Effects of dietary copper supplementation on nutrient digestibility, serum biochemical indices, and growth rate of young female mink (*Neovison vison*)

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ABSTRACT: The objective of this study was to determine whether nutrient digestibility and growth performance of mink were affected by different dietary copper levels. Ninety-six 60-day-old healthy female minks were randomly assigned to 8 treatment groups with 12 animals in each group and fed a diet supplemented with either 0, 4, 8, 16, 32, 64, 128 or 256 mg/kg copper as $CuSO_4 \times 5H_2O$, respectively. Our data showed that body weight and average daily gain increased (linear and quadratic, $P < 0.05$) as Cu increased in the diet, the highest body weight and average daily gain were seen in the Cu32 group. Feed : gain ratio responded in a linear ($P = 0.0025$) fashion with increasing level of Cu, the lowest feed : gain ratio was seen in the Cu64 group. Digestibility of ether extract responded in a linear ($P = 0.0190$) fashion with increasing level of Cu. There were no differences in apparent digestibility of dry matter, CP, and gross energy among groups ($P > 0.05$). N retention linearly ($P = 0.0363$) responded to increasing levels of Cu. Glutamic-oxalacetic transaminase and glutamic-pyruvic transaminase increased (linear and quadratic, $P < 0.05$) as Cu increased in the diet; the highest glutamic-oxalacetic transaminase and glutamic-pyruvic transaminase were seen in the Cu256 group. Total protein of the minks was similar among the treatments, however, albumin in serum responded in a linear ($P = 0.0370$) and quadratic ($P = 0.0049$) fashion with increasing level of Cu. The activity of ceruloplasmin responded in a linear ($P = 0.0001$) and quadratic ($P = 0.0203$) fashion with increasing level of Cu. The activity of Cu-Zn superoxide dismutase responded in a linear ($P = 0.0010$) fashion with increasing level of Cu. Our results indicate that supplemental Cu plays an important role in the growth performance of mink, helping young female mink digest and efficiently utilize added dietary fat.

Keywords: minks; copper sulfate; fat digestibility; growth performance; serum traits

INTRODUCTION

Copper is a key trace mineral in mink nutrition in terms of its role in hemoglobin formation and normal pigmentation of fur (Ajayi 2005; Ishizaki et al. 2010). The element is also an essential component of many enzyme systems such as cytochrome C oxidase, amino oxidase, polyphenol oxidase, ferroxidase, and copper-zinc superoxiode dismutase (Gaetke and Chow 2003). The National Research Council (1982) recommended 4.5–6.0 ppm Cu for mink diets. However, studies by Aulerich and Ringer (1976) indicated superior weight gains in male mink fed supplemental Cu at the 50 mg/kg level. Besides being an essential nutrient, Cu has received considerable attention due to its growth promoting and antimicrobial properties in animals when it has been fed at prophylactic levels (Zhou et al. 1994b; Pang et al. 2009; Lu et al. 2010; Karimi et al. 2011). Studies by Dove and Haydon (1992)

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and Dove (1995) have indicated that addition of 250 ppm Cu improved digestibility and utilization of the fat of weaned pigs. However, dietary Cu supplementation may have beneficial effects on digestibility and utilization of the fat of mink since typical mink diets usually contain considerable amounts of fat.

Little work has been done on the effects of Cu in mink. Therefore, the objectives of the present study were to determine the effect of dietary Cu levels on growth, feed conversion ratio, and digestibility of nutrients in mink. The optimum dietary Cu requirement for mink was also estimated.

MATERIAL 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 (CAAS). Animals were maintained and processed in accordance with the CAAS Guide for the Care and Use of Laboratory Animals.

Animals and diets. Ninety-six healthy young standard dark female minks were randomly assigned to eight groups with the following dietary treatments: Control – basal diet, no supplemental Cu; Cu4 – basal diet + 4 mg/kg Cu; Cu8 – basal diet + 8 mg/kg Cu; Cu16 – basal diet + 16 mg/kg Cu; Cu32 – basal diet + 32 mg/kg Cu; Cu64 – basal diet + 64 mg/kg Cu; Cu128 – basal diet + 128 mg/kg Cu; Cu256 – basal diet + 256 mg/kg Cu (from copper sulfate in all treatments). The final Cu content of diets was 7.63, 11.06, 14.98, 23.79, 40.51, 72.34, 133.68, and 259.16 mg/kg dry matter (DM), respectively. The randomization was based on age and the initial body weight (BW). Littermates were assigned to different groups in an effort to minimize genetic influence on reproduction and the variation in response to the dietary treatments. The average (± SD) age of the animals at the start was 60 ± 3 days. 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 feed and tap water *ad libitum* throughout the study. Diets were provided twice daily at 8:00 and 16:00 h. The ingredients and chemical composition of the basal diet, formulated according to National Research Council (1982) and Mertin et al. (2000), are given in Table 1.

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

Items	Content
Ingredients	
Extruded corn	36.2
Soybean meal	7.0
Corn gluten meal	8.0
Fish meal	14.0
Bone meat meal	18.0
Cheese meal	5.0
Soybean oil	8.0
Feather meal	1.0
Blood meal	1.0
Premix[1]	1.0
Lysine	0.3
Methionine	0.3
NaCl	0.2
Total	100.0
Nutrient levels[2]	
Crude protein	32.68
Ether extract	13.24
Crude carbohydrate	43.91
Lysine	1.64
Methionine	0.88
Cysteine	0.36
Ca	3.10
Total P	2.07
Cu (mg/kg)	7.63
Metabolizable energy (MJ/kg)	19.14
% from protein	32.10
% from fat	27.53
% from nitrogen-free extract	40.37

[1]contents per kg of premix: vitamin A 1 000 000 IU, vitamin D3 200 000 IU, vitamin E 6000 IU, vitamin B1 600 mg, vitamin B2 800 mg, vitamin B6 300 mg, vitamin B12 10 mg, vitamin K 3100 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

[2]values of CP, Ca, Cu, and total P were measured, other values calculated

Growth trial. The growth trial lasted for 12 weeks in a randomized design during which feed intake was recorded daily and the feed efficiency and average daily gain (ADG) were calculated. The ani-

mals were weighed (with the accuracy of 0.001 kg) biweekly after overnight fasting, and mean BW were used to determine growth rate.

Digestion and N balance experiment. On day 30 of the growing period, eight animals from each treatment group were selected randomly and housed individually in metabolic crates that allowed separation of urine and feces to determine nutrient digestibilities and N balance, based on the method described by Jorgensen and Glem-Hansen (1973). After a 4-day adaptation period in the metabolic cages, the digestive experiment lasted for 3 days and the excretions were collected each day. Feed was sampled for further analysis. After overnight fasting, fecal output was collected daily, weighed, and then 10% of the output was kept for subsequent analysis. Urine samples were collected in plastic containers, weighed, and recorded, and then 20% of the urine samples were kept to evaluate N retention. To avoid ammonium evaporation from the urine, 10 ml sulfuric acid (10% v/v) were added to the urine collection bottles and five drops of methylbenzene were added to prevent decaying. All samples were dried at 55°C in a forced-air oven to reach a constant weight, air equilibrated, and then ground to pass 1 mm screen and kept for further analysis. All samples were stored at –20°C for chemical analysis.

Blood samples. Blood samples were taken via the toe clip from mink after anesthesia in the morning before offering feed at the end of the experiment. Blood samples were collected in two separate tubes, one heparinized and one without an anti-coagulating substance. Samples were immediately transferred to the lab where plasma and serum was obtained subsequently by centrifuging the whole blood samples at 3000 rpm for 15 min. The heparinized plasma samples were frozen at –20°C until analyzed for Cu, Zn, and Fe concentrations. Serum samples were frozen at –80°C until analyzed for serum biochemical indices.

Chemical analysis. The chemical composition of feed and feces was analyzed by standard methods. Samples of feeds and feces were dried in a forced-air oven at 105°C for 24 and 48 h, respectively, and the DM content was calculated. Samples of feeds and feces were analyzed for ash (AOAC 2003; method ID 954.05), crude protein (CP) (AOAC 2003; method ID 954.01), and crude fibre (AOAC 2003; method ID 962.09). Ether extract (EE) was determined by extracting the sample with petroleum ether (AOAC 2003; method ID 920.39) using a Gerhardt Soxtherm 2000 Automatic Soxhlet Extraction System (C. Gerhardt Fabrik und Lager chemischer Apparate GmbH & Co. KG, Königswinter, Germany). Bomb calorimeter (Adiabatic Oxygen Bomb Calorimeter; Par Instrument Co., Moline, USA) was used to determine gross energy (GE). The metabolizable energy (ME) content was calculated on the basis of the concentrations of CP, EE, and carbohydrates, digestibility coefficients were calculated from Danish standard values for the individual feedstuffs, and the following values for the content of ME per unit of digestible nutrients (kJ/g) were detected: CP – 18.8, EE – 39.8, and crude carbohydrates – 17.6 (Hansen et al. 1991). Copper, zinc, iron concentrations of feed and plasma were analyzed by flame atomic absorption spectroscopy (Shimadzu Scientific Instruments, Kyoto, Japan). Plasma samples were prepared according to Solaiman et al. (2001). Total protein (TP), albumin (ALB), glutamic-pyruvic transaminase (GPT), and glutamic-oxalacetic transaminase (GOT) were determined by an automatic biochemistry analyzer Hitachi 7020 (Hitachi High Technologies, Inc., Ibaraki, Japan). Ceruloplasmin (CER) activity was determined using a ceruloplasmin ELISA kit (Nanjing Jiancheng Bioengineering Research Institute, China) and the following procedures were in accordance with instructions in the kit. The Cu-dependent enzyme activities of serum Cu-Zn superoxide dismutase (SOD) were tested by means of commercially available assay kits purchased from Nanjing Jiancheng Bioengineering Institute. The apparent digestibility (AD) of nutrients and energy was calculated as follows:

$$AD = (A - B)/A \times 100$$

where
A = nutrient or energy intake from feed
B = nutrient or energy excretion in feces

Statistical analysis. Data were analyzed using the General Linear Models (GLM) procedure of SAS (Statistical Analysis System, Version 9.0, 2002). The following model was used:

$$Y_{ij} = \mu + d_i + \varepsilon_{ij}$$

where
Y_{ij} = observation
μ = general mean
d_i = effect of Cu level (i = 1, ..., 8)
ε_{ij} = random error

Table 2. Effect of dietary copper levels on body weight and feed efficiency in mink (12 animals per group)

Item		Initial body weight (g)	Final body weight (g)	ADG (g/day)	F/G (g/g)
Group	Control	596.47 ± 37.89	973.40 ± 95.39^{ab}	6.28 ± 1.10^{ab}	13.63 ± 2.33^{b}
	Cu4	596.53 ± 33.67	960.90 ± 56.72^{abc}	6.07 ± 1.18^{ab}	13.84 ± 3.13^{b}
	Cu8	596.00 ± 48.57	968.20 ± 66.27^{ab}	6.20 ± 0.83^{ab}	13.83 ± 2.35^{b}
	Cu16	597.29 ± 54.16	1017.00 ± 58.54^{a}	7.00 ± 1.06^{a}	13.08 ± 1.38^{b}
	Cu32	594.29 ± 53.49	1031.70 ± 92.11^{a}	7.29 ± 1.89^{a}	12.93 ± 2.30^{b}
	Cu64	596.73 ± 67.04	1008.01 ± 114.44^{ab}	6.85 ± 1.62^{ab}	12.36 ± 2.05^{b}
	Cu128	597.84 ± 54.55	929.20 ± 96.68^{bc}	5.52 ± 1.72^{bc}	14.26 ± 4.12^{b}
	Cu256	598.28 ± 46.20	884.10 ± 89.52^{c}	4.76 ± 1.74^{c}	17.52 ± 7.39^{a}
P-value	linear	0.9994	0.0243	0.0190	0.0025
	quadratic	0.9852	0.0128	0.0092	0.0834

ADG = average daily gain, F/G = feed : grain ratio

$^{a–c}$statistically significant difference between groups ($P < 0.05$)

Data were mean ± SD. Statistically significant effects were further analyzed and means were compared using Duncan's Multiple Range Test. Linear and quadratic effects due to Cu level were determined. Probability values less than 0.05 ($P < 0.05$) were considered significant.

RESULTS

Body weight and feed conversion. Effects of different dietary Cu levels on growth performance of mink are shown in Table 2. There were no significant differences in bodyweight of mink at the start of the study ($P > 0.05$). At the end of the experiment, minks in either Cu16 or Cu32 had significantly higher BW than those in Cu128 and Cu256 ($P < 0.05$). BW of minks in either control, Cu8 or Cu64 groups was higher than that in group Cu256 ($P < 0.05$). Furthermore, minks supplemented with 32 mg/kg Cu had higher ADG (7.29 g) than the Cu96 ($P < 0.001$) and Cu256 ($P < 0.001$) groups. BW and ADG increased (linear and quadratic, $P < 0.05$) as Cu increased in the diet, the highest BW and ADG were seen in the Cu32 group. Feed : gain ratio responded in a linear ($P = 0.0025$) fashion with increasing level of Cu.

Nutrient digestibility. The digestibility of DM, CP, EE, and GE are shown in Table 3. Digestibility of EE responded in a linear ($P = 0.0190$) fashion with increasing level of Cu. There were no differences in apparent digestibility of DM, CP, and GE among groups ($P > 0.05$; Table 3).

N metabolism. Effects of different dietary Cu levels on N-balance of mink are shown in Table 4. The Cu32 and Cu128 groups had higher N retention than the Control group or the Cu256 group ($P < 0.05$). N retention linearly ($P = 0.0363$) responded to increasing levels of Cu. Daily N intake, fecal N

Table 3. Effect of dietary copper levels on nutrient digestibilities in mink (8 animals per group) (%)

Item		DM	CP	EE	GE
Group	Control	67.13 ± 3.89	70.44 ± 3.31	83.73 ± 3.17^{ab}	78.17 ± 2.99
	Cu4	67.19 ± 2.41	70.31 ± 1.64	85.10 ± 2.39^{ab}	77.57 ± 2.70
	Cu8	67.86 ± 3.40	69.86 ± 1.28	85.63 ± 3.59^{ab}	77.83 ± 1.33
	Cu16	69.90 ± 2.28	69.17 ± 3.58	87.82 ± 1.60^{a}	80.86 ± 3.72
	Cu32	68.02 ± 2.16	69.16 ± 2.28	88.51 ± 3.45^{a}	80.14 ± 3.80
	Cu64	67.11 ± 2.79	68.78 ± 2.73	88.25 ± 2.81^{a}	78.67 ± 2.07
	Cu128	68.84 ± 2.78	68.36 ± 1.61	87.56 ± 2.64^{a}	78.14 ± 4.88
	Cu256	70.10 ± 3.31	68.18 ± 3.77	82.22 ± 7.57^{b}	76.71 ± 4.57
P-value	linear	0.8738	0.7106	0.0190	0.5080
	quadratic	0.9452	0.9648	0.0580	0.2005

DM = dry matter, CP = crude protein, EE = ether extract, GE = gross energy

^{a,b}statistically significant difference between groups ($P < 0.05$)

Table 4. Effects of dietary copper levels on nitrogen metabolism in mink (8 animals per group) (g/day)

Item		N intake	Fecal N	Urine N	N retention
Group	Control	3.55 ± 0.30	1.10 ± 0.15	2.23 ± 0.26	0.23 ± 0.04^{bc}
	Cu4	3.43 ± 0.61	1.08 ± 0.16	2.10 ± 0.41	0.24 ± 0.06^{abc}
	Cu8	3.35 ± 0.56	1.00 ± 0.16	2.08 ± 0.40	0.27 ± 0.03^{abc}
	Cu16	3.60 ± 0.34	1.11 ± 0.05	2.21 ± 0.35	0.28 ± 0.04^{ab}
	Cu32	3.60 ± 0.34	1.10 ± 0.09	2.21 ± 0.40	0.29 ± 0.02^{a}
	Cu64	3.44 ± 0.34	1.03 ± 0.16	2.14 ± 0.23	0.28 ± 0.05^{ab}
	Cu128	3.45 ± 0.46	1.01 ± 0.15	2.16 ± 0.41	0.28 ± 0.02^{a}
	Cu256	3.11 ± 0.42	0.99 ± 0.18	1.90 ± 0.27	0.22 ± 0.05^{c}
P-value	linear	0.7527	0.9681	0.8794	0.0363
	quadratic	0.7578	0.8226	0.9204	0.1155

$^{a–c}$statistically significant difference between groups ($P < 0.05$)

and urinary N in animals were not significantly different among the treatment groups ($P > 0.05$).

Hematological parameters. The results of clinical chemistry as a further measure of the minks' response to nutritional regimens are depicted in Table 5. GOT and GPT increased (linear and quadratic, $P < 0.05$) as Cu increased in the diet; the highest GOT and GPT were seen in the Cu256 group. TP of the minks was similar among the treatments, however, albumin in serum responded in a linear ($P = 0.0370$) and quadratic ($P = 0.0049$) fashion with increasing level of Cu. The activity of CER responded in a linear ($P = 0.0001$) and quadratic ($P = 0.0203$) fashion with increasing level of Cu. The activity of Cu-Zn SOD responded in a linear ($P = 0.0010$) fashion with increasing level of Cu. Minks supplemented with 256 mg/kg Cu had higher activity of Cu-Zn SOD than the control, Cu4 or Cu8 groups ($P < 0.05$).

Plasma minerals. Plasma Cu, Zn, and Fe concentrations are presented in Table 6. Plasma Cu concentrations increased (linear, $P < 0.05$) as Cu increased in the diet; the highest plasma Cu concentrations were seen in the Cu256 group. Furthermore, Cu supplementation had no effect on plasma Zn concentrations or plasma Fe concentrations ($P > 0.05$).

DISCUSSION

Studies at Cornell University with mink on purified diets indicated that 20 mg/kg Cu was satisfactory for growth and fur development (McCarthy et al. 1966). In this study, we found that growth rate

Table 5. Effects of copper supplementation on hematological parameters and serum biochemical parameters in mink (8 animals per group)

Item		GOT (U/l)	GPT (U/l)	TP (g/l)	ALB (g/l)	CER (U/l)	Cu-Zn SOD (U/ml)
Group	Control	236.80 ± 26.72^{b}	216.04 ± 20.09^{b}	77.66 ± 3.36	34.64 ± 1.80^{ab}	21.76 ± 3.72^{c}	27.63 ± 8.84^{b}
	Cu4	238.07 ± 21.32^{b}	231.47 ± 44.45^{b}	79.18 ± 3.76	35.52 ± 2.11^{a}	22.04 ± 2.86^{c}	29.66 ± 7.84^{b}
	Cu8	227.94 ± 26.84^{b}	226.00 ± 28.69^{b}	79.28 ± 5.01	35.71 ± 2.21^{a}	22.72 ± 2.91^{c}	28.48 ± 5.31^{b}
	Cu16	228.67 ± 22.54^{b}	229.60 ± 53.50^{b}	78.00 ± 5.94	35.03 ± 0.99^{ab}	23.20 ± 3.35^{bc}	31.04 ± 6.40^{ab}
	Cu32	228.51 ± 49.06^{b}	226.17 ± 34.18^{b}	79.12 ± 5.47	35.79 ± 4.69^{a}	23.71 ± 3.35^{bc}	32.50 ± 2.61^{ab}
	Cu64	231.34 ± 35.69^{b}	234.40 ± 44.76^{b}	78.67 ± 5.90	36.59 ± 4.94^{a}	28.16 ± 4.76^{a}	34.25 ± 7.01^{ab}
	Cu128	229.98 ± 38.69^{b}	251.50 ± 95.98^{b}	81.73 ± 3.18	36.65 ± 1.52^{a}	26.92 ± 4.14^{ab}	34.20 ± 1.71^{ab}
	Cu256	310.12 ± 78.45^{a}	320.85 ± 7.63^{a}	76.78 ± 6.91	32.21 ± 2.57^{b}	28.46 ± 4.56^{a}	36.76 ± 5.31^{a}
P-value	linear	0.0001	0.0001	0.7190	0.0370	0.0001	0.0010
	quadratic	0.0094	0.2928	0.0934	0.0049	0.0203	0.1644

GOT = glutamic-oxalacetic transaminase, GPT = glutamic-pyruvic transaminase, TP = total protein, ALB = albumin, CER = ceruloplasmin, Cu-Zn SOD = Cu-Zn superoxide dismutase

$^{a–c}$different superscripts indicate statistically significant difference between groups ($P < 0.05$)

Table 6. Effects of dietary Cu supplementation on plasma Cu, Zn, and Fe concentrations of mink (8 animals per group)

Item		Plasma Cu (mg/l)	Plasma Zn (mg/l)	Plasma Fe (mg/l)
Group	Control	0.60 ± 0.04^{d}	0.97 ± 0.13	1.88 ± 0.19
	Cu4	0.59 ± 0.04^{d}	1.05 ± 0.11	1.87 ± 0.17
	Cu8	0.66 ± 0.07^{cd}	0.96 ± 0.10	1.85 ± 0.20
	Cu16	0.69 ± 0.09^{c}	1.04 ± 0.10	1.79 ± 0.19
	Cu32	0.75 ± 0.12^{c}	0.96 ± 0.11	1.77 ± 0.27
	Cu64	0.74 ± 0.08^{c}	1.05 ± 0.13	1.82 ± 0.15
	Cu128	0.86 ± 0.08^{b}	1.05 ± 0.12	1.81 ± 0.24
	Cu256	1.01 ± 0.11^{a}	1.03 ± 0.18	1.98 ± 0.24
P-value	linear	0.0001	0.3399	0.1565
	quadratic	0.0704	0.4525	0.1249

$^{a–d}$statistically significant difference between groups ($P < 0.05$)

of mink fed diets with over 100 mg/kg added Cu decreased, whereas mink fed the added 32 mg/kg of Cu diet had higher growth rate compared to those fed less than 8 mg/kg of Cu diet. The data from this trial are in agreement with the data from Aulerich and Ringer (1976) in male mink, who reported that there was a greater growth response in male mink fed the higher level of supplemental Cu and a greater increase in weight gains of males fed 25 mg/kg Cu. In another studies, Aulerich et al. (1982) noted that supplemental dietary Cu at levels up to 200 mg/kg did not stimulate mink body weight gains during the post weaning growth period, however, at the end of experiment control-fed minks had the highest BW compared with other groups. It is of interest to note that in the 1982 research work, the control diet contained 60 mg/kg Cu, 330 mg/kg Zn, and 330 mg/kg Fe. Our experiments showing that feed efficiency was affected by Cu levels is consistent with Skrivan et al. (2002). However, the data from this trial are in disagreement with the data of Li et al. (2008) who noticed that an improvement in feed efficiency by Cu supplementation, even though not as great as the improvement in growth, has generally been observed.

The apparent digestibilities of CP and EE in this trial were slightly lower compared to values reported in other trials (Ahlstrom 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 (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 are characterized by lower digestibility than components of fresh feed (Vhile et al. 2005; Gugolek et al. 2010). In addition, another factor influencing the digestibility of nutrients is the fat : carbohydrate (F : C) ratio. The digestibility of CP increased whereas that of carbohydrates decreased with the F : C ratio increasing (Suvegova et al. 2000). The increase in dietary fibre and fat contents may interfere with the apparent digestibility of CP and EE in the present experiments. Suvegova et al. (2001) reported that the digestibility of CP and fats decreased with increasing percentual proportion of poultry shanks in feed rations for minks – for CP from 80.75 to 70.35%, and for fats from 90.14 to 84.24%. In our study, EE digestibility was increased with increasing dietary Cu levels. Similar results were reported in pigs (Dove 1995; Luo and Dove 1996) and in Black Bengal kids (Datta et al. 2007). Luo and Dove (1996) reported that the addition of 250 mg/kg Cu improved digestibility and utilization of fat in weanling pigs. The improvement in apparent fat digestibility due to Cu addition (more than 48 mg/kg) observed in the present study and the previous study may also be partially due to increased lipase and phospholipase A activities in the small intestine (Dove 1995; Luo and Dove 1996). Moreover, Cu functions biochemically as a component of several Cu-dependent enzymes and as a co-factor for numerous other enzymes. The improved fat digestibility resulting from Cu addition could lead to the increased absorption of fatty acids and fat soluble vitamins and affect other aspect of nutrient metabolism in the body and therefore stimulate growth of the mink. It is also possible that high dietary Cu concentrations enhance growth of mink by stimulating activities of the enzymes involved in nutrient utilization.

Studies by Mejborn (1989) on copper sulfate supplementation of mink diets indicated that 75–90% of the Cu intake was excreted in the feces and that urinary Cu excretion was elevated with increased Cu intake. This high excretion of Cu can increase bile excretion (Harada et al. 1993). The primary function of bile is to emulsify fats in the small intestine and correspondingly to promote the fat digestibility. To our knowledge, this field of research is unexplored in mink, and further investigations to better understand the relationships between fat digestibility and Cu addition are needed.

The N retention in this trial was the same as the result of Hellwing et al. (2005). However, the experiment results attained much lower values compared to those reported in other trials (Zhang et al. 2012; Zhang et al. 2013). In the study, collection routine was very precise and avoided the urinary N loss. The N retention was in accordance with average daily gain in the present experiments. Little is known about the effects of dietary Cu on N balance in mink but information is available for pig. For example, Luo and Dove (1996) reported that Cu supplementation significantly ($P < 0.02$) improved apparent N retention in weanling pigs. Zhou et al. (1994a) demonstrated that Cu given by either intravenous injection or oral intake increased serum mitogenic activity (mitogenic peptides, an indicator of blood growth factor activity) in weanling pigs, and increased pituitary growth hormone mRNA concentration. Cu also was shown to stimulate growth hormone secretion from bovine pituitary explants *in vitro* (Yang et al. 2012). Therefore it is possible that Cu enhances protein retention and protein synthesis by stimulating hormone and growth factors in mink.

Research on the effects of dietary Cu on serum characteristics in mink is lacking. Papadimitriou and Loumbourdis (2005) found that frog fed diets of 100 mg Cu/kg for 30 days 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-day period of exposure to five concentrations of copper sulfate (0.25–4.0 mg/l $CuSO_4$) in carp (*Cyprinus caprio* L.), activity of GOT and GPT in serum and gills increased. These transaminases are the most sensitive indicators of hepatic cell injury. The increase in GPT and GOT observed in this study may indicate Cu-related liver injury. Albumin measurements are the most useful in the assessment of impairment severity of the liver synthetic functions. Low levels of albumin may indicate liver damage. TP concentrations in liver disease are often near normal because a decrease of albumin may be offset by an increase of globulins. Fischer studied rats fed a diet containing Cu as $CuCl_2$ (150–600 mg/kg) for 60 days and showed increased activity for both GOT and GPT in serum (Papadimitriou and Loumbourdis 2002). Hwang et al. (1998) found that activities of both aminotransferases increased in rats fed diets of different concentrations of Cu for 2 months as the plasma concentration of Cu increased. The variable amount of Cu stored in liver acts to maintain plasma Cu concentrations even in the case of low Cu supply. Values of plasma Cu or serum CER, although convenient markers of Cu nutritional status, are a relatively insensitive indice of tissue depletion. Copper is the essential part of CER and Cu-Zn SOD (Vivoli et al. 1995; Santi et al. 2011). In most vertebrate species (except birds), CER is the main form of Cu in plasma and is believed to be the major protein that transports Cu to extrahepatic tissues. Our result of CER was similar to that 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 $CuSO_4$. Hussein and Staufenbiel (2012) found CER activity rose with increasing Cu dosage. In our experiment, the activity of Cu-dependent enzymes was enhanced by increasing dietary Cu concentration. The increase in plasma Cu concentrations as a result of Cu supplementation during the course of the study, in all the treatments, is consistent with Powell et al. (1997), who reported an increase in plasma Cu of mink which received different levels of Cu. Our results contradict to Aulerich et al. (1982) who reported no changes in plasma Cu of mink receiving 25, 50, 100, and 200 mg/kg Cu treatments. Mink fed 116 mg/kg Cu during the growth and molting period (July–November) 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.

CONCLUSION

The results of this feeding trial indicate that supplementary Cu plays an important role in the growth performance of mink. The data show that Cu is important in the digestion of dietary fat in young female mink and that after Cu supplemen-

tation growing furring mink can utilize added dietary fat more efficiently. The study indicated improvements in growth performance due to increased fat utilization and increased N retention.

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