

Effects of Dietary Copper Supplementation on Growth Performance and Serum Biochemical Parameters in Angora Rabbits from Weaning to Two Months of Age (Postprint)

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Abstract

This study aimed to investigate the effects of dietary copper supplementation level on growth performance, serum biochemical indices, and serum enzyme activity in Angora rabbits from weaning to 2 months of age. A total of 180 Mengyin fine-wool Angora rabbits were selected and randomly divided into 6 groups with 30 rabbits per group, with individual cage housing. The six groups were fed experimental diets supplemented with 0, 10, 20, 40, 80, and 160 mg/kg copper (as copper sulfate pentahydrate) to the basal diet, with measured copper contents of 3.25, 13.13, 23.40, 43.56, 83.58, and 163.87 mg/kg, respectively. The preliminary period was 7 d, and the formal experimental period was 23 d. The results showed that dietary copper supplementation level had an extremely significant effect on average daily gain (ADG) and feed to gain ratio (F/G) ($P < 0.01$), with the highest ADG and lowest F/G observed at 80 mg/kg copper supplementation. Dietary copper supplementation level had no significant effect on hair yield, hair diameter, or skin area ($P > 0.05$). Dietary copper supplementation level had significant effects on serum total protein (TP), albumin (ALB), globulin (GLB), urea nitrogen (UN) contents, and albumin to globulin ratio (A/G) ($P < 0.05$). Serum UN content showed a trend of first decreasing and then increasing with increasing dietary copper supplementation level, with the lowest serum UN content observed at 80 mg/kg copper supplementation. Dietary copper supplementation level had no significant effect on serum copper-zinc superoxide dismutase (Cu-Zn SOD) activity ($P > 0.05$), but had a significant effect on serum ceruloplasmin (CP) activity ($P < 0.05$). With increasing dietary copper supplementation level, serum ceruloplasmin activity first increased and then decreased, reaching the highest value at 80 mg/kg copper supplementation. Dietary copper supplementation level had a significant effect

on liver index ($P < 0.05$), but had no significant effect on other visceral organ indices ($P > 0.05$). The liver index was highest at 20 mg/kg copper supplementation. Based on the measured indices in this experiment, the appropriate dietary copper supplementation level for Angora rabbits from weaning to 2 months of age is recommended to be 20~80 mg/kg.

Full Text

Effects of Dietary Copper Addition on Performance and Serum Indices of Weaning to 2-Month-Old Long Hairy Rabbits

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Abstract

This study investigated the effects of dietary copper addition on performance, serum biochemical indices, and serum enzymatic activities of weaning to 2-month-old long hairy rabbits. One hundred eighty Mengyin fine wool type long hairy rabbits with similar body weight were randomly assigned to 6 groups (30 rabbits/group) and housed individually. The six groups received experimental diets supplemented with 0, 10, 20, 40, 80, and 160 mg/kg copper (as copper sulfate pentahydrate) based on a basal diet, with measured copper contents of 3.25, 13.13, 23.40, 43.56, 83.58, and 163.87 mg/kg, respectively. The trial included a 7-day adaptation period followed by a 23-day experimental period. Results showed that dietary copper addition had extremely significant effects on average daily gain (ADG) and feed/gain ratio (F/G) ($P < 0.01$), with the highest ADG and lowest F/G observed at 80 mg/kg copper addition. Dietary copper addition had no significant effects on lanugo production, wool diameter, or fur area ($P > 0.05$). Serum total protein (TP), albumin (ALB), globulin (GLB), urea nitrogen (UN) contents, and albumin/globulin ratio (A/G) were significantly affected by dietary copper addition ($P < 0.05$). Serum UN content initially decreased then increased with increasing copper addition, reaching its lowest value at 80 mg/kg copper addition. Dietary copper addition had no significant effect on serum copper-zinc superoxide dismutase (Cu-Zn SOD) activity ($P > 0.05$) but significantly affected serum ceruloplasmin (CP) activity ($P < 0.05$), which increased then decreased with increasing copper addition, peak-

ing at 80 mg/kg copper addition. Dietary copper addition significantly affected liver index ($P < 0.05$) but had no significant effects on other visceral organ indices ($P > 0.05$). The highest liver index was observed at 20 mg/kg copper addition. Based on all measured indices, the appropriate dietary copper addition for weaning to 2-month-old long hairy rabbits is recommended to be 20–80 mg/kg.

Key words: copper; long hairy rabbits; performance; serum indices

As an essential trace element, copper participates in the metabolism of major nutrients and plays a vital role in hematopoiesis, metabolism, growth and reproduction, maintenance of performance, enhancement of immunity, and biological membrane stability[1]. Early research by Bowler et al.[2] demonstrated that 250 mg/kg copper supplementation promoted growth in finishing pigs. Ma Delei et al.[3] reported that dietary copper content significantly affected ADG in weaning to 2-month-old meat rabbits, with ADG increasing as copper content rose. Han Aiyun et al.[4] similarly noted that 75–150 mg/kg copper promoted rabbit growth, while Li Daolin[5] observed growth-promoting effects of high-dose copper in rex rabbits. However, some researchers found no growth-promoting effect of copper in rabbits[6]. Although copper has been extensively studied in meat animals, research on copper requirements for long hairy rabbits remains limited. This experiment aimed to investigate the effects of varying copper addition levels on growth performance, wool production, serum indices, and visceral organ development in long hairy rabbits, to determine whether dietary copper supplementation promotes growth, and to provide a theoretical basis for establishing feeding standards for long hairy rabbits in China.

1.1 Experimental Animals and Management

One hundred eighty 35-day-old weaned Mengyin fine wool type long hairy rabbits with similar body weight and good health were randomly divided into 6 groups (30 rabbits/group) and housed individually in cages under natural ventilation and lighting. Rabbits were fed at 06:00 and 18:00 daily with ad libitum access to feed and water. Immunization and disinfection followed routine farm procedures. The trial consisted of a 7-day preliminary period and a 23-day formal experimental period.

1.2 Experimental Diets

The basal diet was formulated according to the Professional Standard of the People's Republic of China (Draft for Approval 1994) "Angora Rabbit Feeding Standard" (Table 1). Six experimental diets were prepared by supplementing the basal diet with 0, 10, 20, 40, 80, and 160 mg/kg copper as copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), with measured copper contents of 3.25, 13.13, 23.40, 43.56, 83.58, and 163.87 mg/kg, respectively. All diets were mixed according to formulation, pelleted into 4 mm diameter pellets, and stored in a ventilated, dark place.

1.3 Sample Collection and Preparation

At the end of the experiment, rabbits were fasted and weighed to obtain slaughter live weight. After weighing, rabbits were sheared and wool weight was recorded as lanugo production. Six rabbits per group were selected for cardiac blood collection (5 mL), which was allowed to clot at room temperature for 15 minutes, then centrifuged at 3,000 r/min for 10 minutes. The separated serum was aliquoted into centrifuge tubes and stored at -20°C for serum analysis. Rabbits were euthanized by cervical dislocation and slaughtered; visceral organs were excised and weighed, and skins were removed for fur area determination.

1.4 Measurement Indicators

1.4.1 Growth Performance Body weight was recorded at the beginning and end of the formal experimental period. Feed consumption during the experimental period (excluding the preliminary period) was recorded to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed/gain ratio (F/G).

1.4.2 Wool Performance Actual shearing weight was recorded as individual lanugo production. Wool diameter was measured using an optical fiber diameter analyzer and fiber projector. Skins were removed, and lengths at the shoulder, waist, and hip regions were measured; the average value was taken as skin width. The length from the middle of the neck to the tail base was measured as skin length, and skin area was calculated.

1.4.3 Serum Biochemical Indices Serum total protein (TP), albumin (ALB), globulin (GLB), albumin/globulin ratio (A/G), and urea nitrogen (UN) contents were determined using kits from Wako Pure Chemical Industries, Japan, according to the manufacturer's instructions on a Hitachi 7020 automatic analyzer.

1.4.4 Serum Enzyme Activities Serum copper-zinc superoxide dismutase (Cu-Zn SOD) and ceruloplasmin (CP) activities were determined using kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer's instructions, with absorbance values measured using a UV762 UV spectrophotometer.

1.4.5 Visceral Organ Development At the end of the experiment, rabbits were fasted and weighed to obtain slaughter live weight. The liver, heart, kidneys, and spleen were excised and weighed, and organ indices were calculated as follows:

Liver index = liver weight (g) / slaughter live weight (kg)
Heart index = liver weight (g) / slaughter live weight (kg)
Kidney index = kidney weight (g) / slaughter live weight (kg)
Spleen index = spleen weight (g) / slaughter live weight (kg)

1.5 Data Processing

Experimental data were expressed as means \pm standard error. One-way ANOVA was performed using SPSS 13.0 software, and significant differences were tested using Duncan's multiple comparison test. $P < 0.05$ and $P < 0.01$ were considered significant and extremely significant levels, respectively.

2.1 Effects of Dietary Copper Addition on Growth Performance

As shown in Table 2, with no significant difference in initial body weight ($P > 0.05$), dietary copper addition significantly affected ADG and F/G ($P < 0.05$) but had no significant effect on ADFI ($P > 0.05$). ADG increased then decreased, while F/G decreased then increased with increasing copper addition. The highest ADG (20.28 g/d) and lowest F/G were observed at 80 mg/kg copper addition.

2.2 Effects of Dietary Copper Addition on Wool Performance

As shown in Table 3, dietary copper addition had no significant effects on lanugo production, wool diameter, or fur area ($P > 0.05$). The highest lanugo production (34.55 g) occurred at 40 mg/kg copper addition, the maximum wool diameter (13.09 m) at 20 mg/kg copper addition, and the maximum fur area at 80 mg/kg copper addition.

2.3 Effects of Dietary Copper Addition on Serum Biochemical Indices

As shown in Table 4, dietary copper addition significantly affected serum TP, ALB, GLB, and UN contents, as well as A/G ratio ($P < 0.05$). The highest serum TP content (54.80 g/L) was observed at 160 mg/kg copper addition, significantly different from the 20 and 40 mg/kg groups ($P < 0.05$). The highest serum ALB content (38.25 g/L) also occurred at 160 mg/kg copper addition, significantly different from the 20 and 40 mg/kg groups ($P < 0.05$). The highest serum GLB content was observed at 20 mg/kg copper addition, significantly different from all other groups ($P < 0.05$). The highest serum A/G ratio occurred at 20 mg/kg copper addition, significantly different from the 10 and 40 mg/kg groups ($P < 0.05$). The lowest serum UN content (3.35 mmol/L) was observed at 80 mg/kg copper addition, significantly different from the 0 and 10 mg/kg groups ($P < 0.05$).

2.4 Effects of Dietary Copper Addition on Serum Enzyme Activities

As shown in Table 5, dietary copper addition significantly affected serum ceruloplasmin activity ($P < 0.05$), which increased then decreased with increasing copper addition, reaching its highest value (74.48 U/L) at 80 mg/kg copper addition. Dietary copper addition had no significant effect on serum Cu-Zn SOD activity ($P > 0.05$).

2.5 Effects of Dietary Copper Addition on Visceral Organ Development

As shown in Table 6 , dietary copper addition significantly affected liver index ($P < 0.05$). The highest liver index (3.31) was observed at 20 mg/kg copper addition, significantly different from the 0 and 80 mg/kg groups ($P < 0.05$). Dietary copper addition had no significant effects on kidney index, heart index, or spleen index ($P > 0.05$).

3.1 Effects of Dietary Copper Addition on Growth Performance

As an essential trace element, copper plays an important role in promoting animal growth, enhancing immunity, and promoting hemoglobin synthesis[7]. Studies have shown that dietary copper supplementation has obvious growth-promoting and feed-saving effects in young animals[7]. Patten et al.[8] found that adding 40 mg copper per kilogram of diet significantly improved rabbit growth rate and feed utilization. Gan Bozhong[9] reported that adding 10 or 20 mg/kg copper to diets significantly increased rabbit ADG, which increased with dietary copper addition. Li Daolin[5] reported that high doses of copper in diets promoted growth in rex rabbits. However, some scholars believe that copper has no growth-promoting effect on rabbits[6]. Research on copper in meat animal growth and development has been extensive, but studies on copper requirements for long hairy rabbits are limited. This experiment aimed to investigate the effects of different copper addition levels on growth performance, wool production, serum indices, and visceral organ development in long hairy rabbits, to explore whether dietary copper supplementation has growth-promoting effects on long hairy rabbits, and to provide a theoretical basis for establishing feeding standards for long hairy rabbits in China.

3.2 Effects of Dietary Copper Addition on Wool Performance

Copper is closely related to animal hair growth, promotes keratin protein production, indirectly compensates for insufficient sulfur-containing amino acids, increases wool production in fur animals, and copper deficiency can lead to poor growth and disheveled hair[10]. Li Zhen[11] reported that 50 mg/kg copper addition significantly promoted rabbit hair growth. Li Hong et al.[6] studied dietary copper addition in Angora rabbits and found that wool length increased with copper addition, but wool yield was not significantly affected. In this experiment, dietary copper addition had no significant effect on wool performance of Mengyin fine wool type long hairy rabbits, possibly because the experimental period was too short for the dose effect to fully manifest, and wool quality in long hairy rabbits is greatly affected by age. Generally, lanugo production in long hairy rabbits is low and of poor quality, which may explain why copper addition did not significantly promote wool performance.

3.3 Effects of Dietary Copper Addition on Serum Biochemical Indices

Serum TP, ALB, and UN contents reflect protein and amino acid metabolism and normal liver and kidney function, representing the degree of protein digestion, absorption, and anabolism. Zhang Sujiang[12] studied copper effects on serum biochemical indices in 60 Junmu No. 1 growing pigs and found that TP and ALB contents in copper-supplemented groups were higher than in the control group, with significant improvements in the 20 and 250 mg/kg copper groups. This experiment showed that appropriate copper addition significantly increased serum TP, ALB, and GLB contents, indicating that copper can improve immunity by increasing serum ALB content, thereby improving production performance. Serum TP and ALB contents were highest at 160 mg/kg copper addition, suggesting that high copper can accelerate protein synthesis. Serum UN is the main product of protein metabolism, and its content reflects metabolic status; lower content indicates less nitrogen excretion and higher protein synthesis efficiency[13]. In this experiment, serum UN content initially decreased then increased with increasing copper addition, with the lowest value at 80 mg/kg copper addition, suggesting that protein utilization efficiency in long hairy rabbits was highest at this level.

3.4 Effects of Dietary Copper Addition on Serum Enzyme Activities

At least 14 copper-containing enzymes exist in animals, and dietary copper content changes can alter their activities. Literature indicates that dietary copper addition affects serum CuZn-SOD and ceruloplasmin activities[14]. Li Daolin[5] reported that when copper sulfate was used as the copper source at 80 mg/kg, serum CuZn-SOD activity in rex rabbits was extremely significantly different compared to 0 and 10 mg/kg copper groups. However, Gan Bozhong[9] found that dietary copper addition had no significant effects on erythrocyte and liver SOD or serum ceruloplasmin activities in 90-day-old Angora rabbits, although these enzyme activities tended to increase with copper addition. In this experiment, using copper sulfate pentahydrate as the copper source, serum CuZn-SOD activity was highest at 40 mg/kg copper addition, with no significant difference compared to other groups, but showed a trend of increasing then decreasing with increasing copper addition. Ceruloplasmin, also known as copper oxidase, has antioxidant effects and can prevent generation of oxides and free radicals in adipose tissue. In this experiment, serum ceruloplasmin activity was highest at 80 mg/kg copper addition and significantly decreased when copper addition increased from 80 to 160 mg/kg.

3.5 Effects of Dietary Copper Addition on Visceral Organ Development

Visceral organs are the basic facilities of animal life activities and the important material basis for physiological function. Organ indices can reflect visceral function to some extent; it is generally believed that larger organ indices indicate stronger visceral function. Most scholars believe that trace element copper and

its enzymes constitute the body's defense system and enhance immune function. The liver and spleen are important immune organs, and their weights and related indices can reflect immune function. Zhao Deming et al.[15] found that broilers fed low-copper diets showed reduced lymphocyte numbers in major lymphoid organs after 7 weeks, with serious effects on immune organs. Wu Jianshe et al.[16] reported that both copper deficiency and excess can cause immune organ atrophy. However, long-term use of excessive trace elements can cause mild poisoning in rabbits, leading to pathological enlargement of visceral organs. This experiment showed that dietary copper addition had some effects on liver organ development in Mengyin fine wool type long hairy rabbits, but the specific mechanism requires further study. Based on all measured indices, high-copper diets (160 mg/kg copper addition, measured copper content of 163.87 mg/kg) did not have significant growth-promoting effects on weaning to 2-month-old long hairy rabbits. According to the results, the appropriate dietary copper addition for weaning to 2-month-old long hairy rabbits is recommended to be 20–80 mg/kg (measured dietary copper content of 23.40–83.58 mg/kg).

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