Effects of Dietary Iron Level on Laying Performance, Egg Quality, and Liver and Blood Indices of Shanma Ducks (Postprint)

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Date: 2017-10-10T00:00:00+00:00

Abstract

The present study was conducted to determine the dietary iron requirement of laying Shanma ducks during the laying period (17–30 weeks of age) by examining the effects of dietary iron levels on laying performance, egg quality, and hepatic and hematological indices. A total of 432 Shanma ducks at 15 weeks of age were randomly allocated to 6 groups with 6 replicates per group and 12 birds per replicate, and housed individually in cages. Following a 2-week feeding period with a basal diet containing no supplemental iron (52.2 mg/kg iron), ferrous sulfate monohydrate ($FeSO4 \cdot H2O$) was added to the basal diet at levels of 0, 15, 30, 45, 60, and 75 mg/kg, resulting in dietary iron concentrations of 52.2, 67.2, 82.2, 97.2, 112.2, and 127.2 mg/kg, respectively. The experimental period lasted 14 weeks. The results showed: 1) During the early laying phase (17–18 weeks of age), average egg weight exhibited a quadratic response to increasing dietary iron levels, with the 67.2, 82.2, and 97.2 mg/kg groups showing significantly greater average egg weight compared with the 112.2 mg/kg group ($P<0.05$). During the peak laying phase (19–30 weeks of age) and the overall experimental period (17–30 weeks of age), dietary iron level had no significant effects on daily feed intake, laying rate, average egg weight, daily egg mass, or feed-to-egg ratio ($P>0.05$). 2) Egg yolk color increased initially and then decreased with increasing dietary iron levels, with the 97.2 mg/kg group achieving the highest yolk color score. 3) Hepatic iron content exhibited an upward trend with increasing dietary iron levels, with the 127.2 mg/kg group showing significantly higher iron content than the 52.2 mg/kg group ($P<0.05$). 4) Hepatic catalase (CAT) activity increased initially and then decreased with increasing dietary iron levels, with the 67.2 mg/kg group exhibiting significantly higher CAT activity than the 97.2, 112.2, and 127.2 mg/kg groups ($P<0.05$). Dietary iron level had no significant effect on hepatic succinate dehydrogenase (SDH) activity ($P>0.05$). 5) Hemoglobin concentration and hematocrit increased with increasing dietary iron levels; the 97.2, 112.2, and 127.2 mg/kg groups had significantly higher hemoglobin concentration than the 52.2 mg/kg group (P<0.05), and the 112.2 and 127.2 mg/kg groups had significantly higher hematocrit than the 52.2 mg/kg group (P<0.05). 6) Using average egg weight as the response criterion, quadratic regression analysis yielded an optimal dietary iron level of 73.9 mg/kg for Shanma ducks during the early laying phase. Based on comprehensive evaluation, the recommended dietary iron level is 73.9 mg/kg for Shanma ducks during the early laying phase and 52.2 mg/kg during the peak laying phase.

Full Text

Effects of Dietary Iron on Laying Performance, Egg Quality, Liver and Blood Indices of Shanma Laying Ducks

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Abstract

This study investigated the effects of dietary iron levels on laying performance, egg quality, and liver and blood indices of Shanma laying ducks to determine their iron requirement during the laying period (17-30 weeks of age). A total of 432 healthy 15-week-old Shanma laying ducks were randomly allocated to six groups with six replicates of twelve birds each, housed individually in cages. After a 2-week feeding period with a basal diet containing no added iron (52.2 mg Fe/kg), the ducks were fed diets supplemented with 0, 15, 30, 45, 60, or 75 mg/kg of ferrous sulfate monohydrate ($FeSO_4 \cdot H_2O$), resulting in dietary iron levels of 52.2, 67.2, 82.2, 97.2, 112.2, and 127.2 mg/kg, respectively. The experiment lasted for 14 weeks. The results showed that: (1) During the early laying period (17-18 weeks), average egg weight initially increased and then decreased with increasing dietary iron levels, with the 67.2, 82.2, and 97.2 mg/kg groups exhibiting significantly higher average egg weight than the 112.2 mg/kg group (P<0.05). However, during the peak laying period (19-30 weeks) and the entire experimental period (17-30 weeks), dietary iron level had no significant effects on daily feed intake, egg production rate, average egg weight, daily egg mass, or feed-to-egg ratio (P>0.05). (2) Yolk color score increased initially and then decreased with increasing dietary iron levels, peaking at 97.2 mg/kg. (3) Liver iron content increased linearly with dietary iron levels, with the 127.2 mg/kg group showing significantly higher iron content than the 52.2 mg/kg group (P<0.05). (4) Liver catalase (CAT) activity increased initially and

then decreased with increasing dietary iron levels, with the 67.2 mg/kg group exhibiting significantly higher CAT activity than the 97.2, 112.2, and 127.2 mg/kg groups ($P<0.05$). Dietary iron level had no significant effect on liver succinate dehydrogenase (SDH) activity ($P>0.05$). (5) Hemoglobin concentration and hematocrit increased linearly with dietary iron levels; the 97.2, 112.2, and 127.2 mg/kg groups showed significantly higher hemoglobin concentration than the 52.2 mg/kg group ($P<0.05$), while the 112.2 and 127.2 mg/kg groups had significantly higher hematocrit than the 52.2 mg/kg group ($P<0.05$). (6) Using average egg weight as the criterion, quadratic regression analysis estimated the optimal dietary iron level for Shanma ducks during the early laying period to be 73.9 mg/kg. Based on comprehensive consideration of all parameters, the recommended dietary iron level is 73.9 mg/kg for early laying period and 52.2 mg/kg for peak laying period.

Key words: iron; laying ducks; laying performance; egg quality

Introduction

Iron is an essential trace element for normal physiological functions in animals, serving as a critical component of hemoglobin, myoglobin, cytochromes, and various oxidases. It plays vital roles in oxygen transport, exchange, and tissue oxidative metabolism. Approximately 20% of iron in the body is stored in the liver, spleen, and bone marrow as ferritin and hemosiderin, with additional iron present in the form of ferritin. In plant-based diets, iron primarily exists as inorganic iron from feed ingredients and mineral additives. The inorganic iron from feed ingredients is predominantly trivalent iron (Fe^{3+}), which readily forms insoluble complexes with phytic acid, oxalic acid, and phosphoric acid, limiting its bioavailability. Ferrous sulfate ($FeSO_4$) is commonly added to poultry diets due to its lower cost and higher absorption efficiency compared to Fe^{3+}. Both insufficient iron intake and impaired absorption can lead to iron deficiency anemia.

While animals can regulate iron homeostasis to some extent through ferroportin 1, which transports iron from cells into the bloodstream where transferrin distributes it to tissues for utilization or storage as ferritin, excess dietary iron is excreted in feces, representing a waste of resources. Therefore, determining the optimal Fe^{2+} supplementation level for optimal production performance is crucial for animal production. However, research on iron requirements for egg-laying poultry, particularly ducks, remains scarce. Song et al. reported that based on egg production rate, the appropriate iron supplementation level for laying hens fed corn-soybean meal diets during the laying period was 40–42 mg/kg. This study aimed to investigate the effects of dietary iron levels on laying performance, egg quality, iron content in yolk and liver, iron-containing enzyme activities in liver, and blood indices of laying ducks to establish their iron requirement and provide data support for diet formulation.

Materials and Methods

1.1 Experimental Animals and Management A total of 432 healthy 15-week-old Shanma laying ducks with normal feed intake and similar initial body weight (P>0.05) were randomly divided into six groups, each consisting of six replicates of twelve birds. All experimental ducks were individually housed in double-layer stainless steel galvanized cages (27.8 cm × 40.0 cm × 55.0 cm) throughout the trial. The ducks were fed a basal diet without iron supplementation (containing 52.2 mg/kg iron) for 2 weeks, after which they received diets supplemented with 0, 15, 30, 45, 60, or 75 mg/kg ferrous sulfate monohydrate ($FeSO_4 \cdot H_2O$) (calculated as Fe^{2+}), resulting in dietary iron levels of 52.2, 67.2, 82.2, 97.2, 112.2, and 127.2 mg/kg, respectively. The experimental period lasted 14 weeks. During the brooding and growing periods, ducks were vaccinated according to routine immunization procedures against duck viral hepatitis, infectious serositis, and avian influenza. Throughout the experiment, ducks had ad libitum access to purified water treated by iron removal equipment (iron content reduced from 5 mg/L to <0.3 mg/L) and feed. The lighting schedule was 16 h daily (intensity $ $15lx/m\hat{}\{2\}$$), and temperature, humidity, and weather conditions were recorded daily at 06:00, 12:00, and 18:00.

1.2 Experimental Design and Diet Composition A single-factor completely randomized design was employed. The basal diet was a corn-dried distillers grains with solubles (DDGS)-soy protein concentrate type, with nutrient levels determined based on previous studies from our research group. The composition and nutrient levels of the basal diet are presented in , with a measured iron content of 52.2 mg/kg.

1.3 Sample Collection and Analysis

1.3.1 Feed Sample Analysis Dietary iron content was determined using atomic absorption spectroscopy with a Z-2000 AAS (HITACHI, Japan) following modified acid digestion pretreatment according to Huang et al. Briefly, 1 g of sample was placed in a 50 mL beaker, mixed with 10 mL of acid solution (perchloric acid:nitric acid = 1:4, v/v), covered with a watch glass, digested at 120 °C for 1 h, then at 270 °C until clear and transparent. After evaporation to approximately 1 cm height, the solution was cooled to room temperature, diluted to 25 mL with ultrapure water, and mixed thoroughly, with corresponding blank controls prepared simultaneously.

1.3.2 Laying Performance and Egg Quality During the experiment, feed allowance was adjusted based on the previous day's intake to maintain consistent feed provision per replicate, with maximum feed offered while ensuring complete consumption. Feed intake was calculated by accurately recording feed offered and residual amounts. Egg production parameters including egg number and daily egg weight per replicate were recorded to determine average daily feed intake, egg production rate, average egg weight, daily egg mass, and feed-to-egg ratio.

Every four weeks, three eggs per replicate were collected for quality assessment within 48 h of laying. Parameters measured included shell weight, yolk weight, albumen weight, egg shape index, shell thickness, shell strength, yolk color, and Haugh unit, with mean values calculated for statistical analysis. Egg shape index was determined using vernier calipers (Shanghai-made 01120028) as the ratio of longitudinal to transverse diameter. Shell thickness was measured at the blunt end, middle, and sharp end using a digital micrometer (MODEL-1061) and averaged. Shell strength and yolk color were measured using an ORKA eggshell force gauge (EFR-01, Israel) and an automatic egg analyzer (EMT-5200, Israel), respectively.

1.3.3 Iron Content in Yolk and Liver At week 16, two yolk samples per replicate and liver samples from two slaughtered ducks per replicate were collected, freeze-dried at -80 °C, and ground into powder. Yolk and liver samples (2.5 g each) were defatted with ether overnight, air-dried in a fume hood, carbonized on an electric furnace, and ashed in a muffle furnace at 550 °C to constant weight. Iron content in the crude ash was determined by atomic absorption spectroscopy.

1.3.4 Liver Enzyme Activities At week 16, liver samples were collected from two randomly selected ducks per replicate, frozen at -80 °C, and analyzed for succinate dehydrogenase (SDH) and catalase (CAT) activities using commercial assay kits from Nanjing Jiancheng Bioengineering Institute.

1.3.5 Blood Indices At 10:00 during week 16, blood samples (10 mL) were collected from the wing vein of two randomly selected ducks per replicate (after 12 h fasting) using heparinized anticoagulant vacuum tubes. Five milliliters of whole blood were stored at 4 °C for hemoglobin concentration determination (using Nanjing Jiancheng Bioengineering Institute kits) and hematocrit measurement (using capillary tubes). The remaining 5 mL was centrifuged at 3,000 r/min for 20 min at 4 °C to obtain plasma, which was stored at -20 °C for plasma iron content and total iron-binding capacity (TIBC) analysis. Transferrin saturation was calculated as: Transferrin saturation (%) = (plasma iron content/TIBC) × 100.

1.4 Data Processing and Statistical Analysis Experimental data were analyzed using the GLM procedure of SAS 9.0 software for one-way ANOVA. When significant effects were detected, Student-Newman-Keuls multiple comparison tests were performed, with $P<0.05$ considered statistically significant. For the key sensitive indicator of average egg weight during the early laying period, quadratic regression analysis was performed using the REG procedure of SAS 9.0 according to Corzo et al. The quadratic equation ($Y = AX^2 + BX +$

C, where Y represents average egg weight, X represents dietary iron level, A and B are quadratic and linear coefficients, and C is the constant) was established, and the optimal dietary iron level was determined as 95% of the X-coordinate corresponding to the curve' s maximum point.

Results

2.1 Effects of Dietary Iron Level on Laying Performance As shown in , during the early laying period (17-18 weeks), dietary iron level had no significant effects on daily feed intake, egg production rate, daily egg mass, or feed-to-egg ratio ($P>0.05$). However, average egg weight increased initially and then decreased with increasing dietary iron levels, with the 67.2, 82.2, and 97.2 mg/kg groups showing significantly higher average egg weight than the 112.2 mg/kg group ($P<0.05$). During the peak laying period (19-30 weeks) and the entire experimental period (17-30 weeks), dietary iron level had no significant effects on any performance parameters ($P>0.05$).

2.2 Effects of Dietary Iron Level on Egg Quality As presented in , yolk color score increased initially and then decreased with increasing dietary iron levels, reaching the highest value at 97.2 mg/kg. Dietary iron level had no significant effects on other egg quality parameters ($P>0.05$).

2.3 Effects of Dietary Iron Level on Iron Content in Liver and Yolk As shown in , liver iron content increased linearly with dietary iron levels, with the 127.2 mg/kg group exhibiting significantly higher iron content than the 52.2 mg/kg group ($P<0.05$). Dietary iron level had no significant effect on yolk iron content ($P>0.05$).

2.4 Effects of Dietary Iron Level on Liver CAT and SDH Activities As presented in , liver CAT activity increased initially and then decreased with increasing dietary iron levels, with the 67.2 mg/kg group showing the highest activity. Dietary iron level had no significant effect on liver SDH activity ($P>0.05$).

2.5 Effects of Dietary Iron Level on Blood Indices As shown in , hemoglobin concentration and hematocrit increased linearly with dietary iron levels. The 97.2, 112.2, and 127.2 mg/kg groups had significantly higher hemoglobin concentration than the 52.2 mg/kg group ($P<0.05$), while the 112.2 and 127.2 mg/kg groups showed significantly higher hematocrit than the 52.2 mg/kg group ($P<0.05$). Dietary iron level had no significant effects on plasma iron content, TIBC, or transferrin saturation ($P>0.05$).

2.6 Estimation of Dietary Iron Requirement During the early laying period, average egg weight exhibited a significant quadratic response to dietary iron levels ($P<0.05$), while other performance indicators showed no significant quadratic relationships. Using average egg weight as the criterion, quadratic regression analysis estimated the optimal dietary iron level for laying ducks during the early laying period to be 73.9 mg/kg (). During the peak laying period, no significant quadratic relationships were observed between performance indicators or liver enzyme activities and dietary iron levels (P>0.05); therefore, the iron requirement was determined based on the lowest dietary iron level that met production needs.

Discussion

Previous studies have reported that dietary iron level has no significant effect on poultry production performance, with its primary influence observed in blood indices or tissue iron reserves. Ma (2012) found that supplementing corn-soybean meal diets with 0–160 mg/kg $FeSO_4 \cdot 7H_2O$ had no significant effects on feed intake or feed-to-gain ratio in 1-21 day-old broilers. Similarly, Ma (2014) observed no significant effects on feed intake, daily gain, or feed-to-gain ratio in 21–42 day-old broilers fed diets supplemented with 0–100 mg/kg $FeSO_4 \cdot 7H_2O$. To minimize interference from water iron content, this study provided purified water treated by iron removal equipment (iron content reduced from 5 mg/L to <0.3 mg/L). Under these conditions, dietary iron level had no significant effects on daily feed intake, egg production rate, daily egg mass, or feed-to-egg ratio during either the early or peak laying periods. Bess et al. reported that broiler breeder egg production was affected by iron source ($FeSO_4$ vs. amino acid chelate) rather than dietary composition, with production rate decreasing over time. Organic iron is generally considered more bioavailable than inorganic iron, resulting in different effects on performance. Ma (2012) demonstrated that organic protein iron showed positive linear relationships with daily gain and feed intake, and a negative correlation with feed-to-gain ratio in broilers. Ma et al. (2012) confirmed that glycine-chelated iron (0–160 mg/kg) linearly improved daily gain and feed intake in 22–42 day-old broilers, with the 160 mg/kg glycine iron group showing significantly higher daily gain than the 160 mg/kg $FeSO_4$ group. In this study, dietary iron levels of 67.2–97.2 mg/kg resulted in higher average egg weight during the early laying period but showed no significant differences during the peak laying period, suggesting that basal dietary iron (52.2 mg/kg) may be adequate for peak production, with additional supplementation providing no further benefit.

Yolk color score increased initially and then decreased with dietary iron levels, peaking at 97.2 mg/kg. Yolk color reflects pigment deposition, which is derived from dietary lipophilic pigments since poultry cannot synthesize pigments endogenously. Therefore, a dietary iron level of 97.2 mg/kg may enhance lipophilic pigment deposition in yolk.

Approximately 60% of body iron is incorporated into hemoglobin in red blood cells, 2–20% into myoglobin, with the remainder present in iron-containing enzymes (SDH, CAT, cytochrome c oxidase) and stored as ferritin and hemosiderin in liver and spleen. Previous studies have demonstrated significant positive correlations between dietary iron level and hemoglobin concentration. This study found that hemoglobin concentration, hematocrit, and liver iron content increased linearly with dietary iron levels, indicating these parameters are sensitive indicators of dietary iron intake in laying ducks. Liver CAT activity increased initially and then decreased, with peak activity at 67.2 mg/kg, consistent with findings in 1-21 day-old broilers. Iron in egg yolk is tightly bound to phosvitin, similar to other divalent metals. Since vitellogenin and very low-density lipoproteins are synthesized in the liver, hepatic iron reserves could theoretically be transferred to yolk via yolk precursors. However, this study found no significant effect of dietary iron level on yolk iron content. Previous research has shown that egg iron content is less responsive to dietary manipulation compared to other trace elements, though some studies have reported increased yolk iron with organic iron supplementation. The relationship between hepatic iron storage and yolk iron deposition may depend on the quantity and form of iron stored in the liver, warranting further investigation.

Conclusion

Based on comprehensive evaluation of laying performance, egg quality, liver and blood indices, and production costs, the recommended dietary iron level is 73.9 mg/kg for early laying period and 52.2 mg/kg for peak laying period in Shanma ducks.

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