Effects of dietary copper on elemental balance, plasma minerals and serum biochemical parameters of growing-furring male mink (Mustela vison)

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ABSTRACT

The objectives of this study were to study the effects of different levels of dietary copper on copper and zinc balance, plasma minerals and serum biochemical parameters of mink in the growing-furring periods. One hundred and five standard dark male mink were randomly assigned to seven groups with the following dietary treatments: basal diet with no supplemental Cu (Control); basal diet supplemented with either 6, 12, 24, 48, 96, or 192 mg/kg Cu from copper sulfate, respectively. The average daily gain (ADG) linearly ($P = 0.0026, P = 0.0006$) responded to increasing levels of Cu; maximal growth was seen in the Cu24 group. Feed efficiency tended to improve with the increase of dietary copper level (linear $P = 0.0010$, quad, $P = 0.0011$). Fecal copper, urinary copper, retention copper responded in a linear ($P < 0.05$) fashion with increasing level of Cu. The effect of level of Cu was linear ($P < 0.001$) for plasma Cu concentration. The serum glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities were increased linearly ($P < 0.05$) with dose of Cu, but serum total protein (TP) and albumin (ALB) concentrations decreased linearly ($P < 0.05$) as dietary copper levels increased. Effect of level of Cu was linear ($P < 0.001$) for serum ceruloplasmin (CER) concentration or Cu-Zn superoxide dismutase (Cu-Zn SOD) activity. Supplemental dose of Cu linearly decreased serum triglyceride (TG) ($P = 0.011$) and total cholesterol (TC) ($P = 0.007$). Our results indicated that the activity of Cu-dependent enzymes was enhanced by increasing dietary Cu concentration and that supplementation of Cu in the diet of mink could alter the plasma lipid profile and copper concentration.

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1. Introduction

Copper is an essential constituent of the growing number of copper enzymes and copper metalloprotein with functions as diverse as electron transfer (Cobine et al., 2006), pigmentation (Sendovski et al., 2011; Xu et al., 2013) and oxidation resistance (Wang et al., 2013). According to Engle and Spears (2001), copper deficiency causes an increase in plasma cholesterol. Netto et al. (2014) showed that copper supplementation altered lipid metabolism.

The national research council (NRC, 1982) requirement of copper for pigs is only 4.5–6.0 ppm. When fed with 32–64 ppm, this element was determined to be effective for growth promotion in mink (Wu et al., 2012). It has also been observed that such high levels of Cu supplementation result in its high excretion in the feces (Huang et al., 2010). The presence of high Cu concentrations in the feces inhibits the normal fermentation process, and the accumulation of Cu in the soil causes environmental concerns.

Therefore, the aim of the study was to evaluate the effects of dietary copper level on copper excretion, the antioxidant capacity, plasma minerals and lipid metabolism of male mink in the growing-furring period.
2. Materials and methods

The animal protocol for this experiment was approved by the Animal Care Committee of the Institute of Special Economic Animal and Plant Science of the Chinese Academy of Agricultural Sciences (44.02°N, 126.15°E). Animals were maintained and processed in accordance with the Chinese Academy of Agricultural Sciences Guide for the Care and Use of Laboratory Animals.

2.1. Animals, diets and management

One hundred and five 16-week-old standard dark male minks were randomly assigned to seven groups with the following dietary treatments: basal diet with no supplemental Cu (Control); basal diet supplemented with either 6 (Cu6), 12 (Cu12), 24 (Cu24), 48 (Cu48), 96 (Cu96), or 192 (Cu192) mg/kg Cu from copper sulfate, respectively. The basal diet mainly consisted of corn, fish meal, meat bone meal and soybean oil, with no Cu supplementation. The experiment period lasted for 98 d. The composition and chemical analysis of the basal diet are shown in Table 1.

The animals were housed individually in open-sided sheds in mink growing cages (60 cm long × 40 cm wide × 50 cm high) with additional attached nest boxes (30 cm long × 40 cm wide × 30 cm high). All animals were vaccinated with distemper and canine parvovirus before the study started. Animals had access to clean drinking water (contained less than 0.01 mg Cu/L by analysis). Animals had access to feed and tap water ad libitum throughout the study. Measured amount of treatment diets were offered twice daily at 08:00 and 16:00. The minks were weighed in the morning every two weeks before feeding from day 0 to the end of the trial. The left-over food was weighted and recorded daily. The average daily gain (ADG) and feed:gain were calculated for each animal individually.

2.2. Copper, zinc and nitrogen balance experiment

On day 45 of the study, eight animals from each treatment group were selected randomly and housed individually in metabolic crates that allowed separation of urine and feces to determine copper, zinc and nitrogen, based on the method described by Jørgensen and Glem-Hansen (1973). The balance experiment lasted for 3 days and the total feces and urine were collected and recorded.

Feed were sampled for subsequent analysis. The urine samples were collected using plastic bottles and stored at −20°C until they were analyzed. Fecal and feed samples were dried in a forced-air drying oven at 65°C, and then ground to pass the 40 mesh sieve.

2.3. Blood sampling

Blood samples were taken via heart punctures from minks at the end of the experiment. Blood samples were collected in two separate tubes, one heparinized and one without an anticoagulating substance. Samples were immediately transferred to the lab where plasma and serum was harvested subsequently by centrifuging the whole blood samples at 3500 × g for 5 min. The heparinized plasma samples were frozen in −20°C until analyzed for Cu, Zn and Fe concentrations. Serum samples were frozen in −80°C until analyzed.

2.4. Chemical analysis

The chemical composition of the diets and feces were analyzed by standard methods. Dry matter was determined by drying feed or fecal samples at 105°C to constant weight. Diets or fecal samples were analyzed for nitrogen [Method 984.13; Association of Official Analytical Chemists (AOAC), 2005]. The Cu, Fe and Zn contents of feed, organs and plasma samples were estimated in an air-acetylene flame on an atomic absorption spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan; Method 995.10; AOAC, 2005). The total protein (TP), albumin (ALB), urea nitrogen (BUN), glutamic-pyruvic transaminase (GPT), glutamic-oxalacetic transaminase (GOT), alkaline phosphatase (ALP), Triacylglyceride (TG) and Total Cholesterol (TC) were tested by automatic biochemistry analyzer (Hitachi 7020, Hitachi High Technologies, Inc., Ibaraki, Japan). Test kits were purchased from Nanjing Jiancheng Biochemical Corporation (Nanjing Jiancheng Biochemical Corporation, Nanjing, China). The Cu-dependent enzyme activities of serum Cu–Zn superoxide dismutase (Cu–Zn SOD) were tested by means of commercially available assay kits purchased from Nanjing Jiancheng Bioengineering Institute. The ceruloplasmin (CER) level was determined using a CER enzyme linked immunosorbent assay (ELISA) kit (Nanjing Jiancheng Bioengineering Research Institute, China).

2.5. Statistic analysis

Data were analyzed using the general linear models (GLM) procedure of SAS 9.13. The following model was used:

\[ Y_{ij} = \mu + d_i + e_{ij}. \]

Where \( Y_{ij} \) is the observation; \( \mu \) is the general mean; \( d_i \) is the effect of Cu level (1–7); \( e_{ij} \) is the random error.

Tukey tests were used to detect statistical significance between treatment groups. Linear and quadratic effects due to copper level were determined. Significant differences were accepted if \( P \leq 0.05 \).

3. Results

3.1. Growth performance

No minks died during the experimental period. Effect of dietary copper levels on growth performance of minks is shown in Table 2. The initial body weights (BW) of the minks were similar among the treatments, however, final BW and ADG linearly (\( P = 0.0026, P = 0.0006 \)) responding to increasing levels of Cu; maximal growth was seen in the Cu24 group. The effect of Cu on average daily feed

| Table 1 | Ingredient and chemical composition of basal diet. |
|---------|-----------------------------------------------|
| Items   | Contents                                      |
| Extruded corn | 31.2 Metabolizable energya; MJ/kg DM 20.32 |
| Soybean meal  | 6.0 Dry matter 97.60                          |
| Corn gluten meal | 8.0 Crude protein; % of DM 33.05               |
| Fish meal   | 18.0 Crude fat; % of DM 16.70                 |
| Meat and bone meal | 18.0 Crude carbohydrate; % of DM 42.41      |
| Cheese meal | 0.3 Ash; % of DM 8.02                         |
| Soybean oil | 12.0 Lysine; % of DM 1.69                     |
| Feather meal | 1.0 Methionine; % of DM 0.93                   |
| Blood meal  | 1.0 Cysteine; % of DM 0.36                    |
| Premixb   | 1.0 Copper, mg/kg 7.6                         |
| Lysine    | 0.3 Zinc, mg/kg 43.6                         |
| Methionine | 0.3                                             |
| NaCl      | 0.2                                             |
| Total     | 100                                            |

DM = dry matter.

a Metabolizable energy was calculated according to NRC (1982).

b Contained the following per kg of premix: vitamin A, 1,000,000 IU; vitamin D3, 200,000 IU; vitamin E, 6,000 IU; vitamin B12, 600 mg; vitamin B6, 800 mg; vitamin B1, 300 mg; vitamin B12, 10 mg; vitamin K1, 100 mg; vitamin C, 40,000 mg; niacin acid, 4000 mg; pantothenic acid, 1200 mg; biotin, 20 mg; folic acid, 80 mg; choline, 30,000 mg; Fe, 8200 mg; Mn, 1200 mg; Zn, 5200 mg; I, 50 mg; Se, 20 mg; Co, 50 mg.
Data are expressed as least squares means with pooled SEM; n = 15 per treatment.

intake (ADFI) was quadratic \( (P = 0.0001) \); the highest ADFI was seen in the Cu192 group, but ADFI generally followed ADG trends, displaying a linear \( (P = 0.0001) \) response to Cu level. Feed efficiency tended to improve with the increase of dietary copper level (linear \( P = 0.0010 \), quad: \( P = 0.0011 \)).

3.2. Copper, zinc and nitrogen balance

Effects of different dietary copper levels on elemental balance of mink are shown in Table 3. Zinc and N absorption and retention were generally not affected \( (P > 0.10) \) by Cu addition. Intake of Cu and fecal copper increased linearly \( (P < 0.01) \) with the dose of copper, which was expected. Fecal copper, urinary copper, retention copper responded in a linear \( (P < 0.05) \) fashion with increasing level of Cu. The 52–68% of the copper intake was excreted in the feces. However, the urinary excretion constituted only 0.1–3.6% of the total copper intake.

3.3. Plasma minerals

Effects of different dietary copper levels on plasma minerals of mink are shown in Table 4. Copper supplementation had no effect on plasma Zn concentrations or plasma Fe concentrations \( (P > 0.05) \). On the other hand, effect of level of Cu was linear \( (P < 0.0001) \) for plasma Cu concentrations.

3.4. Serum biochemical parameters

The serum GOT and GPT activities were increased linearly \( (P < 0.05) \) with dose of Cu, but serum TP and ALB concentrations decreased linearly \( (P < 0.05) \) as dietary copper levels increased (Table 5). Effect of level of Cu was linear \( (P < 0.001) \) for serum CER concentration or Cu–Zn SOD activity. Supplemental dose of Cu linearly decreased serum TC \( (P = 0.007) \) and TG \( (P = 0.011) \). On the other hand, Copper level had no significant effect on serum ALP activity \( (P > 0.05) \).

4. Discussion

In our study, energy level in each group was designed to be kept consistent in order to eliminate the influence of energy. Hence, in this study, feed intake was mainly affected by the copper levels of diets. Feeding high levels of copper has been observed by a number of researchers to increase feed intake in other species \( (\text{Edmonds et al., 1985; Burnell et al., 1988; Kornegay et al., 1989}) \). However, to what extent Cu-stimulated feed intake contributing to growth stimulation is still an open question. Neuropeptide Y (NPY), an extremely potent stimulator of feeding behavior \( (\text{King et al., 2000}) \) may play an important role in regulating feed consumption. Intravenously injected copper has been shown to stimulate the secretion of neuropeptide Y \( (\text{Zhou et al., 1994}) \), which is a known feed intake stimulant for pigs. In our study, greater improvement in feed efficiency than in growth by copper supplementation has been observed. It is necessary to point out that an increase in feed intake in general will not only stimulate growth rate, but also improve feed efficiency, because the extra nutrients can be used almost exclusively for growth rather than for maintenance. Therefore, it is difficult to separate completely the contribution of increased feed consumption and feed efficiency to growth.

The apparent absorption of nitrogen in this trial were slightly lower compared with values reported in other trials \( (\text{Alhistrom and Skrede, 1998; Hellwing et al., 2005}) \). This is likely due to the composition of the diet. A major factor influencing the digestibility of nutrients \( (\text{in particular protein}) \) is the diet composition. It has been found that in another fur animal species, the Arctic fox, nutrients from diets composed of animal meals were characterized by lower digestibility than components of fresh feed \( (\text{Gugolek et al., 2010; Vhile et al., 2005}) \). In addition, another factor influencing

| Treatments | Intake Cu, mg/d | Intake Zn, mg/d | Intake N, g/d | Fecal excretion Cu, mg/d | Fecal excretion Zn, mg/d | Fecal excretion N, g/d | Urine excretion Cu, mg/d | Urine excretion Zn, mg/d | Urine excretion N, g/d | Retention Cu, mg/d | Retention Zn, mg/d | Retention N, g/d | Apparent absorption, % Cu | Apparent absorption, % Zn | Apparent absorption, % N |
|------------|----------------|----------------|---------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|----------------|----------------|----------------|------------------------|------------------------|------------------------|
| Control    | 0.86           | 15.04          | 5.40          | 0.45                   | 26.38                  | 1.39                   | 0.00                   | 3.72                   | 0.00                   | 0.41           | 3.67           | 0.36           | 48.29                   | 75.06                   | 74.21                   |
| Cu6        | 1.49           | 14.46          | 5.26          | 0.99                   | 25.69                  | 1.32                   | 0.02                   | 3.63                   | 0.47                   | 11.25          | 0.32           | 33.78          | 76.20                   | 75.12                   | 74.47                   |
| Cu12       | 1.98           | 13.36          | 5.09          | 1.32                   | 24.12                  | 1.30                   | 0.04                   | 3.50                   | 0.62                   | 10.79          | 0.29           | 33.81          | 82.70                   | 74.47                   | 74.47                   |
| Cu24       | 3.58           | 14.99          | 5.39          | 2.31                   | 26.71                  | 1.32                   | 0.13                   | 3.77                   | 1.15                   | 11.75          | 0.30           | 35.50          | 79.13                   | 75.55                   | 75.55                   |
| Cu48       | 6.25           | 14.60          | 5.38          | 3.86                   | 28.06                  | 1.35                   | 0.19                   | 3.71                   | 2.21                   | 13.49          | 0.32           | 38.37          | 91.33                   | 74.89                   | 74.89                   |
| Cu96       | 12.29          | 15.66          | 5.55          | 7.19                   | 28.19                  | 1.46                   | 0.29                   | 3.79                   | 4.81                   | 12.55          | 0.30           | 41.36          | 81.80                   | 73.55                   | 73.55                   |
| Cu192      | 23.93          | 15.33          | 5.46          | 14.54                  | 30.93                  | 1.41                   | 0.12                   | 3.75                   | 9.27                   | 15.12          | 0.31           | 39.06          | 97.90                   | 74.28                   | 74.28                   |
| SEM        | 1.06           | 0.27           | 0.09          | 0.64                   | 0.62                   | 0.03                   | 0.01                   | 0.00                   | 0.42                   | 0.51           | 0.01           | 0.94           | 3.50                    | 0.39                    | 0.39                    |
| P value     | Linear         | 0.8682         | 0.0026        | 0.0006                 | 0.0001                 | 0.0010                 | 0.8754                 | 0.0058                 | 0.0001                 | 0.5223         | 0.5509         | 0.5509         | 0.5223                   | 0.5509                   | 0.5509                   |

Data are expressed as least squares means with pooled SEM; n = 8 per treatment.
the digestibility of nutrients is the fat:carbohydrate (F:C) ratio. The digestibility of crude protein increased whereas that of carbohydrates decreased with the F:C ratio increasing (Suvegova et al., 2001). The increase in dietary fat and fiber contents may interfere with the apparent digestibility of crude protein and ether extract in the present experiments.

Assessing the absorption of minerals is a complex matter, considering the numerous interacting factors that can influence the results. The endogenous contribution, such as biliary and gastrointestinal secretions and sloughed mucosal cells (Sandstrom et al., 1993), should also be taken into consideration. To date there are few reports on mineral absorption and homeostasis in terrestrial carnivorous species such as the mink. In the present study, the data of minerals showed large individual variation, which is in line with other studies on species such as polar foxes (Szmyeczko et al., 2010) and minks (Denstadli et al., 2010). A gradual decrease in the apparent absorption of Cu with increased levels of copper agrees with results reported previously by Mejborn (1989) on copper sulfate supplementation of mink diets indicated that 75–90% of the copper intake was excreted in the feces and that urinary copper excretion was elevated with increased copper intake. However, the excretion constituted only 0.3–1.5% of the total copper intake which is consistent with the present findings.

Organic Cu are claimed to be preferentially absorbed and metabolized and provide less chance for antagonism with other minerals or substances (Gheisari et al., 2011). However, some authors reported the absence of such effect with Cu sources (Cheng et al., 2011; Miles et al., 2003). The data from this trial indicate copper methionine and copper sulfate are better than trivalent copper chloride. In the present study the apparent Zn absorption was highly negative, probably due to contamination from Zn galvanized toy equipment in the cages. Our results showed that Cu supplementation had no effect on Zn balance, which is in agreement with Eckert et al. (1999).

The variable amount of copper stored in liver acts to maintain plasma copper concentrations even in the face of low copper supply. Measurements of plasma copper or serum CER, although they are convenient markers of copper nutritional status, are relatively insensitive indices of tissue depletion. Cu levels were reflected in plasma Cu concentrations within the physiological range. Minks fed 116 mg/kg Cu during the growth and molting period (July–Nov) had a mean serum Cu concentration of 0.7 ± 0.4 mg/L (Mejborn, 1989) which is the same as the level detected in the present study. At the end of the trial, all the treatments consistently increased plasma Cu concentrations, suggesting that the absorption of Cu from the diets was sufficient to meet or exceed the requirement during this period of time. Assessment of copper status is complicated by the fact that normal ranges for plasma copper differ greatly between species. These plasma Cu concentrations were, however, within the normal range (0.4–1.5 mg/L) for mink (Aulerich et al., 1982; Powell et al., 1997; Wang, 2012).

Research on the effects of dietary copper on serum characteristics in mink is lacking. Papadimitriou and Loubbourdis (2005) found that frogs fed with diets of 100 mg Cu/kg of Cu for 30 d had GPT activity about twice as high at the end of the experiment as compared with the activity of this enzyme at the beginning of exposure. Karan et al. (1998) found that after a 14 d period of exposure to five concentrations of copper sulfate (0.25–4.0 mg/L CuSO4) in carp (Cyprinus caprio L.), activity of GOT and GPT in serum and gills increased. The increase in GPT and GOT observed in this study may indicate copper-related injury to the liver. Some researchers studied rats fed a diet containing Cu as CuCl2 (150–600 mg/kg) for 60 d, showed increased activity for both GOT and GPT in serum (Papadimitriou and Loubbourdis, 2002; Sugawara et al., 1995). Hwang et al. (1998) found that the activities of both aminotransferases increased in rats fed with diets of different concentrations of Cu for 2 months as the plasma concentration of Cu increased. Serum ALB is the most abundant blood plasma protein and is produced in the liver and forms a large proportion of all plasma protein (Farrugia, 2010). The decrease in serum ALB observed in this study also showed that copper-related injury to the liver.

Copper is the essential part of CER and Cu–Zn SOD (Santi et al., 2011; Vivoli et al., 1995; Wu et al., 2014). In most vertebrates species (except birds), CER is the main form of copper in plasma and is believed to be the major protein that transports copper to extrahepatic tissues. Our result of CER was similar to those found by Feng et al. (2007) who indicated that the activity of plasma CER was higher in pigs fed a diet supplemented with 250 mg Cu from CuSO4. DiSilvestro (1990) found rat CER activity rose with increasing Cu dosage. However, Ma and Li (2009) suggested that the copper levels

### Table 4

| Treatments | Plasma Cu | Plasma Zn | Plasma Fe |
|------------|-----------|-----------|-----------|
| Control    | 0.57      | 1.04      | 1.83      |
| Cu6        | 0.63      | 1.02      | 1.79      |
| Cu12       | 0.89      | 0.98      | 1.72      |
| Cu24       | 0.73      | 1.04      | 1.84      |
| Cu48       | 0.84      | 1.04      | 1.83      |
| Cu96       | 0.89      | 1.04      | 1.84      |
| Cu192      | 0.99      | 1.02      | 1.80      |
| SEM        | 0.02      | 0.02      | 0.03      |

*Data are expressed as least squares means with pooled SEM; n = 8 per treatment.*

### Table 5

| Treatments | GOT, U/L | GPT, U/L | ALP, U/L | TP, g/L | ALB, g/L | CER, U/L | Cu–Zn SOD, U/mL | TC, mmol/L | TG, mmol/L |
|------------|----------|----------|----------|---------|----------|----------|-----------------|------------|------------|
| Control    | 247      | 232      | 164      | 81.0    | 35.5     | 19.17    | 25.91           | 7.48       | 2.16       |
| Cu6        | 228      | 226      | 152      | 75.8    | 35.7     | 18.97    | 26.46           | 9.27       | 1.93       |
| Cu12       | 237      | 230      | 162      | 78.5    | 35.0     | 23.20    | 28.87           | 7.73       | 2.21       |
| Cu24       | 241      | 266      | 163      | 85.3    | 35.8     | 23.71    | 29.29           | 7.37       | 1.88       |
| Cu48       | 231      | 234      | 155      | 75.2    | 36.6     | 28.16    | 32.16           | 6.77       | 1.75       |
| Cu96       | 230      | 252      | 164      | 78.0    | 35.4     | 26.92    | 32.26           | 6.37       | 1.66       |
| Cu192      | 310      | 321      | 165      | 69.8    | 32.2     | 28.46    | 35.79           | 3.98       | 1.63       |
| SEM        | 6.71      | 8.42      | 3.21      | 1.24     | 0.44      | 0.72     | 0.81            | 0.52       | 0.07       |

*Data are expressed as least squares means with pooled SEM; n = 8 per treatment.*
in meat rabbit diet had no significant effect on the activities of Cu-Zn SOD and CER in serum. In our experiment, the activity of Cu-dependent enzymes was enhanced by increasing dietary Cu concentration. Serum TC and TG concentrations have been decreased due to Cu supplementation in other animals (Engle and Spears, 2000; Engle et al., 2000), consistent with our research.

5. Conclusions

The results of this feeding trial indicate that supplemental Cu plays an important role in the growth performance of mink, and the suitable supplemental level is 24 mg/kg. Results infer that supplementation of Cu in the diet of mink can alter the plasma lipid profile and copper concentration. Our results indicate that the activity of Cu-dependent enzymes is enhanced by increasing dietary Cu concentration and supplemental of Cu in the diet of mink can decrease serum TC and TG concentration.

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