

Effects of Dietary Copper Level on Serum Lipid Metabolism Indices, Blood 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 study aimed to investigate the effects of dietary copper levels on serum lipid metabolism indices, hematological parameters, intestinal digestive enzyme activities, and bile trace element concentrations in minks during the winter fur growth period. A total of 140 healthy minks ((110±3) days of age, half male and half female) were selected and randomly assigned to 7 groups with 20 replicates per group and one mink per replicate. The minks in each group were fed experimental diets based on a basal diet (copper content of 7.68 mg/kg) 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. The experiment consisted of a 7-day preliminary period followed by a 90-day formal feeding period. The results demonstrated that: 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 was no significant difference 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 ($P < 0.05$), while 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 ($P < 0.01$). In conclusion, copper supplementation in mink diets

has an important regulatory role in serum cholesterol and bile trace element concentrations.

Full Text

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 aged (110 ± 3) days (half male and half female) were randomly allocated 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-test period lasted 7 days, followed by a 90-day formal experimental period. 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. Male minks had significantly higher serum TC and triglyceride (TG) contents than female minks ($P < 0.05$). 2) Dietary copper level had no significant effect on hemoglobin content or hematocrit ($P > 0.05$), and no significant differences were observed between male and female minks in these parameters ($P > 0.05$). 3) Male minks exhibited significantly higher jejunum lipase activity ($P < 0.05$) and markedly higher trypsin activities in jejunum and ileum and amylase activity in jejunum ($P < 0.01$) compared to female minks. 4) Bile copper content in minks increased linearly with dietary copper level ($P < 0.05$), while bile manganese and iron contents decreased linearly or quadratically ($P < 0.01$). In conclusion, dietary copper supplementation significantly regulates serum cholesterol and bile trace element contents in minks.

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

Introduction

Copper is an essential trace element for animals that plays crucial roles in energy metabolism, hemoglobin synthesis, and lipid metabolism [1-2]. Copper deficiency can impair iron absorption and subsequently affect hematopoietic function [3-4], while appropriate copper supplementation in animal diets can enhance intestinal lipase activity [5-7] and improve apparent fat digestibility [8-11]. Furthermore, numerous studies have demonstrated that dietary copper supplementation can reduce plasma total cholesterol (TC) and triglyceride (TG) contents [12-16]. Although extensive research has been reported on the biological functions of copper in livestock and poultry, studies on the effects of dietary copper supplementation on lipid metabolism, hemoglobin synthesis, and intestinal digestive enzyme activities in minks remain scarce. Therefore, this

experiment used minks during the winter fur-growing period as subjects to investigate the effects of different dietary copper levels on serum lipid metabolism parameters, blood parameters, intestinal digestive enzyme activities, and bile trace element contents. The aim was to elucidate the theoretical mechanisms by which copper promotes and improves animal growth performance and lipid metabolism regulation, providing a scientific basis for the rational application of copper.

1. Materials and Methods

1.1 Experimental Design and Management

The experiment was conducted at the fur animal production base of the Ministry of Agriculture's Changbai Mountain Wildlife Resources Key Field Scientific Observation and Testing Station. One hundred and forty healthy minks aged (110 ± 3) days with similar body weight (half male and half female) were randomly selected. A single-factor randomized experimental design was employed, with the 140 minks divided into seven groups, each containing 20 replicates of one mink per replicate. Copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) served as the copper source. The groups were fed experimental diets supplemented with 0 (control), 6 (Cu6), 12 (Cu12), 24 (Cu24), 48 (Cu48), 96 (Cu96), and 192 mg/kg (Cu192) copper, respectively, based on a basal diet containing 7.68 mg/kg copper. The pre-test period lasted 7 days, followed by a 90-day formal experimental period.

All experimental minks were housed individually in cages ($40 \text{ cm} \times 40 \text{ cm} \times 60 \text{ cm}$) and fed twice daily at 07:30 and 15:30 with ad libitum access to feed and water under conventional immunization protocols. As no unified feeding standards currently exist 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 .

1.2 Sample Collection

Blood samples: 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 coagulation-promoting and anticoagulant tubes. After centrifugation 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 subsequent analysis.

Tissue and organ samples: At the end of the feeding trial, 16 minks from each group were fast-weighed after fasting, then 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 opened with dissecting scissors to scrape intestinal chyme into

cryovials, which were stored at -80°C for determination of trypsin, lipase, and amylase activities in intestinal chyme.

1.3 Analytical Methods

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

1.3.2 Blood Parameters Hemoglobin content: Determined using the cyanmethemoglobin (HICN) colorimetric method with assay kits from Nanjing Jiancheng Bioengineering Institute.

Hematocrit: 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 content was weighed and mixed with 0.86% physiological saline at a 1:9 mass-to-volume ratio, homogenized, and centrifuged at 2,500 r/min for 10 min in a low-temperature centrifuge. The supernatant was collected. Following the instructions of Nanjing Jiancheng Bioengineering Institute assay kits, 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 superior 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 parameters as dependent factors. $P < 0.05$ was considered statistically significant, and $P < 0.01$ was considered highly significant.

2. Results

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

The effects of dietary copper level on serum lipid metabolism parameters are presented 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 ($P < 0.05$).

The control group of male minks showed significantly higher serum TC content than the Cu96 and Cu192 groups ($P < 0.05$). The control group of male minks exhibited highly significantly higher serum TG content than all other groups ($P < 0.01$), with no significant differences among the other groups ($P > 0.05$). No significant differences were observed in serum HDL-C and LDL-C contents among all groups of 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 ($P > 0.05$). The control, Cu6, and Cu12 groups of female minks showed highly significantly higher serum TC content than the Cu96 and Cu192 groups ($P < 0.01$). The control and Cu6 groups of female minks had significantly higher serum TC content than the Cu48 group ($P < 0.05$). The Cu6 group of female minks exhibited significantly higher serum TG content than the Cu48 group ($P < 0.05$), with no significant differences among other groups ($P > 0.05$). The control group of female minks showed significantly higher serum LDL-C content than the Cu48 group ($P < 0.05$), with no significant differences among other groups ($P > 0.05$). Male minks had significantly higher serum TC and TG contents than female minks ($P < 0.05$).

2.2 Effects of Dietary Copper Level on Blood Parameters in 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 no significant differences were observed between male and female minks in these parameters ($P > 0.05$).

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

The effects of dietary copper level on intestinal digestive enzyme activities are presented 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,

male minks showed highly significantly higher trypsin activities in jejunum and ileum and amylase activity in jejunum ($P < 0.01$), and significantly higher lipase activity in jejunum ($P < 0.05$) compared to female minks.

2.4 Effects of Dietary Copper Level on Bile Trace Element Contents in 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 in minks increased linearly with dietary copper level ($P < 0.05$), while bile manganese and iron contents decreased linearly or quadratically ($P < 0.01$).

3. Discussion

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 mechanisms of copper's effects on lipid metabolism remain unclear and require 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 low-copper diets 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 low-copper diets to adult rats 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 HDL-C content was unaffected by dietary copper level.

Serum TC content is influenced by various factors including age, gender, and diet, generally being higher in male animals than in females. The current results showed that male minks had significantly higher serum TC and TG contents than female minks, possibly due to higher subcutaneous fat and body weight in male minks during the winter fur-growing period. Additionally, as strict carnivores, minks have higher serum TC content than humans and livestock such as pigs, cattle, and sheep.

3.2 Effects of Dietary Copper Level on Blood Parameters in Minks

Copper in blood exists primarily as erythrocyte ceruloplasmin and plasma ceruloplasmin [21]. Ceruloplasmin can convert ferric iron to ferrous iron, promoting iron absorption from the gastrointestinal tract and hemoglobin and porphyrin

synthesis [3-4]. Copper also promotes the maturation and release of immature erythrocytes [3]. Copper deficiency reduces plasma ceruloplasmin activity, causing impaired iron conversion, anemia, blocked hemoglobin synthesis, iron metabolism disorders, and reduced erythrocytes, leading to “hypocupremia” [22]. This study found that dietary copper level had no significant effect on hemoglobin content or hematocrit in minks, consistent with the findings of Aulerich et al. [23]. These results indicate that feeding the basal diet did not cause severe copper deficiency sufficient to alter hemoglobin content or hematocrit. Furthermore, no significant gender differences were observed in hemoglobin content or hematocrit.

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

Numerous studies have shown that dietary copper supplementation can increase intestinal lipase activity [5-7] and improve apparent fat digestibility [8-11]. Enhanced intestinal digestive enzyme activity directly improves nutrient digestibility and consequently growth performance [6]. The present study found that dietary copper level had no significant effect on trypsin, lipase, or amylase activities in the jejunum or ileum of minks, though lipase activity tended to increase initially then decrease with dietary copper level. This may be because low dietary copper levels increased intestinal lipase activity, while excessive copper levels could cause copper toxicity [24], thereby reducing lipase activity. Many factors influence intestinal digestive enzyme activity, including species differences, growth stage, diet composition, and environmental temperature [25].

The results also showed that male minks had significantly higher trypsin, lipase, and amylase activities in the jejunum than female minks. Previous studies have demonstrated that male minks have higher crude protein and crude fat digestibility than females, which is objectively confirmed by these findings. However, the underlying mechanisms of gender effects on intestinal digestive enzyme activity in minks remain unclear and require further investigation.

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

Bile is one of the primary excretory pathways for endogenous copper [26-27]. Studies have shown that increasing dietary copper level elevates bile copper content, though not proportionally, while bile iron, manganese, and zinc contents decrease substantially. Research on the effects of dietary copper level on bile iron, manganese, and zinc contents remains limited. Based on the present results, it can be inferred that feeding minks diets with different copper levels may increase bile secretion, 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 exceeding 250 mg/kg. Armstrong et al. [29] found that dietary supplementation with 225 mg/kg copper (as copper sulfate) resulted in higher bile copper content, while

lower levels (33, 66, 100 mg/kg) did not elevate bile copper content compared to the control group.

4. Conclusion

1. With increasing dietary copper level, serum TC, TG, and LDL-C contents showed a decreasing trend, while HDL-C content was unaffected by dietary copper level.
2. Dietary copper level did not affect hemoglobin content or hematocrit in minks.
3. Jejunal and ileal lipase activities in minks tended to increase initially then decrease with dietary copper level.
4. Gender differences existed in serum lipid metabolism parameters and intestinal digestive enzyme activities, with male minks showing significantly higher serum TC and TG contents and higher trypsin, lipase, and amylase activities in the jejunum than female minks.

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