Effects of Dietary Copper Level on Serum Lipid Metabolism Indices, Hematological Parameters, Intestinal Digestive Enzyme Activity, and Bile Trace Element Content in Mink during the Winter Fur Growth Period (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary copper levels on serum lipid metabolism indices, blood parameters, intestinal digestive enzyme activities, and bile trace element contents in minks during the winter fur period. One hundred and forty healthy minks aged (110±3) days (half male and half female) were selected and randomly divided into 7 groups with 20 replicates per group and one mink per replicate. Minks in each group were fed experimental diets supplemented with 0 (control group), 6 (Cu6 group), 12 (Cu12 group), 24 (Cu24 group), 48 (Cu48 group), 96 (Cu96 group), and 192 mg/kg (Cu192 group) copper to a basal diet (containing 7.68 mg/kg copper). The pre-trial period was 7 d and the formal trial period was 90 d. The results showed: 1) Serum total cholesterol (TC) content in minks decreased linearly (: P<0.01, : P<0.01) or quadratically (: P<0.05, : P<0.01) with increasing dietary copper levels. Serum TC and triglyceride (TG) contents in male minks were significantly higher than those in female minks (P<0.05). 2) Dietary copper levels had no significant effect on hemoglobin content and hematocrit in minks (P>0.05), and there were no significant differences in hemoglobin content and hematocrit between male and female minks (P>0.05). 3) Jejunal lipase activity in male minks was significantly higher than that in female minks (P<0.05), and jejunal and ileal trypsin activities and jejunal amylase activity were highly significantly higher than those in female minks (P<0.01). 4) Bile copper content in minks increased linearly with increasing dietary copper levels (P<0.05), while bile manganese and iron contents decreased linearly or quadratically with increasing dietary copper levels (P<0.01). Therefore, copper supplementation

in mink diets has important regulatory effects on serum cholesterol and bile trace element contents.

Full Text

Effects of Dietary Copper Level on Serum Lipid Metabolism Parameters, Blood Parameters, Intestinal Digestive Enzyme Activities and Bile Trace Element Contents of Minks during Winter Fur-Growing Period

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Abstract

This experiment was conducted to investigate the effects of dietary copper level on serum lipid metabolism parameters, blood parameters, intestinal digestive enzyme activities, and bile trace element contents in minks during the winter fur-growing period. One hundred and forty healthy minks at (110±3) days of age were randomly assigned to seven groups with 20 replicates per group and one mink per replicate. The seven groups were fed experimental diets supplemented with 0 (control group), 6 (Cu6 group), 12 (Cu12 group), 24 (Cu24 group), 48 (Cu48 group), 96 (Cu96 group), and 192 mg/kg (Cu192 group) copper, respectively, based on a basal diet containing 7.68 mg/kg copper. The pre-trial period lasted 7 days, and the formal trial period lasted 90 days. The results showed: 1) Serum total cholesterol (TC) content in minks decreased linearly (: $P<0.01$, : $P<0.01$) or quadratically (: $P<0.05$, : $P<0.01$) with increasing dietary copper level. Serum TC and triglyceride (TG) contents in male minks were significantly higher than those in female minks ($P<0.05$). 2) Dietary copper level had no significant effect on hemoglobin content or hematocrit ($P > 0.05$), and there were no significant differences in hemoglobin content or hematocrit between male and female minks ($P > 0.05$). 3) Jejunal lipase activity in male minks was significantly higher than that in female minks ($P<0.05$), while jejunal and ileal trypsin activities and jejunal amylase activity were extremely significantly higher in male minks than in female minks ($P<0.01$). 4) Bile copper content in minks increased linearly with increasing dietary copper level ($P<0.05$), whereas bile manganese and iron contents decreased linearly or quadratically with increasing dietary copper level ($P<0.01$). In conclusion, dietary copper supple-

mentation in minks has important regulatory effects on serum cholesterol and bile trace element contents.

Keywords: copper; minks; lipid metabolism; bile; digestive enzyme activity

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Copper is an essential trace element for animals and plays important roles in physiological processes such as energy metabolism, hemoglobin synthesis, and lipid metabolism [1-2]. Copper deficiency affects iron absorption and subsequently impairs hematopoietic function [3-4], while appropriate copper supplementation in animal diets can increase intestinal lipase activity [5-7] and improve apparent fat digestibility [8-11]. Furthermore, numerous studies have shown that dietary copper supplementation can reduce plasma total cholesterol (TC) and triglyceride (TG) contents [12-16]. To date, numerous reports have investigated the biological functions of copper in livestock and poultry, but few studies have examined the effects of dietary copper supplementation on lipid metabolism, hemoglobin synthesis, and intestinal digestive enzyme activity in minks. Therefore, this experiment used minks during the winter fur-growing period as subjects to investigate the effects of adding different copper levels to diets on serum lipid metabolism parameters, blood parameters, intestinal digestive enzyme activities, and bile trace element contents, aiming to reveal the theoretical mechanisms by which copper promotes and improves animal growth performance and lipid metabolism regulation, and to provide a scientific basis for the rational application of copper.

1.1 Experimental Design and Management

The experiment was conducted at the fur animal production base of the Key Field Scientific Observation and Experiment Station of Changbai Mountain Wildlife Resources of the Ministry of Agriculture. One hundred and forty healthy minks at (110±3) days of age with similar body weight were randomly

selected (half male and half female). A single-factor randomized experimental design was adopted, with the 140 experimental minks randomly divided into seven groups, each containing 20 replicates with one mink per replicate. Using copper sulfate pentahydrate (CuSO ·5H O) as the copper source, the groups were fed experimental diets supplemented with 0 (control group), 6 (Cu6 group), 12 (Cu12 group), 24 (Cu24 group), 48 (Cu48 group), 96 (Cu96 group), and 192 mg/kg (Cu192 group) copper, respectively, based on a basal diet containing 7.68 mg/kg copper. The pre-trial period lasted 7 days, and the formal trial period lasted 90 days.

All experimental minks were housed individually in cages (40 cm × 40 cm × 60 cm) and fed twice daily at 07:30 and 15:30 with *ad libitum* access to feed and water under conventional immunization protocols. As there is currently no unified feeding standard for minks, the basal diet for the winter fur-growing period was formulated based on recent domestic research on mink nutritional requirements, with its composition and nutrient levels shown in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (DM basis)

Ingredients	Content (%)	Nutrient levels[2])	Content
Extruded corn		ME/(MJ/kg)	
Soybean meal		DM	
Corn gluten meal		CP	
Fish meal		EE	
Bone meat meal		CC	
Cheese meal		Ash	
Soybean oil		Lys	
Feather meal		Met	
Blood meal		Cys	
Premix[1])		TP	
L-lysine		Cu/(mg/kg)	7.68
DL-methionine			
NaCl			
Total			

[1]) Per kilogram of premix contained: vitamin A retinol 1,000,000 IU, cholecalciferol 200,000 IU, tocopheryl acetate 6,000 IU, thiamine 600 mg, riboflavin 800 mg, cobalamin 10 mg, menadione 100 mg, ascorbic acid 40,000 mg, nicotinic acid 4,000 mg, pantothenic acid 1,200 mg, biotin 20 mg, folic acid 80 mg, choline 30,000 mg, Fe 8,200 mg, Mn 1,200 mg, Zn 5,200 mg, I 50 mg, Se 20 mg, Co 50 mg.

[2]) ME was a calculated value, while the other nutrient levels were measured values.

1.2 Sample Collection

Blood sample collection: At the end of the feeding trial, 16 minks were selected from each group. Blood (8–10 mL) was collected from the toe tip and placed equally into procoagulant and anticoagulant tubes. After transfer to the laboratory, samples were centrifuged at 3,500 r/min for 10 min at 4 °C. The separated serum was aliquoted into 1.5 mL Eppendorf tubes and stored at –80 °C for later analysis.

Tissue and organ sample collection: At the end of the feeding trial, 16 minks from each group were selected. After fasting and weighing, the minks were fed their respective diets. Sixty minutes later, they were euthanized by injection with succinylcholine chloride. Rapid dissection was performed to collect approximately 10 cm segments of jejunum and ileum. Both ends of the intestinal segments were tied with fine thread, snap-frozen in liquid nitrogen, then quickly removed. The intestinal segments were cut open with dissecting scissors to scrape intestinal chyme into cryovials, which were stored at –80 °C for subsequent determination of trypsin, lipase, and amylase activities in intestinal chyme.

1.3 Measurement Indicators and Methods

1.3.1 Serum Lipid Metabolism Parameters Serum TG content was measured using the phosphoglycerol oxidase-peroxidase (GPO-PAP) method, serum TC content was measured using the cholesterol oxidase-peroxidase (COD-PAP) method, and low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) contents were measured using selective clearance methods. All these indicators were determined using assay kits purchased from Zhongsheng Beikong Biotechnology Co., Ltd.

1.3.2 Blood Parameters **Hemoglobin content determination:** Blood hemoglobin content was determined using the cyanmethemoglobin (HICN) colorimetric method with assay kits purchased from Nanjing Jiancheng Bioengineering Institute.

Hematocrit determination: Hematocrit was determined using the Wintrobe method.

1.3.3 Intestinal Digestive Enzyme Activities Before thawing, a 定量 (0.5–1.0 g) of intestinal chyme was weighed and mixed with 0.86% physiological saline at a mass-to-volume ratio of 1:9, homogenized, and centrifuged in a low-temperature centrifuge at 2,500 r/min for 10 min. The supernatant was collected. Following the instructions of the assay kits from Nanjing Jiancheng Bioengineering Institute, absorbance was measured using a SPECORD 50 UV spectrophotometer to calculate trypsin, lipase, and amylase activities.

1.3.4 Bile Trace Element Contents Two milliliters of bile sample was pipetted into a 100 mL conical flask, mixed with 10 mL of ultra-pure nitric acid, sealed for 2 h, then digested on an electric furnace at low temperature until nearly dry. The solution was transferred without loss to a 10 mL volumetric flask. Copper, zinc, manganese, and iron contents were determined using a VARIAN SpectrAA-240 atomic absorption spectrophotometer.

1.4 Data Analysis

Experimental data were statistically analyzed using the GLM procedure of SAS 9.13 software. Gender differences were tested for significance using one-way ANOVA, and differences among groups were tested using Duncan' s multiple comparison test. Using the REG procedure of SAS 9.13 software, linear and quadratic regression analyses were performed with dietary copper level as the independent variable and various indicators as dependent variables. $P<0.05$ was considered statistically significant, and $P<0.01$ was considered extremely significant.

2.1 Effects of Dietary Copper Level on Serum Lipid Metabolism Parameters of Minks during Winter Fur-Growing Period

The effects of dietary copper level on serum lipid metabolism parameters are shown in Table 2 . In male minks, serum TC (linear, $P<0.01$; quadratic, $P<0.05$) and TG (linear, $P<0.01$; quadratic, $P<0.01$) contents decreased linearly or quadratically with increasing dietary copper level. Dietary copper level had no significant effect on serum HDL-C and LDL-C contents in male minks ($P>0.05$). Serum TC content in the control group was significantly higher than that in the Cu96 and Cu192 groups ($P<0.05$). Serum TG content in the control group was extremely significantly higher than that in all other groups ($P<0.01$), with no significant differences among the other groups ($P > 0.05$). There were no significant differences in serum HDL-C and LDL-C contents among all groups in male minks ($P > 0.05$).

In female minks, serum TC content decreased linearly and quadratically with increasing dietary copper level ($P<0.01$), while serum HDL-C content increased linearly with dietary copper level ($P<0.05$). Dietary copper level had no significant effect on serum TG and LDL-C contents in female minks ($P > 0.05$). Serum TC content in the control, Cu6, and Cu12 groups was extremely significantly higher than that in the Cu96 and Cu192 groups ($P<0.01$). Serum TC content in the control and Cu6 groups was significantly higher than that in the Cu48 group ($P<0.05$). Serum TG content in the Cu6 group was significantly higher than that in the Cu48 group ($P<0.05$), with no significant differences among other groups ($P > 0.05$). Serum LDL-C content in the control group was significantly higher than that in the Cu48 group ($P<0.05$), with no significant differences among other groups ($P > 0.05$). Serum TC and TG contents in male minks were significantly higher than those in female minks ($P<0.05$).

Table 2 Effects of dietary copper level on serum lipid metabolism parameters of minks during winter fur-growing period (mmol/L)

Items	Male							Female						
Groups	Control	Cu6	Cu12	Cu24	Cu48	Cu96	Cu192	Control	Cu6	Cu12	Cu24	Cu48	Cu96	Cu192
TC	8.60[a]	7.74	7.17	6.89	6.71	6.40	5.92	7.54	7.29	6.96	6.40	5.94	5.18	5.20
TG	2.50	2.10	2.04	1.98	1.85	1.89	1.83	2.03	2.24	1.97	1.80	1.73	1.76	1.78
HDL-C	1.47	1.31	1.34	1.36	1.23	1.35	1.34	-	-	-	-	-	-	-
LDL-C	-	-	-	-	-	-	-	-	-	-	-	-	-	-

In the same column and the same item, values with different small letter superscripts mean significant difference (P<0.05), and with different capital letter superscripts mean significant difference (P<0.01), while with the same or no letter superscripts mean no significant difference (P>0.05). The same as below.

2.2 Effects of Dietary Copper Level on Blood Parameters of Minks during Winter Fur-Growing Period

The effects of dietary copper level on blood parameters are shown in Table 3 . Dietary copper level had no significant effect on hemoglobin content or hematocrit in minks (P > 0.05), and there were no significant differences in hemoglobin content or hematocrit between male and female minks (P > 0.05).

Table 3 Effects of dietary copper level on blood parameters of minks during winter fur-growing period

Items	Male				Female			
Groups	Control–Cu192	Linear	Quadratic	P-value	Control–Cu192	Linear	Quadratic	P-value
Hb (g/L)	-	-	-	-	-	-	-	-
Hematocrit (%)	-	-	-	-	-	-	-	-

2.3 Effects of Dietary Copper Level on Intestinal Digestive Enzyme Activities of Minks during Winter Fur-Growing Period

The effects of dietary copper level on intestinal digestive enzyme activities are shown in Table 4 . Dietary copper level had no significant effect on digestive enzyme activities in the jejunum or ileum of minks (P > 0.05). However, trypsin and amylase activities in the jejunum and trypsin activity in the ileum were extremely significantly higher in male minks than in female minks (P<0.01),

while jejunal lipase activity was significantly higher in male minks than in female minks (P<0.05).

Table 4 Effects of dietary copper level on intestinal digestive enzyme activities of minks during winter fur-growing period (U/mg prot)

Items	Male				Female				Male vs Female
Jejunum	Control	Cu192	Linear	P-value	Control	Cu192	Linear	P-value	
Trypsin	8,913	8,464	-	-	-	-	-	-	P<0.01
Lipase	4,705	4,559	-	-	-	-	-	-	P<0.05
Amylase	572.8	548.1	-	-	-	-	-	-	P<0.01
Ileum									
Trypsin	8,243	7,746	-	-	-	-	-	-	P<0.01

2.4 Effects of Dietary Copper Level on Bile Trace Element Contents of Minks during Winter Fur-Growing Period

The effects of dietary copper level on bile trace element contents are shown in Table 5 . Bile copper content increased linearly with increasing dietary copper level (P<0.05), while bile manganese and iron contents decreased linearly or quadratically with increasing dietary copper level (P<0.01).

Table 5 Effects of dietary copper level on bile trace element contents of minks during winter fur-growing period

Items	Control	Cu192	Linear	Quadratic	P-value
Copper	-	-	-	-	P<0.05
Manganese	-	-	-	-	P<0.01
Iron	-	-	-	-	P<0.01
Zinc	-	-	-	-	-

-: not detected.

3.1 Effects of Dietary Copper Level on Serum Lipid Metabolism Parameters in Minks

Numerous studies have demonstrated a close relationship between copper and blood lipid metabolism, showing that dietary copper supplementation primarily reduces plasma TC and TG levels [12-13,15-16]. However, the underlying mechanism of copper's effect on lipid metabolism remains unclear and requires further investigation. Cholesterol in animals is mainly synthesized by the liver, with a portion derived from food. 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme in hepatic cholesterol synthesis [17].

Kim et al. [18] found that feeding a low-copper diet increased HMG-CoA reductase activity in mouse liver cells. Cholesterol 7 -hydroxylase is the rate-limiting enzyme for cholesterol conversion to bile acids in the liver [19]. Tang et al. [20] reported that feeding adult rats a low-copper diet reduced cholesterol 7 -hydroxylase gene expression by 80% and decreased its activity, impairing cholesterol conversion and leading to hypercholesterolemia. The present study showed that serum TC, TG, and LDL-C contents tended to decrease with increasing dietary copper level, while dietary copper level did not affect serum HDL-C content.

Serum TC content is influenced by various factors including age, sex, and diet, and is generally higher in male animals than in female animals. The results of this study showed that serum TC and TG contents in male minks were significantly higher than those in female minks, possibly because male minks have higher subcutaneous fat and body weight than female minks during the winter fur-growing period. Additionally, as strict carnivores, minks have higher serum TC content than humans and animals such as pigs, cattle, and sheep.

3.2 Effects of Dietary Copper Level on Blood Parameters in Minks

Copper in blood exists primarily in two forms: erythrocyte ceruloplasmin and plasma ceruloplasmin [21]. Ceruloplasmin can convert ferric iron to ferrous iron, promoting iron absorption in the gastrointestinal tract and synthesis of hemoglobin and porphyrin [3-4]. Additionally, copper can promote the maturation and release of immature erythrocytes [3]. When animals are copper-deficient, plasma ceruloplasmin activity decreases, leading to impaired iron valence conversion, anemia, blocked hemoglobin synthesis, iron metabolism disorders, and reduced erythrocytes, resulting in "hypocupremia" [22]. This study found that dietary copper level had no significant effect on hemoglobin content or hematocrit in minks, which is similar to the results reported by Aulerich et al. [23]. These findings indicate that feeding the basal diet did not cause severe copper deficiency sufficient to alter hemoglobin content or hematocrit in minks. Moreover, there were no significant sex differences in hemoglobin content or hematocrit.

3.3 Effects of Dietary Copper Level on Intestinal Digestive Enzyme Activities in Minks

Many studies have shown that dietary copper supplementation can increase intestinal lipase activity [5-7] and improve apparent fat digestibility [8-11]. Increased intestinal digestive enzyme activity directly improves nutrient digestibility and consequently enhances growth performance [6]. The present study showed that dietary copper level had no significant effect on trypsin, lipase, or amylase activities in the jejunum or ileum of minks, although lipase activity tended to increase initially and then decrease with increasing dietary copper level. This may be because feeding low-copper diets increased intestinal lipase activity, while excessive dietary copper levels could cause copper toxicity [24]

and subsequently reduce intestinal lipase activity. Many factors affect intestinal digestive enzyme activity in animals, including species differences, growth stage, diet composition, and environmental temperature [25].

The results also showed that jejunal trypsin, lipase, and amylase activities were significantly higher in male minks than in female minks. Previous studies have shown that male minks have higher digestibility of crude protein and crude fat than female minks, which is objectively confirmed by these results. However, the underlying mechanism of sex effects on intestinal digestive enzyme activity in minks remains unclear and requires further investigation.

3.4 Effects of Dietary Copper Level on Bile Trace Element Contents in Minks

Bile is one of the main excretory pathways for endogenous copper [26-27]. Studies have shown that bile copper content increases with dietary copper level, but not proportionally, while bile iron, manganese, and zinc contents decrease proportionally. Research on the effects of dietary copper level on bile iron, manganese, and zinc contents is still limited. Based on the present results, it can be inferred that feeding minks diets with different copper levels may increase bile secretion volume, thereby reducing bile iron, manganese, and zinc contents. Czarnecki et al. [28] reported that bile copper content did not increase when dietary copper level was below 250 mg/kg, but increased when dietary copper level exceeded 250 mg/kg. Armstrong et al. [29] showed that dietary supplementation with 225 mg/kg copper (as copper sulfate) resulted in higher bile copper content, while low copper levels (33, 66, 100 mg/kg) did not increase bile copper content compared with the control group.

4 Conclusion

With increasing dietary copper level, serum TC, TG, and LDL-C contents showed a decreasing trend, while dietary copper level did not affect serum HDL-C content.

Dietary copper level did not affect hemoglobin content or hematocrit in minks.

Jejunal and ileal lipase activities in minks tended to increase initially and then decrease with increasing dietary copper level.

There were sex differences in serum lipid metabolism parameters and intestinal digestive enzyme activities in minks, with serum TC and TG contents and jejunal trypsin, lipase, and amylase activities being significantly higher in male minks than in female minks.

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