Effects of Bacillus subtilis Supplementation in Diets with Different Iron Supplementation Levels on Growth Performance, Hematopoietic Function, Iron Metabolism, and Kidney Function in Goslings (Postprint)

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Abstract

This experiment aimed to investigate the effects of Bacillus subtilis supplementation in diets with different iron addition levels on growth performance, hematopoietic function, iron metabolism, and kidney function in goslings. A total of 360 1-day-old Wulong geese were selected and randomly divided into 6 groups with 6 replicates per group and 10 geese per replicate. Group I was the control group, receiving 100 mg/kg iron in the basal diet without Bacillus subtilis; Groups II-VI were experimental groups, receiving 0, 25, 50, 75, and 100 mg/kg iron respectively in the basal diet, with Bacillus subtilis supplementation at 250 mg/kg for all groups. The experimental period lasted 4 weeks. The results showed: 1) Compared with Group I, Group VI goslings exhibited increased body weight, average daily gain, dressing percentage, semi-eviscerated yield, eviscerated yield, breast muscle percentage, leg muscle percentage, blood hemoglobin content, hematocrit, mean corpuscular volume, serum iron content, total iron binding capacity, and transferrin saturation ($P>0.05$), and decreased average daily feed intake, feed conversion ratio, abdominal fat percentage, and serum uric acid, urea nitrogen, and creatinine content ($P>0.05$). 2) The body weight and average daily gain of Groups IV and V were significantly or extremely significantly higher than those of Groups II, III, and VI ($P<0.05$ or $P<0.01$), and the feed conversion ratio of Group IV was significantly or extremely significantly lower than that of Groups II, III, and VI ($P<0.05$ or $P<0.01$). 3) The eviscerated yield of Groups IV and V was significantly higher than that of Group II ($P<0.05$); the breast muscle percentage of Group IV was significantly or extremely significantly higher than that of Groups II and III ($P<0.05$ or $P<0.01$), and the breast muscle percentage of Group V was significantly higher than that of Group II (P<0.05). 4) The blood hemoglobin content of Groups V and VI was significantly or extremely significantly higher than that of Groups II, III, and IV (P<0.05 or P<0.01), the blood red blood cell count of Group V was significantly or extremely significantly higher than that of Groups II, III, and IV (P<0.05 or P<0.01), the blood hematocrit of Group V was significantly or extremely significantly higher than that of Groups II, III, IV, and VI (P<0.05 or P<0.01), the blood mean corpuscular volume of Group IV was significantly or extremely significantly higher than that of Groups II, III, V, and VI (P<0.05 or P<0.01), and the blood mean corpuscular hemoglobin of Group V was significantly or extremely significantly higher than that of Groups II, III, IV, and VI (P<0.05 or P<0.01). 5) The serum total iron binding capacity of Groups IV and V was significantly higher than that of Groups III and VI (P<0.05), and the serum uric acid content of Group IV was significantly or extremely significantly lower than that of Groups II and VI (P<0.05 or P<0.01). These results indicate that the iron nutritional requirement of goslings has a certain threshold, and excessively high or low dietary iron addition levels are not conducive to growth and development. Bacillus subtilis can synergistically promote growth performance, hematopoietic function, iron metabolism, and kidney function in goslings with iron, and reduce dietary iron addition levels; it is recommended that under the condition of adding 250 mg/kg Bacillus subtilis to the basal diet of goslings, the appropriate iron addition level is 60.34-80.50 mg/kg.

Full Text

Effects of Bacillus subtilis Supplementation in Different Iron Supplemental Level Diets on Growth Performance, Hematopoietic Function, Iron Metabolism and Kidney Function of Goslings

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Abstract

This study investigated the effects of Bacillus subtilis supplementation in diets with varying iron levels on growth performance, hematopoietic function, iron metabolism, and kidney function in goslings. A total of 360 one-day-old Wulong geese were randomly allocated into 6 groups, each consisting of 6 replicates with 10 goslings per replicate. Group served as the control, receiving a basal diet supplemented with 100 mg/kg iron without Bacillus subtilis. Groups through were experimental groups receiving the basal diet supplemented with 0, 25, 50, 75, and 100 mg/kg iron, respectively, all with 250 mg/kg Bacillus subtilis. The experiment lasted for 4 weeks.

The results showed: 1) Compared with group , group exhibited increased body weight, average daily gain, slaughter rate, half-eviscerated rate, all-eviscerated rate, chest muscle rate, leg muscle rate, blood hemoglobin content, hematocrit, mean corpuscular volume, serum iron content, total iron binding capacity, and transferrin saturation (P>0.05), while average daily feed intake, feed-to-gain ratio, abdominal fat rate, and serum uric acid, urea nitrogen, and creatinine contents decreased (P>0.05). 2) The body weight and average daily gain of groups and were significantly or extremely significantly higher than those of groups , , and (P<0.05 or P<0.01), while the feed-to-gain ratio of group was significantly or extremely significantly lower than that of groups , , and (P<0.05 or P<0.01). 3) The all-eviscerated rate of groups and was significantly higher than that of group (P<0.05); the chest muscle rate of group was significantly or extremely significantly higher than that of groups and (P<0.05 or P<0.01), and the chest muscle rate of group was significantly higher than that of group (P<0.05). 4) The blood hemoglobin content of groups and was significantly or extremely significantly higher than that of groups , , and (P<0.05 or P<0.01). The blood red blood cell count of group was significantly or extremely significantly higher than that of groups , , and (P<0.05 or P<0.01). The blood hematocrit of group was significantly or extremely significantly higher than that of groups , , , and (P<0.05 or P<0.01). The blood mean corpuscular volume of group was significantly or extremely significantly higher than that of groups , , , and (P<0.05 or P<0.01). The blood mean corpuscular hemoglobin of group was significantly or extremely significantly higher than that of groups , , , and (P<0.05 or P<0.01). 5) The serum total iron binding capacity of groups and was significantly higher than that of groups and (P<0.05), while the serum uric acid content of group was significantly or extremely significantly lower than that of groups and (P<0.05 or P<0.01).

These findings indicate that the iron nutritional requirement of goslings has a specific threshold, as both excessive and insufficient dietary iron levels are detrimental to growth and development. Bacillus subtilis can synergistically interact with iron to promote growth, hematopoietic function, iron metabolism, and kidney function while reducing dietary iron requirements. It is recommended that under conditions of 250 mg/kg Bacillus subtilis supplementation, the appropriate dietary iron level for goslings is 60.34–80.50 mg/kg.

Keywords: iron; Bacillus subtilis; goslings; growth performance; hematopoietic function; iron metabolism; kidney function

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Iron is an essential mineral element in animals that plays a crucial role in normal growth and development by participating in the synthesis of various functional proteins and iron-containing enzymes, as well as hematopoietic function [1-3]. Iron deficiency damages red blood cell volume, shape, and structure, ultimately leading to iron deficiency anemia, while iron excess primarily causes oxidative damage [4-5]. With the rapid development of animal production in China, environmental pollution from livestock operations has become increasingly severe and attracted societal attention. Heavy metal pollution represents one of the most prominent issues in soil contamination, as heavy metals accumulate and transform in biological organisms after absorption, posing potential threats to human and animal health. Due to the cumulative, irreversible, and long-term consequences of heavy metal pollution, research in this field has remained a hotspot in environmental and ecological sciences both domestically and internationally [6]. Iron is a significant pollutant among heavy metals, necessitating more in-depth research on technologies to reduce dietary iron supplementation levels.

The nutritional status of trace elements, particularly iron, directly affects animal growth and health. The growth-promoting effects of iron in livestock and poultry have been confirmed in numerous studies. Appropriate iron supplementation can improve growth rate and feed utilization in pigs [7-8]. Iron deficiency reduces growth performance in broiler chickens [9], while increasing dietary iron levels (0–50 mg/kg) enhances weight gain and feed intake [10]. However, high iron levels (400–800 mg/kg) significantly decrease body weight and feed intake in broilers, indicating impaired growth performance [11].

As a probiotic, Bacillus subtilis effectively improves intestinal environment and promotes growth. Studies have confirmed that Bacillus subtilis can inhibit Clostridium perfringens-induced necrotic enteritis in broilers and improve intestinal health [12], significantly increase feed intake, feed conversion ratio, and egg production in laying hens [13], and substantially improve growth performance in broilers [14]. Additionally, Bacillus subtilis strains possess strong metal adsorption capacity, as metal ions can be immobilized through interaction with anions on the bacterial cell surface [15]. Previous research by Ma Chuanxing et al. [16] in our laboratory investigated iron requirements in Wulong goslings, determining that the appropriate supplementation level for 1-4-week-old goslings was 99.56 mg/kg, which differs from parameters proposed by other countries and warrants further verification. Research on appropriate iron supplementation levels in gosling diets containing Bacillus subtilis remains unexplored. Therefore, this study used Wulong geese to investigate the effects of Bacillus subtilis supplementation in diets with varying iron levels on growth performance, hematopoietic function, iron metabolism, and kidney function, aiming to explore the synergistic relationship between iron and Bacillus subtilis and the feasibility of reducing iron supplementation in poultry diets.

1.1 Experimental Animals and Design

A total of 360 healthy one-day-old Wulong geese were randomly divided into 6 groups using a random allocation numbering method, with 6 replicates per group and 10 goslings per replicate (half male and half female). The experimental design is shown in Table 1 . Group was the control group, receiving a basal diet supplemented with 100 mg/kg iron without Bacillus subtilis. Groups through were experimental groups receiving the basal diet supplemented with 0, 25, 50, 75, and 100 mg/kg iron, respectively, all containing 250 mg/kg Bacillus subtilis. The experiment lasted for 4 weeks. The experimental goslings were provided by Gaomi City Yinhe Runyan Goose Industry Co., Ltd., a breeding base of the National Waterfowl Industry Technical System. The iron source used was ferrous sulfate monohydrate (FeSO ·H O) with 91.4% active ingredient. The Bacillus subtilis was a freeze-dried powder with viable bacteria count of 2×10 CFU/kg, both purchased from Qingdao Puxing Biological Technology Co., Ltd.

1.2 Experimental Diets

The basal diet was formulated according to NRC (1994) poultry nutrient requirements. The composition and nutrient levels of the basal diet are shown in Table 2 .

Table 2 Composition and Nutrient Levels of the Basal Diet (Air-Dry Basis)

Note: 1) Multivitamin and trace element provided the following per kg of diet: VA 1,500 IU, VD 200 IU, VE 12.5 mg, VK 1.5 mg, VB 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 other nutrient levels were calculated values.

1.3 Management

Before the experiment, the goose house was thoroughly disinfected. Throughout the experiment, goslings were raised indoors in net-floor pens with separate sections. They had free access to water and feed, with feed provided in small amounts frequently. The growth status of the flocks was monitored closely.

1.4 Measurements

1.4.1 Growth Performance Indices At the end of week 4, goslings were weighed after fasting to calculate average daily gain (ADG). Daily feed consumption was recorded to calculate average daily feed intake (ADFI) and feed-to-gain ratio (F/G). Mortality and culling were recorded daily to calculate mortality rate (MR).

1.4.2 Slaughter Performance Indices At the end of week 4, two goslings (one male and one female) with body weight close to the group average were selected from each replicate, totaling 36 goslings per group. After 12 hours of fasting, they were slaughtered. Carcass weight, half-eviscerated weight, all-eviscerated weight, abdominal fat weight, chest muscle weight, and leg muscle weight were measured according to "Poultry Production Performance Terminology and Measurement Methods" (NY/T 823—2004). Slaughter rate (SR), half-eviscerated rate (HER), all-eviscerated rate (AER), abdominal fat rate (AFR), chest muscle rate (CMR), and leg muscle rate (LMR) were calculated.

1.4.3 Blood and Serum Biochemical Indices At week 4, two goslings (one male and one female) with body weight close to the group average were selected from each replicate, totaling 36 goslings per group. After weighing, blood was collected via cardiac puncture using heparinized tubes and stainless steel needles. Whole blood was stored at 4°C and analyzed using an automatic biochemical analyzer (Hitachi 7600-020) for hemoglobin (HGB) content, red blood cell (RBC) count, hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). Serum was obtained by centrifugation at 3,000 r/min and stored at -20°C for determination of total iron binding capacity (TIBC), transferrin saturation (TS), and contents of iron, uric acid (UA), urea nitrogen (UN), and creatinine (Crea).

1.5 Statistical Analysis

Data were analyzed using one-way ANOVA with LSD method and paired t-test in SPSS 22.0 software. Results were expressed as "mean ± standard error." Linear or curvilinear responses of indices to dietary iron levels were analyzed using unrelated comparison methods. Curve fitting was used to determine appropriate dietary iron supplementation levels. $P<0.05$ and $P<0.01$ were considered significant and extremely significant levels, respectively.

2.1 Effects of Bacillus subtilis Supplementation in Different Iron Level Diets on Growth Performance of Goslings

As shown in Table 3 , paired t-test analysis revealed that compared with group (without Bacillus subtilis), group (with Bacillus subtilis) showed increased body weight and ADG ($P>0.05$), and decreased ADFI and F/G ($P>0.05$).

Table 3 shows the effects of Bacillus subtilis on gosling growth performance.

As shown in Table 4 , dietary iron level had extremely significant effects on body weight, ADG, and F/G ($P<0.01$). With increasing dietary iron levels, body weight and ADG initially increased then decreased, while F/G initially decreased then increased. Specifically, the body weight and ADG of groups and were extremely significantly higher than those of group ($P<0.01$) and significantly higher than those of groups and ($P<0.05$). The F/G of group was extremely significantly lower than that of groups and ($P<0.01$) and significantly lower than that of group ($P<0.05$), with no significant difference from group ($P>0.05$). No significant differences were observed in ADFI and MR among groups ($P>0.05$).

Through curve fitting, under the premise of 250 mg/kg Bacillus subtilis supplementation, quadratic curve regression equations between ADG (Y) and F/G (Y) with dietary iron level (X) were well fitted:

$Y = 41.718 + 0.161X - 0.001X^2$ ($R^2 = 0.722$, $P = 0.000$)
$Y = 2.428 - 0.008X + 6.629E\text{-}5X^2$ ($R^2 = 0.884$, $P = 0.000$)

These equations indicate that with 250 mg/kg Bacillus subtilis in the basal diet, ADG was maximized at 80.50 mg/kg iron supplementation, and optimal F/G was achieved at 60.34 mg/kg iron supplementation.

These results demonstrate that both Bacillus subtilis and iron promote gosling growth and development. However, under Bacillus subtilis supplementation, basal diet iron levels reaching 100 mg/kg tended to decrease growth performance indices and increase MR, indicating excessive iron supplementation has no biological benefit.

2.2 Effects of Bacillus subtilis Supplementation in Different Iron Level Diets on Slaughter Performance of Goslings

As shown in Table 5 , paired t-test analysis revealed that compared with group , group showed increased SR, HER, AER, CMR, and LMR ($P>0.05$), and decreased AFR ($P>0.05$).

Table 5 shows the effects of Bacillus subtilis on gosling slaughter performance.

As shown in Table 6 , dietary iron level significantly affected AER and CMR ($P<0.05$). With increasing dietary iron levels, SR, HER, AER, CMR, and LMR initially increased then decreased, while AFR initially decreased then increased. Specifically, the CMR of group was extremely significantly higher than that of group ($P<0.01$) and significantly higher than that of group ($P<0.05$), with no significant difference from groups and ($P>0.05$). The CMR of group was significantly higher than that of group ($P<0.05$), with no significant difference from groups and ($P>0.05$).

These results indicate that appropriate dietary iron and Bacillus subtilis supplementation can improve goose slaughter performance and reduce abdominal fat deposition. However, under Bacillus subtilis supplementation, basal diet iron levels reaching 100 mg/kg tended to decrease slaughter performance indices and increase AFR, confirming that excessive iron supplementation lacks biological significance.

2.3 Effects of Bacillus subtilis Supplementation in Different Iron Level Diets on Hematopoietic Function of Goslings

As shown in Table 7 , paired t-test analysis revealed that compared with group , group showed increased blood HGB content ($P>0.05$), RBC count ($P<0.05$), HCT ($P>0.05$), MCV ($P>0.05$), and MCH ($P<0.05$).

Table 7 shows the effects of Bacillus subtilis on gosling hematopoietic function.

As shown in Table 8 , dietary iron level had extremely significant effects on blood HGB content, RBC count, HCT, MCV, and MCH ($P<0.01$). With increasing dietary iron levels, these indices initially increased then decreased. Specifically, the HGB content of groups and was extremely significantly higher than that of groups and (P<0.01) and significantly higher than that of group (P<0.05). The RBC count of group was extremely significantly higher than that of groups and (P<0.01) and significantly higher than that of group (P<0.05), with no significant difference from group (P>0.05). The HCT of group was extremely significantly higher than that of groups , , and (P<0.01) and significantly higher than that of group (P<0.05). The MCV of group was extremely significantly higher than that of groups and (P<0.01) and significantly higher than that of groups and (P<0.05). The MCH of group was extremely significantly higher than that of groups , , and (P<0.01) and significantly higher than that of group (P<0.05).

These results demonstrate that both Bacillus subtilis and iron improve gosling hematopoietic function, with particularly significant increases in RBC count and MCH. Appropriate dietary iron and Bacillus subtilis supplementation extremely significantly improved hematopoietic function, with all indices superior to the control group (100 mg/kg iron). This indicates synergistic effects between Bacillus subtilis and iron. Iron supplementation directly promotes HGB and RBC synthesis and indirectly enhances RBC synthesis by improving kidney function and erythropoietin production [15]; the mechanism by which Bacillus subtilis improves hematopoietic function requires further investigation.

2.4 Effects of Bacillus subtilis Supplementation in Different Iron Level Diets on Iron Metabolism and Kidney Function of Goslings

As shown in Table 9 , paired t-test analysis revealed that compared with group , group showed increased serum iron content, TIBC, and TS (P>0.05), and decreased serum UA, UN, and Crea contents (P>0.05).

Table 9 shows the effects of Bacillus subtilis on gosling iron metabolism and kidney function.

As shown in Table 10 , dietary iron level had extremely significant effects on serum TIBC and UA content (P<0.01). With increasing dietary iron levels, serum iron content, TIBC, and TS initially increased then decreased, while serum UA, UN, and Crea contents initially decreased then increased. Specifically, the TIBC of groups and was significantly higher than that of groups and (P<0.05), with no significant difference from group (P>0.05). The UA content of group was extremely significantly lower than that of group (P<0.01) and significantly lower than that of group (P<0.05), with no significant difference from groups and (P>0.05). No significant differences were observed in serum TS, iron, UN, and Crea contents among groups (P>0.05).

These results indicate that appropriate dietary iron and Bacillus subtilis supplementation can improve iron metabolism and kidney function in goslings, particularly serum TIBC and UA content, with all indices superior to the control group (100 mg/kg iron). This demonstrates synergistic effects between Bacillus subtilis and iron in improving iron metabolism and kidney function.

3.1 Effects on Growth Performance

Iron, as an essential mineral element, plays important roles in maintaining normal growth, metabolism, and reproduction. Iron content directly affects phosphorus levels and consequently DNA synthesis in cells, while regulating mitochondria and microsomes through iron-containing enzymes to influence protein synthesis. Vahl et al. [17] found that broiler weight gain increased with iron supplementation (0, 20, 60 mg/kg ferrous sulfate) in corn-soybean diets from 1-39 days of age. Lin Yingcai et al. [18] reported that compound iron supplementation (0, 150, 250, 350 g/t) significantly increased ADG and decreased F/G in growing pigs. Bacillus subtilis, approved by China' s Ministry of Agriculture as one of 12 feed-grade microbial additives, effectively improves animal growth performance and feed conversion [19]. Hooge et al. [20] confirmed growth-promoting effects of Bacillus subtilis preparations in broilers and geese. Molnár et al. [21] found that Bacillus subtilis increased ADG and improved feed conversion in broilers. This study demonstrates that Bacillus subtilis supplementation increased body weight and ADG while decreasing F/G in goslings. Appropriate iron and Bacillus subtilis supplementation extremely significantly improved body weight and ADG while extremely significantly reducing F/G, with optimal values superior to the control group (100 mg/kg iron), indicating synergistic effects.

3.2 Effects on Slaughter Performance

Slaughter performance reflects poultry meat production performance and serves as an important basis for evaluating growth performance and processing efficiency. Wang Yanwen et al. [22] reported that live weight affects slaughter meat yield, and appropriate iron supplementation increases growth rate and slaughter performance. Zhang Renyi et al. [23] found that Bacillus licheniformis supplementation (50, 100, 200 mg/kg) did not significantly affect SR, AER, or HER in broilers but improved CMR and LMR, with no significant differences from antibiotic groups. This study shows that Bacillus subtilis improved gosling slaughter performance, and appropriate iron and Bacillus subtilis supplementation enhanced slaughter performance, particularly AER and CMR, with all indices superior to the control group (100 mg/kg iron), demonstrating synergistic effects.

3.3 Effects on Hematopoietic Function

Changes in blood and serum biochemical indices typically indicate altered physiological functions, as pathological changes in any blood component affect systemic tissues and organs. Blood RBC count, HGB content, and HCT are important indicators of iron metabolism and nutritional status [24,3]. This study shows that Bacillus subtilis improved gosling hematopoietic function, particularly RBC count and MCH. Appropriate iron and Bacillus subtilis supplementation extremely significantly improved hematopoietic function, with all indices superior to the control group (100 mg/kg iron), indicating synergistic effects.

Iron supplementation directly promotes HGB and RBC synthesis and indirectly enhances RBC synthesis by improving kidney function and erythropoietin production [15]; the mechanism of Bacillus subtilis requires further study.

3.4 Effects on Iron Metabolism and Kidney Function

Serum iron and ferritin contents are important indicators of iron metabolism, growth, and metabolic status [25-26]. Serum TIBC represents the maximum iron amount that can bind to all transferrin in 100 mL serum. Serum iron content determines nutritional status and reflects liver and body iron stores. Insufficient serum iron causes low transferrin saturation, inadequate iron supply to hematopoietic tissue, and anemia [27-28].

Blood non-protein nitrogen includes UA, UN, and Crea—end products of purine, protein, and muscle metabolism excreted through kidneys, directly reflecting kidney function [29]. UA, the main metabolic product in birds and reptiles, is slightly water-soluble and easily crystallizes. Failure to excrete UA leads to crystal accumulation and kidney failure. UN, a protein catabolism product, is excreted primarily through kidneys (over 90%), with the remainder via intestine and skin. Elevated serum UN indicates kidney dysfunction. Crea, a muscle metabolism product (both exogenous and endogenous), is relatively constant under stable conditions and primarily filtered by glomeruli, serving as a glomerular filtration function indicator.

Insufficient serum iron causes low transferrin saturation, inadequate iron supply, and anemia—a major complication of kidney failure. Clinical studies show oral iron supplementation effectively improves kidney function [30]. In this study, serum iron content trends were opposite to UA, UN, and Crea trends, indicating close relationships between iron metabolism and kidney function, consistent with Meara et al. [31].

This study demonstrates that Bacillus subtilis improved gosling iron metabolism and kidney function, and appropriate iron and Bacillus subtilis supplementation improved these functions, particularly serum TIBC and UA, with all indices superior to the control group (100 mg/kg iron), indicating synergistic effects.

4. Conclusions

1. Appropriate dietary iron and Bacillus subtilis supplementation can improve growth and slaughter performance while reducing abdominal fat deposition in goslings.
2. Appropriate dietary iron and Bacillus subtilis supplementation can improve hematopoietic and kidney function in goslings.
3. Bacillus subtilis supplementation can reduce dietary iron requirements, demonstrating synergistic effects with iron.
4. Goslings have a specific threshold for iron nutritional requirements; excessive or insufficient levels are detrimental to growth and development.

5. For optimal growth performance, the recommended dietary iron level is 60.34-80.50 mg/kg when supplementing with 250 mg/kg Bacillus subtilis.

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