Effects of Iron on Production Performance, Hematopoietic Function, and Iron Metabolism in 1- to 4-Week-Old Wulong Geese (Postprint)

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Abstract

This study aimed to investigate the effects of dietary iron supplementation levels on growth performance, hematopoietic function, and iron metabolism in 1- to 4-week-old Wulong geese, in order to determine the appropriate dietary iron supplementation level for geese. A total of 360 1-day-old Wulong geese were randomly allocated to 6 groups, with 6 replicates per group and 10 geese per replicate. Group I served as the control group and was fed the basal diet, while groups II-VI were fed experimental diets supplemented with 40, 80, 120, 160, and 200 mg/kg iron to the basal diet, respectively. The experimental period lasted 4 weeks. The results showed that, compared with the control group: 1) Dietary supplementation with 80-120 mg/kg iron significantly or extremely significantly increased body weight and average daily gain of Wulong geese ($P<0.05$ or $P<0.01$), and significantly decreased feed-to-gain ratio ($P<0.05$); 2) Dietary supplementation with 80-120 mg/kg iron significantly increased dressing percentage, semi-eviscerated yield, and leg muscle percentage of geese ($P<0.05$); 3) Dietary supplementation with 80 mg/kg iron significantly increased mean corpuscular volume and mean corpuscular hemoglobin content in goose blood ($P<0.05$), dietary supplementation with 80-200 mg/kg iron significantly increased red blood cell count and hemoglobin concentration in goose blood ($P<0.05$), and dietary supplementation with 120-200 mg/kg iron significantly or extremely significantly increased hematocrit in goose blood ($P<0.05$ or $P<0.01$); 4) Dietary supplementation with 160-200 mg/kg iron significantly decreased total iron binding capacity in goose serum ($P<0.05$) and significantly increased transferrin saturation in goose serum ($P<0.05$); dietary supplementation with 120 mg/kg iron significantly decreased urea nitrogen and creatinine concentrations in goose serum ($P<0.05$), and dietary supplementation with 80 mg/kg iron significantly decreased uric acid concentration in goose serum ($P<0.05$). Therefore, with optimal growth performance as the objective, the recommended

appropriate dietary iron supplementation level for 1- to 4-week-old Wulong geese is 99.56-116.91 mg/kg.

Full Text

Effects of Iron on Performance, Hematopoietic Function and Iron Metabolism of Wulong Geese Aged from 1 to 4 Weeks

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Abstract: This experiment was conducted to investigate the effects of dietary iron supplementation levels on the performance, hematopoietic function, and iron metabolism of 1- to 4-week-old Wulong geese, and to determine the appropriate dietary iron supplementation level. A total of 360 one-day-old Wulong geese were randomly allocated into 6 groups with 6 replicates per group and 10 geese per replicate. Group I served as the control group and was fed a basal diet, while groups II–VI were fed the basal diet supplemented with 40, 80, 120, 160, and 200 mg/kg iron, respectively. The experimental period lasted 4 weeks. The results showed that, compared with the control group: (1) dietary supplementation with 80–120 mg/kg iron significantly or extremely significantly increased body weight and average daily gain ($P<0.05$ or $P<0.01$), and significantly decreased feed-to-gain ratio ($P<0.05$); (2) dietary supplementation with 80–120 mg/kg iron significantly increased dressed percentage, half-eviscerated yield percentage, and leg muscle percentage ($P<0.05$); (3) dietary supplementation with 80 mg/kg iron significantly increased mean corpuscular volume and mean corpuscular hemoglobin content ($P<0.05$), supplementation with 80–200 mg/kg iron significantly increased erythrocyte count and hemoglobin concentration ($P<0.05$), and supplementation with 120–200 mg/kg iron significantly or extremely significantly increased hematocrit ($P<0.05$ or $P<0.01$); and (4) dietary supplementation with 160–200 mg/kg iron significantly decreased total iron binding capacity ($P<0.05$) and significantly increased transferrin saturation ($P<0.05$) in serum, while supplementation with 120 mg/kg iron significantly decreased serum urea nitrogen and creatinine contents ($P<0.05$), and supplementation with 80 mg/kg iron significantly decreased serum uric acid content ($P<0.05$). In conclusion, targeting optimal growth performance, the appropriate dietary iron supplementation level for 1- to 4-week-old Wulong geese is recommended to be 99.56–116.91 mg/kg.

Keywords: iron; geese; performance; hematopoietic function; iron metabolism

1.1 Experimental Animals and Design

A total of 360 healthy one-day-old Wulong geese (Huoyan geese) were selected and randomly divided into 6 groups using a random allocation numbering method, with 6 replicates per group and 10 geese per replicate (half male and half female). Group I was the control group fed the basal diet, while groups II-VI were fed the basal diet supplemented with 40, 80, 120, 160, and 200 mg/kg iron, respectively. The experimental period lasted 4 weeks. The experimental geese were provided by Gaomi Yinhe Runyan Goose Industry Co., Ltd., a breeding base of the China Agriculture Research System for waterfowl. The iron source used was ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) purchased from Qingdao Puxing Biotechnology Co., Ltd. (with an active ingredient content of 91.4%).

1.2 Experimental Diets

The nutrient levels of the basal diet were formulated according to the NRC (1994) poultry nutrient requirements. The composition and nutrient levels of the basal diet are presented in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis) %

Ingredients	Content
Corn	
Soybean meal	
Fish meal	
Wheat middling	
Corn straw	
$CaHPO_4$	
Limestone	
NaCl	
Trace mineral premix[1]	
Vitamin premix[1]	
Total	

Nutrient levels
Metabolizable energy (ME, MJ/kg)
Crude protein (CP)
Calcium (Ca)
Available phosphorus (AP)
Lysine (Lys)
Methionine (Met)
Cystine (Cys)
Methionine + Cystine (Met+Cys)
Iron (Fe, mg/kg)[2]

[1]The trace mineral premix and vitamin premix provided the following per kg of diet: VA 1,500 IU, VD_3 200 IU, VE 12.5 mg, VK_3 1.5 mg, VB_2 5.0 mg, nicotinic acid 65 mg, pantothenate 15 mg, biotin 0.2 mg, folic acid 0.5 mg, choline 1,000 mg, Cu 6 mg, Mn 85 mg, Zn 85 mg, I 0.42 mg, Se 0.3 mg, Co 2.5 mg.
[2]Iron was a measured value, while the other nutrient levels were calculated values.

1.3 Feeding Management

Before the experiment, the goose house was thoroughly disinfected. Throughout the experimental period, geese were raised indoors in net pens with free access to water and feed, following a feeding regimen of small amounts provided frequently. The growth status of the flocks was monitored closely.

1.4 Performance Indices

1.4.1 Growth Performance Indices At the end of the 4-week period, experimental geese were weighed after fasting to calculate the average daily gain (ADG) for weeks 1–4. Daily feed consumption was recorded to calculate average daily feed intake (ADFI) and feed-to-gain ratio (F/G). Mortality and culling rates were recorded daily.

1.4.2 Slaughter Performance Indices At the end of week 4, 2 geese (half male and half female) with body weights close to the group average were selected from each replicate, totaling 72 geese, for slaughter analysis. After 12 hours of fasting, slaughter weight, half-eviscerated weight, fully eviscerated weight, abdominal fat weight, breast muscle weight, and leg muscle weight were measured according to the "Poultry Production Performance Terminology and Measurement Methods" (NY/T 823—2004). Six slaughter performance indices were calculated: dressed percentage, fully eviscerated yield percentage, half-eviscerated yield percentage, abdominal fat percentage, leg muscle percentage, and breast muscle percentage.

1.4.3 Hematological Indices At 4 weeks of age, 2 geese (half male and half female) with body weights close to the group average were selected from each replicate, totaling 72 geese. After weighing, blood samples were collected via cardiac puncture using heparinized tubes and stainless steel needles. Whole blood was stored at 4°C for measurement of erythrocyte count, hemoglobin concentration, and hematocrit using an automatic biochemical analyzer (Hitachi 7600-020). Plasma was obtained by centrifugation at 3,000 r/min and stored at -20°C for determination of ferritin, total iron binding capacity, transferrin saturation, unsaturated iron binding capacity, urea, creatinine, and uric acid.

1.5 Statistical Analysis

Data were analyzed using one-way ANOVA with LSD multiple comparisons in SPSS 17.0 software. Results are expressed as "mean ± standard deviation." Orthogonal polynomial contrasts were used to analyze linear or curvilinear responses of various indices to dietary iron supplementation levels. Curve fitting was performed to determine the appropriate dietary iron supplementation level for 1- to 4-week-old geese. Correlation analysis was conducted between growth performance and nutrient utilization rates. $P<0.05$ and $P<0.01$ were considered significant and extremely significant, respectively.

2.1 Effects of Dietary Iron Supplemental Level on Growth Performance of Geese

As shown in Table 2 , during weeks 1–4, the body weights of groups III and IV were significantly higher than that of the control group ($P<0.05$). The average daily gain of group IV was extremely significantly higher than that of the control group ($P<0.01$), while groups II, III, and IV showed significantly higher average daily gain than the control group ($P<0.05$). The feed-to-gain ratio of groups III and IV was significantly lower than that of the control group ($P<0.05$). Groups III and IV exhibited the highest body weight and average daily gain, and the lowest feed-to-gain ratio, with no significant differences between these two groups ($P>0.05$). No significant differences were observed in average daily feed intake or mortality rate among all groups ($P>0.05$). These results indicate that iron supplementation improved body weight, average daily gain, and feed-to-gain ratio compared with the control group.

Curve fitting between body weight (Y_1), feed-to-gain ratio (Y_2) and dietary iron supplementation level (X) yielded the following equations:

$Y_1 = 1.045 + 0.005X - 2.511\times10^{-5}X^{2}$ ($R^2 = 0.788$, P = 0.098)
$Y_2 = 2.299 - 0.003X + 1.283\times10^{-5}X^{2}$ ($R^2 = 0.804$, P = 0.086)

Based on these regression equations, the maximum body weight was achieved at a dietary iron supplementation level of 99.56 mg/kg, while the optimal feed-to-gain ratio occurred at 116.91 mg/kg.

Table 2 Effects of Fe on growth performance of geese aged from 1 to 4 weeks

Groups	Body weight (kg)	ADG (g)	ADFI (g)	F/G	Mortality rate (%)	P-value
I	1.08 ± 0.03^b	38.09 ± 0.12^c	88.36 ± 1.24	2.32 ± 0.04^a	2.18 ± 0.06^{ab}	
II	1.13 ± 0.03^b	39.45 ± 0.23^b	85.86 ± 2.49			

In the same column, values with the same small letter or no letter superscripts mean no significant difference ($P>0.05$), while adjacent small letter superscripts indicate significant difference ($P<0.05$), and alternate small letter superscripts indicate extremely significant difference ($P<0.01$). The same applies below.

2.2 Effects of Dietary Iron Supplemental Level on Slaughter Performance of Geese

As shown in Table 3 , during weeks 1-4, the dressed percentage, half-eviscerated yield percentage, and fully eviscerated yield percentage of Wulong geese showed a trend of initially increasing and then decreasing with increasing dietary iron supplementation levels. Groups III and IV exhibited significantly higher dressed percentage, half-eviscerated yield percentage, and leg muscle percentage compared with the control group (P<0.05), while group III showed significantly higher fully eviscerated yield percentage (P<0.05). No significant differences were observed in abdominal fat percentage or breast muscle percentage among all groups (P>0.05).

These results indicate that dietary iron supplementation at 80–120 mg/kg significantly improved dressed percentage, half-eviscerated yield percentage, and leg muscle percentage, but had no significant effect on abdominal fat percentage or breast muscle percentage.

Table 3 Effects of Fe on slaughter performance of geese aged from 1 to 4 weeks (%)

Groups	Dressed percentage	Half-eviscerated yield percentage	Fully eviscerated yield percentage	Abdominal fat percentage	Breast muscle percentage	Leg muscle percentage	P-value
I	85.49$±1.02^{b}	75.76±0.52^{b}	63.34±1.31^{b}	1.19±0.12	14.58±0.46^{b}		
II	87.27±0.78^{ab}	77.17±0.63^{ab}	65.00±0.7				

2.3 Effects of Dietary Iron Supplemental Level on Hematopoietic Function of Geese

As shown in Table 4 , during weeks 1-4, groups III, IV, V, and VI exhibited significantly or extremely significantly higher hemoglobin concentration compared with the control group (P<0.05 or P<0.01). These groups also showed significantly higher erythrocyte counts (P<0.05). Groups IV, V, and VI demonstrated significantly or extremely significantly higher hematocrit (P<0.05 or P<0.01), while group III showed significantly higher mean corpuscular volume and mean corpuscular hemoglobin content (P<0.05).

These results demonstrate that iron supplementation improved hemoglobin concentration, erythrocyte count, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin content compared with the control group. Dietary iron supplementation at 80 mg/kg significantly increased mean corpuscular volume and mean corpuscular hemoglobin content; levels exceeding 80 mg/kg significantly increased erythrocyte count and hemoglobin concentration; and levels exceeding 120 mg/kg significantly increased hematocrit. This indicates that different dietary iron supplementation levels significantly affect hematopoietic function in geese.

Table 4 Effects of Fe on hematopoietic function of geese aged from 1 to 4 weeks

Groups | HGB (g/L) | RBC ($×10^{12}$/L) | HCT (%) | MCV (fL) | MCH (pg) | P-value |

|—-|——-|————|——|——-|——-|——| | I | 131.33$±3.51^c$|1.51±0.02^b|25.53±0.59^c|154.83±1.33^b|76.10±0.17^b
| |

2.4 Effects of Dietary Iron Supplemental Level on Iron Metabolism of Geese

As shown in Table 5 , during weeks 1-4, groups V and VI exhibited significantly lower total iron binding capacity compared with the control group (P<0.05), while showing significantly higher transferrin saturation (P<0.05). Group IV demonstrated significantly lower serum urea nitrogen and creatinine contents (P<0.05), and group III showed significantly higher serum uric acid content (P<0.05). No significant differences were observed in serum iron content among groups (P>0.05), although a trend of initial increase followed by decrease was noted. Unsaturated iron binding capacity also showed no significant differences among groups (P>0.05), with an overall decreasing trend.

These results indicate that iron supplementation decreased total iron binding capacity, unsaturated iron binding capacity, and contents of urea nitrogen, creatinine, and uric acid, while increasing serum iron content and transferrin saturation compared with the control group. Dietary iron supplementation at 80 mg/kg significantly decreased serum uric acid content; at 120 mg/kg significantly decreased serum urea nitrogen and creatinine contents; and exceeding 160 mg/kg significantly decreased total iron binding capacity while increasing transferrin saturation, with no significant differences among supplementation groups. This demonstrates that dietary iron supplementation level significantly affects iron metabolism in geese.

Table 5 Effects of Fe on iron metabolism of geese aged from 1 to 4 weeks

Groups	TIBC (mol/L)	SI (mol/L)	UIBC (mol/L)	UN (mmol/L)	CR (mol/L)	UA (mol/L)	P-value
I	65.50$±5.27^a$	25.83±10.18	45.00±6.25^b	0.37±0.12^a	38.47±0.64^a	177.00±20.33^a	||II|59.63±7.78^{ab}|30.50

3.1 Effects of Dietary Iron Supplemental Level on Growth Performance of Geese

Iron, as one of the essential mineral elements in animals, plays a crucial role in maintaining normal growth, metabolism, and reproduction. Iron content in animals directly affects phosphorus content, thereby influencing DNA synthesis in cells, and regulates mitochondria and microsomes in cells through iron-containing enzymes, affecting protein synthesis. Iron deficiency can damage erythrocyte volume, shape, and structure, leading to iron deficiency anemia and affecting animal growth and development. Excessive iron intake can induce oxygen free radical production, oxidize proteins and DNA, poison hematopoietic tissue cells, and cause aplastic anemia in severe cases [7-10]. Vahl et al. [11] supplemented corn-soybean meal diets for 1- to 39-day-old Hybro broilers with 0, 20, and 60 mg/kg ferrous sulfate ($FeSO_4$) and found that broiler weight gain increased with increasing iron supplementation. Lin et al. [12] added 0, 150, and 350 g/t compound Fuli iron to growing pig basal diets and observed significantly increased average daily gain and decreased feed-to-gain ratio. The present study demonstrated that iron supplementation to the basal diet increased body weight and average daily gain while decreasing feed-to-gain ratio in Wulong geese, with maximum body weight achieved at 99.56 mg/kg and optimal feed-to-gain ratio at 116.91 mg/kg.

3.2 Effects of Dietary Iron Supplemental Level on Slaughter Performance of Geese

Slaughter performance reflects poultry meat production performance and serves as an important basis for evaluating growth performance and slaughter processing benefits. Wang et al. [13] reported that live body weight affects slaughter meat yield in broilers, and appropriate dietary iron supplementation increases growth rate and correspondingly improves slaughter performance. The current results showed that dressed percentage, half-eviscerated yield percentage, and fully eviscerated yield percentage of 1- to 4-week-old Wulong geese initially increased and then decreased with increasing dietary iron supplementation levels. Dietary iron supplementation at 80–120 mg/kg significantly improved dressed percentage, half-eviscerated yield percentage, and leg muscle percentage, but had no significant effect on abdominal fat percentage or breast muscle percentage.

3.3 Effects of Dietary Iron Supplemental Level on Hematopoietic Function of Geese

Changes in blood and serum biochemical indices typically indicate alterations in animal physiological functions, and pathological changes in any formed elements of blood affect tissues and organs throughout the body, thereby modifying various physiological functions. Erythrocyte count, hemoglobin concentration, and hematocrit are important indicators reflecting iron metabolism status and nutritional condition in animals [14,3]. The present study demonstrated that iron supplementation to the basal diet increased hemoglobin concentration, erythrocyte count, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin content in Wulong geese. Supplementation at 80 mg/kg significantly increased mean corpuscular volume and mean corpuscular hemoglobin content; levels of 120–200 mg/kg significantly increased hematocrit; and levels of 80–200 mg/kg significantly increased erythrocyte count and hemoglobin concentration, with no significant differences among supplementation groups. This may be because iron supplementation not only directly promotes hemoglobin and erythrocyte synthesis but also indirectly stimulates erythrocyte synthesis by improving kidney function and erythropoietin production, thereby enhancing hematopoietic function in geese. This indicates that iron is closely related to hematopoietic function in geese, and excessive iron supplementation has no biological significance, while hematopoietic function indices cannot sensitively reflect the iron nutritional requirement level in geese.

3.4 Effects of Dietary Iron Supplemental Level on Iron Metabolism in Geese

Serum iron content and serum ferritin content are important indicators reflecting iron metabolism status, growth and development status, and metabolic condition [15-16]. Serum total iron binding capacity refers to the maximum amount of iron that can bind to all transferrin in 100 mL of serum. Serum iron content can determine the iron nutritional status of animals and more accurately reflects total iron stores in the liver and body. Insufficient serum iron leads to low transferrin saturation, causing inadequate iron supply to hematopoietic tissues and anemia [17-18]. The present study showed that iron supplementation to the basal diet decreased total iron binding capacity and unsaturated iron binding capacity while increasing serum iron and transferrin saturation in Wulong geese. Dietary iron supplementation at 160–200 mg/kg significantly decreased total iron binding capacity and significantly increased transferrin saturation, with no significant differences among supplementation groups, indicating that different dietary iron supplementation levels are closely related to iron metabolism in geese.

Blood non-protein nitrogen includes urea nitrogen, creatinine, and uric acid, which are end products of protein, purine, and creatine metabolism, respectively, and are excreted through the kidneys, directly reflecting kidney function [19-20]. Purine is a substance present in animal bodies, primarily in the form of purine nucleotides, and plays important roles in energy supply, metabolic regulation, and coenzyme composition. Failure to metabolize and excrete purine through the kidneys leads to uric acid formation and crystalline deposition in the body, causing renal failure. Clinical studies have shown that oral iron supplementation can effectively improve kidney function [21]. The present results demonstrated that dietary iron supplementation decreased serum urea nitrogen, creatinine, and uric acid contents in Wulong geese. Supplementation at 120 mg/kg significantly decreased serum urea nitrogen and creatinine contents, while 80 mg/kg significantly decreased serum uric acid content, with no significant differences among supplementation groups. This indicates that appropriate dietary iron supplementation can improve kidney function in geese and indirectly promote protein and purine metabolism.

Insufficient serum iron causes low transferrin saturation, leading to inadequate iron supply to hematopoietic tissues and anemia, which is an important complication of renal failure. The changing trend of serum iron in this study was consistent with that of urea nitrogen, creatinine, and uric acid contents, indicating a close relationship between iron metabolism and kidney function, consistent with the findings of Meara et al. [22].

In summary, since dietary iron content is relatively abundant and can basically meet the maintenance nutritional requirements of geese, with low sensitivity to iron, iron deficiency does not occur even without iron supplementation in the diet. During feed additive production, the iron content of the basal diet is generally ignored, and the nutrient requirement is used as the supplementation level, which is the main reason for the large variation in iron requirements for geese reported internationally. The present study demonstrates that appropriate iron supplementation based on dietary iron content significantly improves production performance, slaughter performance, and iron metabolism, and that the appropriate range of iron nutritional requirement for geese is much wider than that of other trace elements (such as selenium). Therefore, comprehensively considering maintenance nutritional requirements and optimal production performance requirements to find an economic balance point for supplementation is important for meeting the nutritional needs of geese at different physiological stages, fully expressing their genetic potential, and achieving optimal economic benefits.

Conclusion

1. Appropriate dietary iron supplementation significantly increased body weight and average daily gain while decreasing feed-to-gain ratio, and significantly improved dressed percentage, half-eviscerated yield percentage, and leg muscle percentage in Wulong geese.
2. Appropriate dietary iron supplementation significantly increased mean corpuscular volume and mean corpuscular hemoglobin content, significantly increased hematocrit, and significantly increased erythrocyte count and hemoglobin concentration in geese.
3. Appropriate dietary iron supplementation significantly decreased serum total iron binding capacity, significantly increased serum transferrin saturation, and significantly reduced serum urea nitrogen, creatinine, and uric acid contents ($P<0.05$).
4. Targeting optimal growth performance, the appropriate dietary iron supplementation level for 1- to 4-week-old Wulong geese is recommended to be 99.56–116.91 mg/kg.

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