Effects of Dietary Iron Supplementation Level on Growth Performance, Slaughter Performance, and Blood Parameters of New Zealand Meat Rabbits Aged 60-90 Days (Postprint)

Authors: Liu Gongyan, Zhang Wei, Li Lei, Gao Qin, Sun Yingchao, Li Fuchang

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Abstract

This experiment was conducted to investigate the effects of dietary iron supplementation levels on growth performance, slaughter performance, and blood parameters of New Zealand meat rabbits aged 60-90 days. A total of 104 New Zealand meat rabbits at 53 days of age with similar body weight were randomly allocated into 4 groups, with 26 replicates per group and 1 rabbit per replicate. The control group was fed a basal diet, while the experimental groups were supplemented with 50, 100, and 200 mg/kg iron in the basal diet, respectively, in the form of ferrous sulfate heptahydrate ($FeSO4 \cdot 7H2O$) with a purity of 98%. The pre-trial period lasted 7 days, and the formal trial period lasted 30 days. The results showed: 1) Dietary iron supplementation level had significant effects on average daily gain (ADG) and feed to gain ratio (F/G) of New Zealand meat rabbits aged 60-90 days ($P < 0.05$), but had no significant effect on average daily feed intake (ADFI) ($P > 0.05$). With increasing dietary iron supplementation levels, ADG first increased and then decreased, while F/G first decreased and then increased; ADG was highest and F/G was lowest when dietary iron supplementation level was 100 mg/kg. 2) Dietary iron supplementation level had significant effects on eviscerated carcass yield and semi-eviscerated carcass yield of New Zealand meat rabbits aged 60-90 days ($P < 0.05$); with increasing dietary iron supplementation levels, eviscerated carcass yield and semi-eviscerated carcass yield first increased and then decreased, and were highest when dietary iron supplementation level was 50 mg/kg. 3) Dietary iron supplementation level had no significant effect on liver index of New Zealand meat rabbits aged 60-90 days ($P > 0.05$), but had significant effects on kidney index and spleen index ($P < 0.05$). When dietary iron supplementation levels were 50, 100, and 200 mg/kg, kidney index was significantly higher than that of the control group ($P < 0.05$); when dietary iron supplementation levels were 100 and 200 mg/kg,

spleen index was significantly higher than that of the control group ($P < 0.05$). 4) Dietary iron supplementation level had no significant effect on iron, copper, calcium, zinc, and magnesium contents in blood of New Zealand meat rabbits aged 60-90 days ($P > 0.05$); dietary iron supplementation level had significant effects on platelet distribution width (PDW), mean platelet volume (MPV), and platelet-large cell ratio (P-LCR) ($P < 0.05$). Based on the measured indices in this experiment, the appropriate dietary iron supplementation level for New Zealand meat rabbits aged 60-90 days is 50-100 mg/kg.

Full Text

Effects of Dietary Iron Supplemental Level on Growth Performance, Slaughter Performance and Blood Indices of 60 to 90-Day-Old New Zealand Meat Rabbits

LIU Gongyan[1], ZHANG Wei[2], LI Lei[3], GAO Qin[1], SUN Yingchao[1], LI Fuchang[1]*

[1]College of Animal Science and Technology, Shandong Agricultural University, Tai' an 271018, China
[2]Jining Polytechnic, Jining 272000, China
[3]Linyi Animal Husbandry and Veterinary Inspection Center, Linyi 276000, China

Abstract: This experiment was conducted to investigate the effects of dietary iron supplemental level on growth performance, slaughter performance, and blood indices of 60 to 90-day-old New Zealand meat rabbits. One hundred and four 53-day-old New Zealand meat rabbits with similar body weight were randomly divided into four groups with twenty-six replicates per group and one rabbit per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 50, 100, and 200 mg/kg iron, respectively. The supplemental form was ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) with 98% purity. The trial consisted of a 7-day pre-trial period followed by a 30-day formal trial period. The results showed: 1) Dietary iron supplemental level had significant effects on average daily gain (ADG) and feed-to-gain ratio (F/G) ($P < 0.05$), but no significant effect on average daily feed intake (ADFI) ($P > 0.05$). As dietary iron supplemental level increased, ADG first increased then decreased, while F/G first decreased then increased; ADG was highest and F/G was lowest when dietary iron supplemental level was 100 mg/kg. 2) Dietary iron supplemental level had significant effects on all eviscerated slaughter ratio and half eviscerated slaughter ratio ($P < 0.05$). Both ratios first increased then decreased with increasing dietary iron supplemental level, reaching their maximum at 50 mg/kg. 3) Dietary iron supplemental level had no significant effect on liver index ($P > 0.05$), but had significant effects on kidney index and spleen index ($P < 0.05$). Kidney index was significantly higher than the control group when dietary iron supplemental level was 50, 100, and 200 mg/kg ($P < 0.05$); spleen index was significantly higher than the con-

trol group when dietary iron supplemental level was 100 and 200 mg/kg ($P < 0.05$). 4) Dietary iron supplemental level had no significant effects on blood iron, copper, calcium, zinc, or magnesium content ($P > 0.05$), but had significant effects on platelet distribution width (PDW), mean platelet volume (MPV), and platelet-large cell ratio (P-LCR) ($P < 0.05$). Based on the comprehensive analysis of all measured indices, the appropriate dietary iron supplemental level for 60 to 90-day-old New Zealand meat rabbits is 50–100 mg/kg.

Keywords: iron; New Zealand rabbit; growth performance; slaughter performance; blood indices

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Iron is an essential trace element for animal life activities and one of the indispensable basic elements for nutrient metabolism, growth, development, and reproduction in biological organisms. It participates in life activities and metabolism in various forms, including functional proteins and iron-containing enzymes (cytochrome oxidase, catalase, xanthine oxidase, etc.), playing an important role in the normal growth and development of animals [1-3]. Iron deficiency in animals damages blood cell volume, morphology, and structure, ultimately leading to nutritional anemia and affecting growth and development. Similarly, excess iron primarily induces oxidative damage [4-5], failing to promote animal growth and instead causing waste of trace element feed additives. With the increasing awareness of ecological and environmental protection, reducing trace element emissions into the environment is imperative, making research on trace element requirements for different animals at different physiological stages particularly important. Elvehjem et al. [6] reported that when dietary iron levels in poultry were below 15 mg/kg, hypochromic microcytic anemia would occur, with pale mucosa, slow or stagnant growth, and decreased appetite; severe deficiency would lead to anemia [7]. Iron deficiency in piglets not only reduces hemoglobin synthesis but also decreases the activity of certain enzymes in red blood cells, causing obstacles in lipid, protein, and carbohydrate synthesis [8]. High iron content in animals leads to iron deposition in tissues and cells, causing pathological damage to cells and affecting normal physiological functions [9]. Cao et al. [10] found that high-iron diets could reduce feed intake and growth performance in chickens.

Currently, China has established complete feeding standards for chickens, pigs, and cattle. However, rabbit research started relatively late, and no rabbit feeding standards have been formulated to date. Most regions in China still follow the nutrient requirements recommended by NRC (1977) [11], which, due to its early publication date and considering China' s regional differences and feed ingredient factors, may not be suitable for China' s rabbit production. Therefore, there is an urgent need to develop rabbit feeding standards that align with China' s production reality. This study used New Zealand growing meat rabbits as experimental subjects and ferrous sulfate as the iron supplement form to investigate the effects of different iron supplemental levels on growth performance, slaughter performance, and blood indices of New Zealand growing meat

rabbits, aiming to determine the appropriate iron supplemental level for 60 to 90-day-old New Zealand meat rabbits and provide a reasonable basis for the formulation of meat rabbit feeding standards in China.

1.1 Experimental Design

One hundred and four 53-day-old New Zealand meat rabbits with similar body weight [(1,110 ± 35) g] were selected (purchased from Shandong Academy of Agricultural Sciences, conventional grade), with equal numbers of males and females. They were randomly divided into four groups according to the principle of consistent sex and body weight, with twenty-six replicates per group and one rabbit per replicate. There was no significant difference in body weight among groups ($P > 0.05$). The basal diet was formulated according to the NRC (1977) [11] and De Blas et al. [12] standards for growing rabbits. Its composition and nutrient levels are shown in Table 1 . The control group was fed the basal diet, while the experimental groups were fed the basal diet supplemented with 50, 100, and 200 mg/kg iron, respectively. The supplemental form was ferrous sulfate heptahydrate (FeSO ·7H O, purchased from Jinan Jiehui Chemical Co., Ltd., with 98% purity). The measured iron levels in the diets were 10.65, 62.39, 115.28, and 210.71 mg/kg, respectively. The pre-trial period lasted 7 days, and the formal trial period lasted 30 days.

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

Ingredients	Content	Nutrient levels[2])	Content
Corn		DE/(MJ/kg)	
Wheat bran		CP	
Peanut vine		CF	
Soybean meal		EE	
NaCl		Met+Cys	
CaHPO		Lys	
Premix[1])		Ca	
Lys		TP	
Total			

[1]) The premix provided the following per kilogram of diet: VA 8,000 IU, VD 1,000 IU, VE 50 mg, Lys 1.5 g, Met 1.5 mg, Cu 40 mg, Zn 50 mg, Mn 30 mg, Mg 150 mg, I 0.1 mg.
[2]) DE was a calculated value, while the others were measured values.

1.3 Feeding Management

The experimental rabbits were housed individually in cages and fed twice daily with ad libitum access to feed and water. Natural lighting and ventilation were provided, and conventional feeding management and immunization procedures were adopted.

1.4 Sample Collection and Preparation

On the morning of day 30 after the experiment began, the rabbits were weighed after fasting. Ten rabbits from each group with body weight close to the group average were selected, and 10 mL of blood was collected from the heart under dim light and placed in EDTA anticoagulant tubes for determination of blood routine indices and iron, copper, calcium, zinc, and magnesium content. After blood collection, the experimental rabbits were immediately slaughtered, and the weights of carcass, liver, kidney, and spleen were recorded.

1.5.1 Determination of Growth Performance Indices

The initial body weight (IBW) of the experimental rabbits at the beginning of the trial and the final body weight (FBW) at the end of the trial were measured and recorded. Feed consumption during the trial period was also recorded to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G) during the experimental period (excluding the pre-trial period).

1.5.2 Determination of Slaughter Performance Indices

Pre-slaughter weight: the fasting weight of experimental rabbits after 12 hours of feed restriction before slaughter.
Half-eviscerated carcass weight: the weight after bleeding, skinning, and removal of hooves, trachea, esophagus, gastrointestinal tract and contents, spleen, pancreas, bladder, and reproductive organs, while retaining lungs, thymus, heart, liver, kidneys, and perirenal fat.
All-eviscerated carcass weight: the weight of half-eviscerated carcass after removal of lungs, thymus, heart, liver, and head (decapitated at the first cervical vertebra), while retaining kidneys and perirenal fat.

The liver, kidneys, and spleen were carefully separated and weighed, and their ratios to pre-slaughter live weight were calculated.

1.5.3 Determination of Blood Indices

Blood routine analysis: EDTA-anticoagulated whole blood was analyzed using a hematology analyzer (Mindray BC-2800 three-class automatic hematology analyzer).
Determination of iron, copper, calcium, zinc, and magnesium content in diets and blood: atomic absorption spectrometry was used (Beijing Bohui Innovation Co., Ltd.; BH5100 five-channel atomic absorption spectrometer).

1.6 Data Processing

Data were analyzed using the GLM procedure in SAS 9.1.3 statistical software. Duncan' s multiple range test was used for multiple comparisons. Results are

expressed as mean ± standard deviation, with $P < 0.05$ considered statistically significant.

2.1 Effects of Dietary Iron Supplemental Level on Growth Performance of 60 to 90-Day-Old New Zealand Meat Rabbits

As shown in Table 2 , with no significant difference in IBW ($P > 0.05$), dietary iron supplemental level had significant effects on FBW, ADG, and F/G ($P < 0.05$), but no significant effect on ADFI ($P > 0.05$). The FBW of all experimental groups was significantly higher than that of the control group ($P < 0.05$). As dietary iron supplemental level increased, ADG first increased then decreased, while F/G first decreased then increased. ADG was highest and significantly higher than the control group ($P < 0.05$), and F/G was lowest and significantly lower than the control group ($P < 0.05$) when dietary iron supplemental level was 100 mg/kg.

Table 2 Effects of dietary iron supplemental level on growth performance of 60 to 90-day-old New Zealand meat rabbits (n=26)

Items	Dietary iron supplemental level/(mg/kg)	P-value
	0	50
IBW/g	1,564.26±36.84	1,556.02±33.63
FBW/g	2,614.56±56.45	2,689.19±51.60
ADFI/g	140.26±2.78	145.02±3.63
ADG/g	35.01±1.56	36.10±2.16
F/G	4.19±0.15	4.02±0.26

In the same row, values with different small letter superscripts indicate significant difference ($P < 0.05$), while values with the same or no superscripts indicate no significant difference ($P > 0.05$). The same applies below.

2.2 Effects of Dietary Iron Supplemental Level on Slaughter Performance of 60 to 90-Day-Old New Zealand Meat Rabbits

As shown in Table 3 , dietary iron supplemental level had significant effects on pre-slaughter weight, all eviscerated slaughter ratio, and half eviscerated slaughter ratio ($P < 0.05$). Pre-slaughter weight, all eviscerated slaughter ratio, and half eviscerated slaughter ratio first increased then decreased with increasing dietary iron supplemental level. Pre-slaughter weight was highest and significantly higher than the control group ($P < 0.05$) when dietary iron supplemental level was 100 mg/kg. All eviscerated slaughter ratio and half eviscerated slaughter ratio were highest and significantly higher than the control group ($P < 0.05$) when dietary iron supplemental level was 50 mg/kg. Dietary iron supplemental level had no significant effect on liver index ($P > 0.05$), but had significant effects on kidney index and spleen index ($P < 0.05$). Kidney index was significantly higher than the control group when dietary iron supplemental level was

50, 100, and 200 mg/kg ($P < 0.05$). Spleen index was significantly higher than the control group when dietary iron supplemental level was 100 and 200 mg/kg ($P < 0.05$).

Table 3 Effects of dietary iron supplemental level on slaughter performance of 60 to 90-day-old New Zealand meat rabbits (n=10)

Items	Dietary iron supplemental level/(mg/kg)	P-value
	0	50
Pre-slaughter weight/g	2,626.34±26.54	2,694.27±21.05
All eviscerated ratio/%	46.32±0.78	48.48±0.52
Half eviscerated ratio/%	50.62±0.45	51.30±0.54
Liver ratio/%	2.98±0.16	2.93±0.12
Kidney ratio/%	0.50±0.03	0.63±0.03
Spleen ratio/%	0.04±0.01	0.06±0.01

2.3 Effects of Dietary Iron Supplemental Level on Trace Element Content in Blood of 60 to 90-Day-Old New Zealand Meat Rabbits

As shown in Table 4 , dietary iron supplemental level had no significant effects on blood iron, copper, calcium, zinc, or magnesium content in New Zealand meat rabbits ($P > 0.05$).

Table 4 Effects of dietary iron supplemental level on the content of Fe, Cu, Zn, Ca and Mg in blood of 60 to 90-day-old New Zealand meat rabbits (n=10)

Items	Dietary iron supplemental level/(mg/kg)	P-value
	0	50
Fe/(mmol/L)	6.59±0.73	7.01±0.82
Cu/(μmol/L)	20.35±2.82	20.51±2.75
Zn/(μmol/L)	82.45±8.18	81.33±9.21
Ca/(mmol/L)	2.57±0.32	2.26±0.56
Mg/(mmol/L)	2.26±0.59	2.64±0.32

2.4 Effects of Dietary Iron Supplemental Level on Blood Routine Indices of 60 to 90-Day-Old New Zealand Meat Rabbits

As shown in Table 5 , dietary iron supplemental level had no significant effects on white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB) content, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), small cell percentage (W-SCR), small cell absolute value (W-SCC), or red cell distribution width (RDW-CV) ($P > 0.05$). When dietary iron supplemental level was 50 mg/kg, platelet distribution width (PDW),

mean platelet volume (MPV), and platelet-large cell ratio (P-LCR) were all significantly higher than the control group and the 200 mg/kg group ($P < 0.05$), but showed no significant difference from the 100 mg/kg group ($P > 0.05$).

Table 5 Effects of dietary iron supplemental level on blood routine index of 60 to 90-day-old New Zealand meat rabbits (n=10)

Items	Dietary iron supplemental level/(mg/kg)	P-value
	0	50
WBC/(×10 /L)	9.76±2.09	8.18±1.26
RBC/(×10^{12}/L)	4.76±0.31	4.53±0.42
HGB/(g/L)	95.50±6.95	99.07±6.06
HCT	0.43±0.02	0.54±0.01
MCV/fL	67.47±0.42	68.35±0.67
MCH/Pg	20.32±0.65	20.47±0.45
MCHC/(g/L)	303.17±10.25	300.20±2.65
PLT/(×10 /L)	581.53±67.40	499.65±76.54
W-SCR	0.54±0.02	0.68±0.69
W-SCC/(×10 /L)	3.12±0.62	3.32±0.60
RDW-CV	0.14±0.19	0.11±0.16
PDW/fL	7.83±0.36	8.78±0.75
MPV/fL	6.57±0.35	7.07±0.64
P-LCR	0.14±0.02	0.14±0.02

3.1 Effects of Dietary Iron Supplemental Level on Growth Performance of 60 to 90-Day-Old New Zealand Meat Rabbits

Iron, as an essential mineral element for animal organisms, plays an important role in maintaining animal growth, metabolism, and reproduction. Iron content in animal bodies affects DNA synthesis in cells and regulates mitochondria and microsomes in cells through iron-containing enzymes, influencing protein synthesis and thereby improving animal growth performance. Vahl et al. [13] added 0, 20, and 60 mg/kg ferrous sulfate to corn-soybean meal diets and found that weight gain of 1–39-day-old Hybro broilers increased with dietary iron supplemental level. Lin et al. [14] reported that adding compound iron to basal diets significantly increased ADG and decreased F/G in growing pigs. Wang et al. [15] found that appropriate dietary iron supplemental level significantly increased body weight and ADG while significantly decreasing F/G in Wulong geese. Most research results show that dietary iron supplementation can improve animal growth performance, which is also confirmed by this experiment that appropriate dietary iron supplemental level is beneficial for the growth of New Zealand meat rabbits.

3.2 Effects of Dietary Iron Supplemental Level on Slaughter Performance of 60 to 90-Day-Old New Zealand Meat Rabbits

The nutritional status of livestock and poultry diets directly relates to and affects slaughter traits. Slaughter ratio is a primary indicator for measuring meat production performance. New Zealand meat rabbits are a typical meat-type breed, and slaughter ratio is an important indicator reflecting meat rabbit production performance. All eviscerated slaughter ratio and half eviscerated slaughter ratio are specific manifestations of slaughter ratio; it is generally considered that meat production performance is good when half eviscerated slaughter ratio is above 80% and all eviscerated slaughter ratio is above 60% [16]. Wang et al. [15] found that appropriate dietary iron supplemental level significantly increased slaughter ratio, half eviscerated ratio, and leg muscle ratio in Wulong geese. In this study, dietary iron supplemental level significantly affected pre-slaughter weight of New Zealand meat rabbits. The all eviscerated slaughter ratio and half eviscerated slaughter ratio of experimental rabbits showed a trend of first increasing then decreasing with increasing dietary iron supplemental level, with the best effect observed at 50 mg/kg. The effect of dietary iron supplemental level on slaughter ratio was due to iron improving rabbit growth performance, which was confirmed by the final weight data. Visceral organs are the basic "facilities" for animal life activities and the important material basis for physiological function. Organ index can reflect visceral function to a certain extent; it is generally believed that larger organ index indicates stronger visceral function. Immune organs are the foundation of animal immune function and the important material basis for physiological function. Immune index can reflect immune function to a certain extent; it is generally believed that larger immune index in healthy animals indicates stronger immunity, decreased immune organ weight indicates immunosuppression, while increased immune organ weight indicates immune enhancement [17]. However, long-term use of diets with excessive trace elements can cause slight poisoning in rabbits, leading to pathological enlargement of visceral organs. This study showed that dietary iron supplemental level had certain effects on visceral organ development in New Zealand meat rabbits, but no pathological enlargement caused by poisoning was observed, possibly due to enhanced metabolism resulting from improved growth performance. However, the specific mechanism requires further investigation.

3.3 Effects of Dietary Iron Supplemental Level on Blood Indices of 60 to 90-Day-Old New Zealand Meat Rabbits

Trace elements play important roles in animal metabolism, enzyme composition, and activation. They have complex relationships of mutual promotion and restriction. Significant competitive interactions exist among metal elements such as iron, copper, zinc, and magnesium. High dietary zinc reduces iron absorption. Ceruloplasmin can release iron from ferritin and convert ferrous ions (Fe^{2}) to ferric ions (Fe^{3}); therefore, insufficient dietary copper hinders iron absorption. In hemoglobin synthesis, copper deficiency prevents iron release from ferritin, caus-

ing microcytic anemia. Hill et al. [18] reported that reducing dietary iron level or increasing dietary calcium level exacerbated copper poisoning in pigs. High copper can cause conditional iron or zinc deficiency in pigs, leading to decreased blood hemoglobin content, anemia, and growth stagnation. However, supplementing iron and zinc in high-copper diets can enhance the growth-promoting effect of copper and protect pigs from copper poisoning. In this study, dietary iron supplemental level had no significant effects on blood iron, copper, calcium, magnesium, or zinc content in 60 to 90-day-old New Zealand meat rabbits, possibly related to the maintenance of animal body fluid balance. Hemoglobin is one of the biologically functional iron compounds in animals, with heme as its main component. Heme is an iron porphyrin derivative, and the structure of amino acid chelated iron is very similar to heme. Blood RBC, HGB, and HCT are important indicators reflecting iron metabolism status and nutritional status in animals. Hemoglobin and MCV are sensitive indicators for evaluating biochemical responses to iron digestion and utilization [19]. This study found that dietary iron supplemental level had no significant effects on RBC, HGB, HCT, or MCV, possibly because the basal diet contained sufficient iron to prevent typical iron deficiency anemia symptoms in rabbits.

When dietary iron supplemental level was 100 mg/kg, 60 to 90-day-old New Zealand meat rabbits showed the highest ADG, lowest F/G, and best growth performance. When dietary iron supplemental level was 50 mg/kg, the rabbits showed the highest slaughter ratio. Based on comprehensive analysis of all measured indices, the appropriate dietary iron supplemental level for 60 to 90-day-old New Zealand meat rabbits is 50–100 mg/kg.

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