supplementation group was significantly higher than that of the 60% and 70% supplementation groups (P<0.05). 4) The serum glucose content of the 60% and 70% supplementation groups was significantly lower than that of the 80%, 90%, and 100% supplementation groups (P<0.05), the serum globulin content of the 60% and 70% supplementation groups was significantly higher than that of the 80%, 90%, and 100% supplementation groups (P<0.05), the blood hemoglobin content of the 80% supplementation group was significantly higher than that of the 90% and 100% supplementation groups (P<0.05), and the blood hematocrit of the 70% supplementation group was significantly higher than that of the 80%, 90%, and 100% supplementation groups (P<0.05). It can be concluded that the appropriate supplementation level for free-range Luhua laying hens under Chinese scholar trees is 70%-80% of ad libitum diet.

Full Text

Effects of Different Supplementary Feeding Levels on Performance, Egg Quality, Visceral Organ Development and Blood Parameters of Free-Range Barred Luhua Laying Hens in Sophora japonica Grove

XIONG Mangli, WU Xujin, ZHU Xiaofu, ZHANG Wenjuan, ZHANG Zhaoshun

(Xianyang Vocational College, Xianyang 712000, China)

Abstract

This experiment investigated the effects of different supplementary feeding levels on production performance, egg quality, visceral organ development, and blood parameters of free-range barred Luhua laying hens in Sophora japonica groves to determine the optimal supplementary feeding level. Two hundred healthy 45-week-old Luhua laying hens with similar laying performance were randomly allocated to 5 groups with 4 replicates per group and 10 hens per replicate. Birds were free-ranged in Sophora japonica groves with supplementary feeding levels of 60%, 70%, 80%, 90%, and 100% of ad libitum intake, where 100% supplementation corresponded to 90 g of experimental diet per day. The pre-experimental period lasted 7 days, followed by a 70-day experimental period. The results showed: (1) The laying rate and average egg weight of the 60% supplementation group were significantly lower than those of other groups (P<0.05). (2) No significant differences were observed in egg shape index, eggshell thickness, eggshell strength, or eggshell ratio among groups (P>0.05). The Haugh unit of the 80%, 90%, and 100% supplementation groups was significantly higher than

that of the 60% and 70% groups (P<0.05). The yolk ratio of the 70% group was significantly higher than that of the 90% and 100% groups (P<0.05), and the yolk color of the 60%, 70%, and 80% groups was significantly higher than that of the 90% and 100% groups (P<0.05). (3) The liver index and heart index of the 60% and 70% groups were significantly lower than those of other groups (P<0.05), while their gizzard index and cecum index were significantly higher (P<0.05). The proventriculus index of the 80% group was significantly higher than that of the 70%, 90%, and 100% groups (P<0.05). The oviduct weight of the 80% group was significantly higher than that of the 60% group (P<0.05), and the total follicle number of the 80% group was significantly higher than that of the 60% and 70% groups (P<0.05). (4) The serum glucose content of the 60% and 70% groups was significantly lower than that of the 80%, 90%, and 100% groups (P<0.05), while their serum globulin content was significantly higher (P<0.05). The blood hemoglobin content of the 80% group was significantly higher than that of the 90% and 100% groups (P<0.05), and the blood hematocrit of the 70% group was significantly higher than that of the 80%, 90%, and 100% groups (P<0.05). In conclusion, the suitable supplementary feeding level for free-range barred Luhua laying hens in Sophora japonica groves is 70%–80% of ad libitum diet intake.

Keywords: supplementary feeding levels; free range in grove; Luhua laying hens; performance; egg quality; visceral organ indexes; serum biochemical parameters

Introduction

With improving living standards, consumers increasingly prioritize food safety, showing greater preference for organic, green, and pollution-free premium livestock products [1]. To enhance the health status and welfare of laying hens while improving egg flavor and quality, free-range ecological systems have emerged. Ecological free-range systems utilize orchards, hillsides, and forest environments for large-scale chicken production, allowing birds to forage freely for grass, insects, seeds, earthworms, and other natural feedstuffs. This approach results in greater activity space, robust physique, strong disease resistance, and high egg quality, making it increasingly popular [2-3]. Research indicates that free-range laying hens can reduce concentrated feed consumption and lower feed costs. Wu et al. [4] reported that combining free-range with supplementary feeding reduced concentrate feed by approximately 30%. Li et al. [5] found that under free-range conditions, different supplementary feeding levels significantly affected the laying rate, egg weight, and egg quality of Hebei local chickens. Zhao et al. [6] reported that under mountain forest ecological grazing conditions, artificial supplementation significantly influenced laying rate, egg quality, and nutrient composition. Su [7] suggested that ecologically free-range laying hens could save 26% of feed. Several experts have also studied supplementary feeding levels for free-range laying hens [8]. However, comprehensive research on

the effects of different supplementary feeding levels on production performance, egg quality, visceral organ development, and blood parameters of barred Luhua laying hens in Sophora japonica groves remains scarce, particularly in northwest China. Therefore, this study aimed to determine the optimal supplementary feeding level for free-range barred Luhua laying hens in Sophora japonica groves, providing a theoretical foundation for the development of forest-based free-range egg production.

---

1. Materials and Methods

1.1 Experimental Design This experiment employed a single-factor randomized block design. Two hundred healthy 45-week-old barred Luhua laying hens with similar body weight and laying performance were randomly divided into 5 groups with 4 replicates per group and 10 hens per replicate. Birds were free-ranged in Sophora japonica groves with supplementary feeding levels of 60%, 70%, 80%, 90%, and 100% of ad libitum intake, where 100% supplementation corresponded to 90 g of experimental diet per day.

1.2 Experimental Diet The supplementary concentrate diet was formulated according to NRC (1994) standards for laying hens and adjusted based on the actual nutritional requirements of Luhua chickens. The composition and nutrient levels of the experimental diet are presented in .

1.3 Management Practices The experiment was conducted from May to July 2017 in Yongshou County, Xianyang City, Shaanxi Province. Hens were free-ranged in Sophora japonica groves at a stocking density of 6.67 m^2 per bird, with 3-meter-high nets separating replicates. Each group received half of their daily supplementary feed allowance in the morning and half in the evening, with ad libitum access to water and natural forage materials in the grove. Lighting consisted of natural daylight supplemented with artificial light to achieve 16 hours daily. Routine management practices were followed. The pre-experimental period lasted 7 days with all groups receiving 100% of the experimental diet, followed by a 70-day experimental period with treatments applied according to designated supplementation levels.

1.4 Measurement Indicators 1.4.1 Production Performance
During the experimental period, daily records were kept for egg number and weight per group. Weekly calculations included laying rate, average egg weight, and actual feed consumption. Average egg weight was calculated as the mean weight of all eggs per replicate. Laying rate (%) = (total eggs laid / (number of hens × experimental days)) × 100. Feed-to-egg ratio = total feed consumption / total egg weight.

1.4.2 Egg Quality
On day 42 of the experimental period, 6 eggs were randomly collected from

each replicate and stored at 4°C for quality analysis. Egg shape index was calculated as the ratio of longitudinal to transverse diameter. Eggshell strength was measured using an eggshell force gauge (ORKA, Israel). Eggshell and yolk ratios were calculated as percentages of egg weight. Eggshell thickness was measured using an eggshell thickness gauge (ORKA, Israel). Haugh unit was determined using an EMT-7300 egg quality system.

1.4.3 Visceral Organ Indexes and Reproductive System Parameters
Visceral organ indexes were calculated as the ratio of organ weight to body weight, including gizzard, proventriculus, duodenum, jejunum, ileum, cecum, rectum, liver, spleen, and heart. Oviduct weight and length, ovary weight, follicle weight, and total follicle number (counting follicles >2 mm diameter) were measured.

1.4.4 Serum Biochemical and Hematological Parameters
Four milliliters of blood were collected from the wing vein. After 3 hours of coagulation, serum was separated by centrifugation at 3,000 rpm for 15 minutes and stored at -20°C. Serum total protein, albumin, globulin, glucose, high-density lipoprotein, low-density lipoprotein, and triglycerides were analyzed using an AU2700 automatic biochemical analyzer (Beckman Coulter Olympus). Another 4 mL of blood with anticoagulant was used for hematological analysis using an XE2100 automatic hematology analyzer (Sysmex) to determine white blood cell count, red blood cell count, hemoglobin content, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, and mean corpuscular hemoglobin.

1.5 Statistical Analysis Data were analyzed using SPSS 15.0 statistical software. Multiple comparisons were performed using the least significant difference (LSD) method. Results are expressed as means ± standard deviation. Significance was determined by t-test with $P<0.05$ considered statistically significant.

---

2. Results

2.1 Effects on Production Performance As shown in , the laying rate and average egg weight of free-range barred Luhua laying hens increased with increasing supplementary feeding levels. The laying rate of the 60% group was 26.00%, 27.49%, 29.30%, and 33.00% lower than the 70%, 80%, 90%, and 100% groups, respectively ($P<0.05$). The average egg weight of the 60% group was 12.61%, 14.49%, 14.93%, and 16.59% lower than the 70%, 80%, 90%, and 100% groups, respectively ($P<0.05$). No significant differences in laying rate or average egg weight were observed among the 70%, 80%, 90%, and 100% groups ($P>0.05$), and feed-to-egg ratio did not differ significantly among all groups ($P>0.05$).

2.2 Effects on Egg Quality As presented in , no significant differences were found in egg shape index, eggshell thickness, eggshell strength, or eggshell ratio among groups (P>0.05). The 80% group showed the highest Haugh unit, which was 8.31% and 5.32% higher than the 60% and 70% groups, respectively (P<0.05), while no significant differences were observed among the 80%, 90%, and 100% groups (P>0.05). The 70% group exhibited the highest yolk ratio and yolk color, with yolk ratio 7.96% and 11.11% higher than the 90% and 100% groups (P<0.05), and yolk color 25.19% and 21.33% higher than the 90% and 100% groups (P<0.05). No significant differences in yolk ratio or yolk color were found among the 60%, 70%, and 80% groups (P>0.05).

2.3 Effects on Visceral Organ Indexes and Reproductive System As shown in , the 70% group had the lowest liver index, which was 24.79%, 28.27%, and 28.48% lower than the 80%, 90%, and 100% groups, respectively (P<0.05). The 60% group had the lowest heart index, which was 15.55%, 16.32%, and 14.15% lower than the 80%, 90%, and 100% groups (P<0.05). Conversely, the 70% group showed the highest gizzard and cecum indexes, with gizzard index 12.18%, 24.67%, and 25.73% higher than the 80%, 90%, and 100% groups (P<0.05), and cecum index 57.14%, 72.33%, and 76.28% higher (P<0.05). The proventriculus index of the 80% group was 27.84%, 35.83%, and 31.89% higher than the 70%, 90%, and 100% groups (P<0.05). No significant differences were observed in duodenum, jejunum, ileum, rectum, or spleen indexes among groups (P>0.05).

The 80% group exhibited the greatest oviduct weight, which was 75.21% higher than the 60% group (P<0.05), though no significant differences were found among the 70%, 80%, 90%, and 100% groups (P>0.05). The 80% group also had the highest total follicle number, which was 29.52% and 30.97% higher than the 60% and 70% groups (P<0.05), while no significant differences were observed among the 80%, 90%, and 100% groups (P>0.05). No significant differences were detected in oviduct length, ovary weight, or follicle weight among all groups (P>0.05).

2.4 Effects on Serum Biochemical and Hematological Parameters As shown in , the 60% group had the lowest serum glucose content, which was 30.65%, 29.31%, and 30.98% lower than the 80%, 90%, and 100% groups (P<0.05), though no significant difference was observed between the 60% and 70% groups (P>0.05). The 70% group had the highest globulin content, which was 43.25%, 50.08%, and 58.71% higher than the 80%, 90%, and 100% groups (P<0.05), with no significant difference between the 60% and 70% groups (P>0.05). No significant differences were found in serum total protein, albumin, triglycerides, high-density lipoprotein, or low-density lipoprotein among groups (P>0.05).

As presented in , the 80% group had the highest hemoglobin content, which was 15.11% and 26.17% higher than the 90% and 100% groups (P<0.05). The 70%

group showed the highest hematocrit, which was 17.99%, 21.09%, and 26.87% higher than the 80%, 90%, and 100% groups ($P<0.05$). No significant differences were observed in white blood cell count, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, or mean corpuscular hemoglobin concentration among groups ($P>0.05$).

3. Discussion

3.1 Effects on Production Performance Golden et al. [9] reported that free-range laying hens had significantly lower total egg production and Grade A eggs compared with caged hens. Yang et al. [10] found that under the same stocking density, the 70% supplementation group showed significantly higher egg weight and laying rate than the 50% group. Wang et al. [11] observed that the laying rate of free-range hens initially increased then decreased with reduced supplementary feeding levels, with the 80% group significantly higher than the 60%, 70% groups and control. Ge et al. [12] reported that the laying performance (laying rate and daily egg mass) of Hebei local chickens gradually increased with higher supplementary feeding levels. Yang et al. [13] found that free-range housing resulted in significantly lower laying rates compared with caged systems. Our findings align with these reports, demonstrating that laying rate and average egg weight increased with supplementary feeding level, indicating that nutrient levels (energy, protein, vitamins, and minerals) play a decisive role in determining laying performance.

3.2 Effects on Egg Quality Different supplementary feeding levels showed no significant effects on egg shape index, eggshell thickness, eggshell strength, or eggshell ratio, likely because egg shape index is primarily genetically determined rather than nutritionally influenced. Free-range hens could forage for grass, Sophora leaves and flowers, insects, and soil minerals, which may have compensated for differences in calcium and phosphorus intake among supplementation levels, resulting in similar eggshell quality across groups. These findings are consistent with Li et al. [5].

Dietary lutein and carotenoids are the primary determinants of yolk color [14], and green forage contains abundant natural pigments that enhance yolk color and increase yolk ratio [15-16]. Li et al. [5] reported that reducing supplementary feed significantly increased yolk ratio and yolk color in free-range local chickens. Our results showed that yolk color and ratio increased with decreasing supplementary feeding levels, with the 70% group showing optimal results, consistent with previous reports. This may be attributed to natural yellow pigments and vitamin A in Sophora flowers affecting yolk characteristics, though further investigation is needed.

Previous studies on Haugh unit in free-range hens have yielded inconsistent results [17-19]. Gu et al. [17] found that free-range hens had significantly higher

Haugh units than caged hens, while Su et al. [18] reported significantly higher Haugh units in forest-range systems. Li [16] demonstrated that nutritional level significantly affected Haugh unit. Our results align with Li [16], indicating that higher supplementary feeding levels increase dietary protein content and albumen height, which positively correlates with Haugh unit.

3.3 Effects on Visceral Organ Indexes and Reproductive System Visceral organ indexes reflect both growth development and environmental adaptability [19]. Limited research exists on the effects of supplementary feeding levels on visceral organs. Hetland et al. [20] and Steenfeldt [21] reported that grit and grass stimulate gizzard and proventriculus development. Xu et al. [22] found that free-range laying hens had significantly higher relative weights of gizzard, proventriculus, and liver compared with caged hens. Our results showed that the 70% group had optimal gizzard and cecum indexes, while the 80% group had optimal proventriculus index. This may be because 70%–80% supplementation could not fully meet nutritional requirements, prompting hens to consume grit, soil particles, Sophora flowers and leaves, and grass fiber. Hard substances stimulate proventriculus acid secretion and development, while fiber promotes gizzard and cecum development. Conversely, decreasing supplementary feeding levels reduced liver and heart indexes, possibly because feed-restricted hens increased foraging activity, consuming more energy and affecting liver and heart development, which may reduce fatty liver and heart disease incidence but requires further study. The 80% group showed maximum oviduct weight and total follicle number, indicating optimal reproductive system development, likely related to physiological responses triggered by foraging behavior, consistent with Wang et al. [11].

3.4 Effects on Serum Biochemical and Hematological Parameters Küçükyilmaz et al. [23] reported that free-range housing enhanced Newcastle disease antibody titers and disease resistance. Our results showed that the 70% group had highest serum globulin content and hematocrit, while the 80% group had highest hemoglobin content, likely due to increased activity and diverse forage intake strengthening immunity. Kyawczyk et al. [24] found that free-range housing affected serum triglyceride content. Our study showed that the 70% group had significantly lower serum glucose, and triglycerides decreased with reduced supplementation, probably related to activity levels. Lower supplementation increased foraging activity, reducing serum glucose and triglycerides, which may explain the decreased liver index and reduced fatty liver. However, the minimal effects of supplementation levels on serum biochemical parameters may relate to the nutritional value of Sophora flowers and requires further investigation.

4. Conclusion

The suitable supplementary feeding level for free-range barred Luhua laying hens in Sophora japonica groves is 70%–80% of ad libitum diet intake.

References

[1] Lü JH. Effects of different feeding methods and nutrition levels on growth performance and meat quality of Beijing oil chickens [D]. Master' s thesis. Wuhan: Huazhong Agricultural University, 2005.

[2] Tian L, Xu YS, Lei XQ, et al. Effects of feeding methods on production performance and economic benefits of Lushi green-shell laying hens [J]. Journal of Domestic Animal Ecology, 2012, 33(3): 90–92.

[3] Zhao XY, Liu L, Xu DM, et al. Comparison of production performance of "Nongda No. 3" laying hens under three feeding modes during peak laying period [J]. China Poultry, 2012, 34(13): 23–26.

[4] Wu QJ, Tao YH. Forest-grassland chicken farming is the best way to produce organic broilers [J]. China Poultry, 2006, 28(4): 29–31.

[5] Li W, Huang RL, Wang HC. Effects of supplementary feeding amount on production performance and egg quality of free-range Hebei local chickens [J]. Journal of Hebei Agricultural University, 2005, 28(4): 97–100.

[6] Zhao XY, Zhang XG, Lin Y, et al. Study on artificial supplementary feed amount in diet of free-range laying hens in mountain forest [J]. China Poultry, 2011, 33(24): 12–15.

[7] Su BY. Study on feeding behavior of free-range local chickens and its impact on grassland productivity in sandy grassland [D]. Master' s thesis. Tai' an: Shandong Agricultural University, 2011: 51.

[8] Li P, Mu SQ, Li P, et al. Research progress on free-range laying hen breeding technology in forest [J]. Poultry Science, 2013(3): 49–52.

[9] Golden JB, Arbona DV, Anderson KE. A comparative examination of rearing parameters and layer production performance for brown egg-type pullets grown for either free-range or cage production [J]. The Journal of Applied Poultry Research, 2012, 21(1): 95–102.

[10] Yang Y, Sun BS, Sun Y, et al. Effects of stocking density and supplementary feeding amount on production performance and yolk cholesterol content of laying hens under free-range conditions [J]. Chinese Journal of Eco-Agriculture, 2014, 22(12): 1453–1459.

[11] Wang J, Yang Z, Hou QY, et al. Effects of different supplementary feeding amounts on laying performance, egg quality, and reproductive organs of free-

range laying hens in forest [J]. Jiangsu Agricultural Sciences, 2014, 42(12): 240-242.

[12] Ge J, Gu ZL, Li Y, et al. Effects of different supplementary feeding amounts on production performance, economic benefits, and egg quality of Hebei local chickens at the end of laying period [J]. Heilongjiang Animal Science and Veterinary Medicine, 2005(10): 27-28.

[13] Yang HM, Cao YJ, Zhu XC, et al. Effects of free-range on production performance, egg quality, and reproductive system development of laying hens [J]. Chinese Journal of Animal Nutrition, 2013, 25(8): 1866-1871.

[14] Cheng ZG, Lin YC, Zheng L. Research progress on skin and yolk pigmentation in broilers and laying hens [J]. China Feed, 2001(4): 5-7.

[15] Li SP. Study on factors affecting yolk color and colorants [D]. Master' s thesis. Harbin: Northeast Agricultural University, 2000.

[16] Li W. Effects of supplementary feeding amount on production performance and egg quality of free-range Hebei local chickens [D]. Master' s thesis. Baoding: Hebei Agricultural University, 2004.

[17] Gu R, Wang KH, Shi SR, et al. Effects of different feeding methods on production performance and egg quality of laying hens [J]. Poultry Science, 2010(8): 10-12.

[18] Su SG, Wu YJ, Li JY, et al. Effects of different feeding methods on egg quality of Huainan partridge chickens [J]. Anhui Agricultural Sciences, 2011, 39(21): 12866-12867.

[19] Wang J, Yang Z, Zhang DC, et al. Effects of different supplementary feeding amounts on visceral organs, routine blood parameters, and serum biochemical indicators of free-range laying hens in forest [J]. China Poultry, 2014, 36(23): 33-36.

[20] Hetland H, Svihus B, Krogdahl Å. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat [J]. British Poultry Science, 2003, 44(2): 275-282.

[21] Steenfeldt S, Kjaer JB, Engberg RM. Effect of feeding silages or carrots as supplements to laying hens on production performance, nutrient digestibility, gut structure, gut microflora, and feather pecking behavior [J]. British Poultry Science, 2007, 48(4): 454-468.

[22] Xu YY, Qian K, Su SG, et al. Effects of feeding methods on slaughter performance and meat quality of Huainan partridge chickens [J]. Anhui Agricultural Sciences, 2012, 40(32): 15741-15743.

[23] Küçükyilmaz K, Bozkurt M, Herken EN, et al. Effects of rearing systems on performance, egg characteristics, and immune response of two layer genotypes [J]. Asian-Australasian Journal of Animal Sciences, 2012, 25(4): 559-568.

[24] Kyawczyk J, Sokołowicz Z, Szymczyk B. Effect of housing system on cholesterol, vitamin, and fatty acid content of yolk and physical characteristics of eggs from Polish native hens [J]. Archiv fur Geflugelkunde, 2011, 75(3): 151–157.

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