EFFECTS OF SUPPLEMENTAL DIETARY COPPER ON GROWTH, REPRODUCTIVE PERFORMANCE AND KIT SURVIVAL OF STANDARD DARK MINK AND THE ACUTE TOXICITY OF COPPER TO MINK[1]

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Summary

Natural dark mink kits were fed a diet supplemented with 0, 25, 50, 100 or 200 ppm Cu from $CuSO_4 \cdot 5H_2O$ for 153 or 357 d. The shorter term Cu supplementation had no significant beneficial or adverse effects on mink body weight gains or hemoglobin or hematocrit concentrations, although plasma Cu concentrations were slightly elevated in the mink fed added Cu. Liver Cu concentrations were significantly increased only in the mink fed 200 ppm Cu. Liver Zn and Fe concentrations were not affected by the added Cu. Darker fur was observed in pelted males fed the higher levels of Cu. The reproductive performance of mink on the longer term Cu supplementation was not adversely affected, although greater kit mortality and reduced "litter mass" were a result of the higher Cu concentrations. The acute (21-d) ip LD_{50} concentrations of Cu sulfate and Cu acetate in adult mink were 7.5 and 5.0 mg/kg, respectively.

(Key Words: Mink, Supplemental Copper, Reproductive Performance, Zinc, Mortality.)

Introduction

In recent years early kit losses have become prevalent in certain strains of natural dark mink. Kits from the affected strains are generally less thrifty and smaller, with considerable losses occurring when the kits are handled, such as during vaccination or when litters are "broken down" and separated. Under these circumstances, death appears to be associated with anemia and a stress-induced type of shock. Dietary Fe supplementation has not alleviated the problem, and recent interest has focuses on the possible role of Cu in this disorder.

Numerous studies have demonstrated the beneficial effects of supplemental dietary Cu in excess of the requirement on growth rate in swine (Hawbaker et al., 1961; Barber et al., 1962; Braude, 1965; Castell and Bowland, 1968; Drouliscos et al., 1970), poultry (Smith, 1969; Jenkins et al., 1970; King, 1972) and rabbits (King, 1975). According to Castell and Bowland (1968), Drouliscos et al. (1970) and Jenkins et al. (1970), there is a greater growth response to supplemental Cu in animals fed diets high in fish meal, as opposed to soybean meal diets, because the minerals in fish meals tend to bind Cu. Because typical mink diets usually contain considerable quantities of fish meals, supplemental dietary Cu may prove beneficial.

In a preliminary feeding trial in which 0, 25 or 50 ppm supplemental Cu from $CuSO_4 \cdot 5H_2O$ was fed to dark mink kits from July 22 through pelting (December 8), there was a greater ($P<.01$) growth response in male mink fed the higher level of supplemental Cu and a greater (although not significantly different from the control) increase in the weight gains of the males fed 25 ppm Cu and in the females that received the two diets that contained supplemental Cu (Aulerich and Ringer, 1976).

Based on the involvement of Cu in certain types of anemia and the apparent stimulatory effect of supplemental Cu on growth, this study was conducted to investigate further the role of Cu in the physiology and nutrition of mink. Because Cu toxicity data on mink are lacking in the literature, trials were also conducted to determine an LD_{50} for Cu sulfate and Cu acetate.

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Experimental Procedure

Feeding Study. The experiment was started July 6, 1979. One hundred twenty standard dark mink kits were assigned to five groups (each containing 12 males and 12 females) and placed on the following dietary treatments: (1) basal diet[3], no supplemental Cu (control); (2) basal diet plus 25 ppm Cu from Cu sulfate[4]; (3) basal diet plus 50 ppm Cu from Cu sulfate; (4) basal diet plus 100 ppm Cu from Cu sulfate; (5) basal diet plus 200 ppm Cu from Cu sulfate.

Littermates were divided among the various groups in an effort to minimize genetic influence on reproduction and response to the dietary treatments. From the start of the study through December 1979, the animals were housed individually in open-sided sheds in mink growing cages (61 cm long x 30.5 cm wide x 38 cm high) with additional attached nest boxes (20 cm long x 16.5 cm wide x 29 cm high). During the remainder of the study, the mink were kept in breeder cages (76.2 cm long x 61 cm wide x 45.7 cm high) with attached nest boxes (38.1 cm long x 29.2 cm wide x 26.7 cm high). They were cared for according to routine commercial ranch procedures. Feed and water were provided ad libitum. The animals were weighed biweekly from July 6 through August 31, and monthly thereafter.

Eight males from each group were killed on December 6, 1979[5]. The remaining 12 females and four males in each group were retained on their respective diets through July 1980 for an evaluation of the effects of the supplemental Cu on reproduction and early kit growth and survival.

Before being killed, the eight males in each group were weighed, and blood samples were taken by heart puncture for plasma Cu analysis[6] and by toe clip for hemoglobin[7] and hematocrit determinations. During necropsy, the livers were weighed and samples taken for analysis and histologic examination.

Triplicate liver samples (~1.0 g) were digested in concentrated nitric acid-70% perchloric acid (2:1) and the Cu, Zn and Fe concentrations

[3] The basal diet consisted of 25% mink cereal, 20% ocean fish scrap, 20% whole chicken, 15% beef tripe, 7.5% beef lungs, 7.5% beef trimmings and 5% beef liver; "as fed" contained 35.13% dry matter.

[4] Analytical grade $CuSO_4 \cdot 5H_2O$, Mallinckrodt Chem. Co., Paris, KY 40361.

[5] Seven from the control group. One control male died as a result of a diaphragmatic hernia (MSU Pathology Report No. 202919).

[6] Determined by spectrophotometric method (Tietz, 1976).

[7] Determined by cyanomethemoglobin method with a hemoglobinometer, Coulter Electronics Inc., Hialeah, FL.

TABLE 1. MEAN INITIAL BODY WEIGHTS AND BODY WEIGHTS AT 8 AND 20 WK FOR MINK FED VARIOUS CONCENTRATIONS OF SUPPLEMENTAL COPPER[a]

	Body weights (g)					
Dietary treatment	No.	Initial (7/6/79)	No.	8 wk (8/31/79)	No.	20+ wk (11/26/79)
Male						
Control	12	709	12	1,525	11	1,897
25 ppm supplemental Cu	12	676	12	1,425	12	1,828
50 ppm supplemental Cu	11	701	11	1,438	11	1,831
100 ppm supplemental Cu	12	625	12	1,351	12	1,693
200 ppm supplemental Cu	12	650	12	1,374	12	1,753
SE		36		46		58
Female						
Control	12	517	12	909	12	1,074
25 ppm supplemental Cu	12	563	12	937	12	994
50 ppm supplemental Cu	12	545	12	947	12	1,068
100 ppm supplemental Cu	12	536	12	947	12	1,065
200 ppm supplemental Cu	12	534	12	965	12	1,058
SE		24		34		35

[a] Fed from July 6 to November 26, 1979.

TABLE 2. MEAN HEMOGLOBIN, HEMATOCRIT AND PLASMA COPPER LEVELS IN MINK FED A CONTROL DIET OR SUPPLEMENTAL COPPER

			Mink fed experimental diets from 7/6/79 to 12/6/79					Mink fed experimental diets from 7/6/79 to 6/27/80	
Dietary treatment	No.	Sex	Hemoglobin, g/dl	Hematocrit, %	Plasma Cu, µg/dl	No.	Sex	Hemoglobin, g/dl	Hematocrit, %
Control	7	♂	22.5	56.8	54.4	4	♂	22.0	51.7
						10	♀	20.9	49.5
25 ppm supplemental Cu	8	♂	22.5	55.8	78.6	4	♂	21.0	52.5
						11	♀	21.0	52.5
50 ppm supplemental Cu	8	♂	21.7	54.3	73.2	4	♂	21.2	54.4
						12	♀	21.0	52.0
100 ppm supplemental Cu	8	♂	22.0	55.8	61.8	3	♂	20.8	53.9
						12	♀	22.1	54.9[a]
200 ppm supplemental Cu	8	♂	22.4	54.6	65.6	3	♂	22.4	54.3
						11	♀	20.8	52.4
SE			.3	.8	6.7		♂	.6	1.3
							♀	.6	1.1

[a]Different ($P<.05$) from control value for same sex.

were measured with an atomic absorption spectrophotometer[8]. The liver samples taken for histology were fixed in neutral buffered formalin and embedded, sectioned and stained according to Uzman's (1956) method for Cu detection.

In an effort to evaluate the effects of Cu supplementation on fur quality, we arranged the pelts from the male mink according to color (darkest to lightest) and assigned numerical scores of 1 to 5 as follows: darkest 20%, 5; the next darkest 20%, 4; et cetera.

The females were mated with males within their respective dietary groups between March 1 and 20, 1980. All matings were confirmed by the presence of motile sperm in vaginal smears taken immediately after mating. During the whelping period (April 15 to May 15, 1980), the mated females were checked daily for evidence of whelping. The kits were counted and weighed on the day of birth and at 4 wk of age.

At the end of the feeding trial (June 27, 1980), blood samples were collected from the remaining animals by toe clip for hemoglobin and hematocrit determination.

Body weight comparisons were made with initial body weight as a covariate. Differences between means were determined using Dunnett's test on the adjusted treatment means.

Acute Toxicity. During November, 1979, an attempt was made to determine oral LD_{50} values for Cu sulfate ($CuSO_4 \cdot 5H_2O$) and Cu acetate [$Cu(C_2H_3O_2)_2 \cdot H_2O$] in mink. Oral dosing (by gavage) of mink with either compound, however, was not feasible because the animals consistently vomited immediately after dosing. Thus, an alternate method of dosing consisting of ip injection of the Cu compounds was selected for the LD_{50} trials.

Forty four previously untreated, standard dark, 6-mo-old mink were used in the test. The compounds to be administered were dissolved in distilled water to provide concentrations for injection into the peritoneum of the mink of not more than 1 ml in volume. Intraperitoneal injections of distilled water or sodium sulfate were administered to mink as a control.

[8] Model 453, Instrumentation Laboratory, Inc., Wellington, MA.

Results and Discussion

Feeding Trial. Atomic absorption spectrophotometric analysis revealed that the control diet contained 60.5, 329.7 and 327.7 ppm (dry weight basis) of Cu, Zn and Fe, respectively. No Cu or Zn was detected in the drinking water, although it contained .5 ppm Fe. A Cu requirement for mink has not been established, but according to N. Glem Hansen (personal communication), 4.5 to 6 mg Cu/kg dry feed considered adequate.

Supplemental dietary Cu at levels up to 200 ppm did not stimulate mink body weight gains during the postweaning growth period (table 1), as had previously been observed with male mink, as well as other species. However, the Cu-supplemented diets did not have any observed toxic effects on the adult animals either.

TABLE 3. MEAN LIVER WEIGHT AND MEAN LIVER COPPER, ZINC AND IRON CONCENTRATIONS IN MALE MINK FED VARIOUS LEVELS OF SUPPLEMENTAL COPPER[a]

Dietary treatment	No.	Liver weight, %[b]	Liver concentration (ppm, dry weight)		
			Cu	Zn	Fe
Control	7	2.69	293	507	1,197
25 ppm supplemental Cu	8	2.61	340	504	1,121
50 ppm supplemental Cu	8	2.76	411	488	1,261
100 ppm supplemental Cu	8	2.85	364	516	1,301
200 ppm supplemental Cu	8	2.56	479[c]	530	1,267
Se		.17	35	23	141

[a] Fed from July 6 to December 6, 1979.

[b] Expressed as a percentage of body weight.

[c] Different ($P<.05$) from control value.

TABLE 4. AVERAGE PELT COLOR SCORES FOR MALE MINK FED VARIOUS LEVELS OF SUPPLEMENTAL COPPER[a]

Dietary treatment	No.	Avg pelt color score[b]
Control	7	3.0
25 ppm supplemental Cu	8	2.2
50 ppm supplemental Cu	8	2.9
100 ppm supplemental Cu	8	3.5
200 ppm supplemental Cu	8	3.6

[a]Fed from July 6 to December 6, 1979.

[b]Mink pelts were graded on a scale of 1 (lightest) to 5 (darkest). The darkest 20% were assigned a score of 5; the next darkest 20% a score of 4 . . . et cetera.

which suggests that mink may be among the more Cu-tolerant species. Rats and swine have been reported to tolerate up to 250 ppm dietary Cu, but other species, especially ruminants, are considerably less tolerant of Cu (Buck et al., 1976).

The Cu supplementation of the diets had no influence on the hematocrit or hemoglobin values of the pelted males, although slightly elevated plasma Cu concentrations were detected in the treated mink (table 2). These plasma Cu concentrations were, however, within the normal range (.5 to 1.5 μg/ml) for most species (Bull, 1980).

The liver weights of the pelted males were not affected by supplemental dietary Cu (table 3). Although microscopic examination of liver sections stained with Uzman's stain failed to reveal any significant deposition of Cu in the treated males, atomic absorption spectrophotometry showed that the Cu concentrations in the liver (table 3) were considerably greater than normal for most adult nonruminant animals (10 to 50 ppm Cu, dry basis; Bull, 1980), but were within the broad range reported by Fisher (1975). Because the liver is one of the main organs involved in the storage and metabolism of Cu, liver Cu concentration might be considered indicative of an animal's Cu status. Although only the liver Cu levels of the mink fed 200 ppm Cu were greater ($P<.05$) than those of the controls, there was a positive correlation of .878 between the dietary Cu level and liver Cu levels. These data suggest that mink may be similar to sheep and swine in that liver Cu stores increase in proportion to dietary Cu

TABLE 5. REPRODUCTIVE PERFORMANCE OF FEMALE MINK FED THE CONTROL DIET OR DIETS THAT CONTAINED SUPPLEMENTAL COPPER AND THE AVERAGE WEIGHT, LITTER MASS[a] AND SURVIVAL OF THEIR KITS

Dietary treatment	No. ♀'s whelped/ no. mated	Avg gestation[b], d	No. kits whelped		Avg no. kits whelped/♀		% kit mortality birth to 4 wk	Avg ± SE kit weight, g		Litter mass, g
			Alive	Dead	Whelped	Mated		At birth	At 4 wk	
Control	11/12	47.2	69	8	7.0	6.4	12	8.8 ± .2[c]	136.9 ± 1.4[c]	704.6
25 ppm supplemental Cu	6/11	46.7	33	5	6.3	3.5	9	9.7 ± .3[c]	143.0 ± 3.7[c]	666.5
50 ppm supplemental Cu	12/12	49.6	64	6	5.8	5.8	19	9.4 ± .2[c]	133.3 ± 2.7[c]	582.3
100 ppm supplemental Cu	8/12	49.4	50	4	6.8	4.5	38	8.5 ± .2[c]	116.4 ± 7.1[d]	474.8
200 ppm supplemental Cu	10/12	46.7	57	6	6.3	5.3	32	8.3 ± .3[c]	143.3 ± 4.8[c]	580.5

[a]Average kit body weight gain between birth and 4 wk of age X the average number of kits raised per lactating female.

[b]Based on date of final mating.

[c,d]Body weight means for each age followed by different superscripts differ ($P<.05$).

intake (Suttle and Mills, 1966). Rats on the other hand, are reported to maintain normal liver Cu levels until a high (1,000 ppm) dietary level is reached (Milne and Weswig, 1968).

Although no differences in liver Zn or Fe levels were found among the mink fed the various levels of Cu, Suttle and Mills (1966) noted that Cu supplementation increased liver Zn concentrations in swine. These researchers, and others (Bunch et al., 1963; DeGoey et al., 1971), have also observed that low dietary Zn and Fe levels tend to accentuate Cu toxicity. Thus, the observed tolerance of mink for dietary Cu may be influenced by complicated interactions between Cu and other elements, such as Mo, Zn, Fe and S.

Although the average fur color scores of those males pelted (table 4), suggest that "high" levels of supplemental Cu may have a beneficial effect on intensifying hair color of dark mink, these results are based on a limited number of observations and additional experiments are being conducted to verify this finding.

The reproductive performance of the female mink fed the Cu-supplemented diets is summarized in table 5. In general, the overall performance of the control mink was superior to that of mink fed supplemental Cu. However, except for the trend toward greater kit mortality between birth and 4 wk of age and the reduced litter mass at weaning with increased Cu supplementation, the characteristics measured were, for the most part, within the normal range for mink. Gestation length and kit weight at birth were not adversely affected by the dietary treatments. The greater kit mortality during the nursing period and the reduced litter mass at weaning, however, suggest that the higher levels of supplemental Cu may have had an adverse affect on lactation.

Acute Toxicity. Levels of the compounds administered, mortality rates and length of survival of the mink that died during the 21-d posttreatment period are shown in table 6. Within 2 min after dosing, almost all the Cu-treated mink attempted to vomit. They salivated profusely and became lethargic, laying on either their sides or backs and moving only if forced to do so. The Cu-treated mink that survived were usually "off feed" for several days and full recovery was slow, as indicated by a decrease in body weights during the posttreatment period. Although the sodium sulfate injections administered for control purposes were not lethal, the animals did show a loss in body weight during the 21-d posttreatment observation period. All mink that died from Cu poisoning showed profuse hemorrhaging throughout the body when necropsied.

TABLE 6. ACUTE TOXICITY OF COPPER SULFATE AND COPPER ACETATE

Compound	Dose[a] mg/kg	No. died/ no. treated	Mortality[b], %	Mean length of survival of animals that died, d	Mean body weight gain (or loss) of mink that survived to 21-d posttreatment, g
Copper sulfate	0[c]	0/6	0		48.3
	3.1	0/6	0		(− 54.2)
	6.2	2/6	33.3	7	(− 61.3)
	9.4	4/6	66.7	4.25	(−122.5)
	12.5	6/6	100	<1	
	25.0	6/6	100	<1	
Sodium sulfate	14.2	0/2	0		(− 90)
Copper acetate	5	1/2	50	2	(− 30)
	10	2/2	100	<1	
	20	2/2	100	1.5	

[a] Intraperitoneal.

[b] Up to 21 d posttreatment.

[c] 5 ml distilled water injected ip.

The acute (21-d) ip LD_{50} for Cu sulfate, as determined by the method of Litchfield and Wilcoxon (1949), was 7.5 mg/kg, with a 95% confidence interval of 5.2 to 10.9 mg/kg. The equation for the slope of the regression line was $Y = -33.36 + 10.67\ X$, where Y = percentage mortality and X = dosage in mg/kg body weight. The LD_{50} for the Cu acetate was 5.0 mg/kg, with a 95% confidence interval of 2.1 to 12.0 mg/kg. The regression equation for the slope of the line was $Y = 25.00 + 5.00\ X$.

Literature Cited

Aulerich, R. J. and R. K. Ringer. 1976. Feeding copper sulfate: Could it have benefits in nutrition of mink? U.S. Fur Rancher 56(12):4.

Barber, R. S., R. Braude and K. G. Mitchell. 1962. Copper sulphate and molasses distillers dried solubles as dietary supplements for growing pigs. Anim. Prod. 4:233.

Braude, R. 1965. Copper as a growth stimulant in pigs. In: Cuprum Pro Vita (Transactions of a Symposium). p 55. Copper Development Assoc., London.

Buck, W. B., G. D. Osweiler and G. A. VanGelder. 1976. Clinical and Diagnostic Veterinary Toxicology (2nd Ed.). Kendall-Hunt Publishing Co., Dubuque, IA.

Bull, R. C. 1980. Copper. ANH Mineral Series. Anim. Nutr. Health 35(8):32.

Bunch, R. G., V. C. Speer, V. W Hays and J. T. McCall. 1963. Effects of high levels of copper and chlortetracycline on performance of pigs. J. Anim. Sci. 22:56.

Cantell, A. G. and J. P. Bowland. 1968. Supplemental copper for swine: Growth, digestibility, and carcass measurements. Can. J. Anim. Sci. 48:403.

DeGoey, L. W., R. C. Wahlstrom and R. J. Emerick. 1971. Studies of high level copper supplementation to rations for growing swine. J. Anim. Sci. 33:52.

Drouliscos, N. J., J. P. Bowland and J. I. Elliot. 1970. Influence of supplemental dietary copper on copper concentration of pig blood, selected tissues, and digestive tract contents. Can. J. Anim. Sci. 50:113.

Fisher, G. L. 1975. Function and homeostasis of copper and zinc in mammals. Sci. Total Environ. 4:373.

Hawbaker, J. A., V. C. Speer, V. W. Hays and D. V. Catron. 1961. Effect of copper sulfate and other chemotherapeutics in growting swine rations. J. Anim. Sci. 20:163.

Jenkins, N. T., T. R. Morris and D. Valamotis. 1970. The effect of diet and copper supplementation on chick growth. Brit. Poul. Sci. 11:241.

King, J.O.L. 1972. The feeding of copper sulphate to growing fowls. Brit. Poul. Sci. 13:61.

King, J.O.L. 1975. The feeding of copper sulphate to growing rabbits. Brit. Vet. J. 131:70.

Litchfield, J. T., Jr. and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Therap. 96:99.

Milne, D. B. and P. H. Weswig. 1968. Effect of supplementary copper on blood and liver copper-containing fractions in rats. J. Nutr. 95:429.

Smith, M. S. 1969. Responses of chicks to dietary supplements of copper sulphate. Brit. Poul. Sci. 10:97.

Suttle, N. F. and C. F. Mills. 1966. Studies of the toxicity of copper to pigs. I. Effects of oral supplements of zinc and iron salts on the development of copper toxicosis. Brit. J. Nutr. 20:135.

Tietz, N. W. 1976. Fundamentals of Clinical Chemistry (2nd Ed.). W. B. Sanders Co., Philadelphia, PA.

Uzman, L. L. 1956. Histochemical localization of copper with rubeanic acid. Lab. Invest. 5:299.