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Interactions of copper, cadmium and molybdenum in buffalo calves: the levels of trace element in blood, urine and tissues

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Abstract— Ten male buffalo calves were randomly allotted into five groups of two each. Four groups were fed with cadmium, molybdenum, cadmium–molybdenum, and copper-cadmium-molybdenum respectively for 130 days to determine the elements' metabolic interactions in calves. These results indicated that cadmium and molybdenum could increase the accumulation of molybdenum and cadmium in liver and kidneys in buffalo calves, but copper could not reduce to normal the levels of molybdenum and cadmium in liver and kidneys caused by cadmium-molybdenum. In addition, we found the copper concentrations in liver and kindeys was significantly greater in treatment calves than in controls.

Keywords: trace elements; cadmium; copper; molybdenum; buffalo calves.

Cadmium is a cumulative poison. It accumulates in the body over many years, especially in the liver and kidneys (Doyle, 1978). Cadmium's interaction with other trace metals in the tissues of several species have been studied extensively. Some researchers have reported that tissue concentrations of copper are lowered by relatively high levels of dietary cadmium. The metabolic antagonism between cadmium and copper was shown (Sandstead, 1977). The cadmium concentration in the blood remains low in spite of its high intake (Bartik, 1981).

Molybdenum is one of the essential elements in animals. It is also present in the tissues of organs. Molybdenum is eliminated fairly rapidly via the kidneys and in the bile, hence, under normal conditions the element does not accumulate in the body (Bartik, 1981). There has been enough evidence that copper may act as a powerful metabolic antagonist of molybdenum (Ward, 1978; Underwood, 1977; Blood, 1979). Urine is one of the major excretive routes of molybdenum.

As no data on the metabolic interrelations between cadmium and molybdenum, copper and cadmium-plus-molybdenum have been reported. We exposed buffalo calves to cadmium, molybdenum and copper alone or together orally for the following objectives: To determine the interaction of cadmium and molybdenum in blood, urine and various tissues; to determine the effect of copper on the levels of cadmium and molybdenum in blood, urine, and tissues; to

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determine the concentrations of cadmium, molybdenum and copper in blood, urine and some tissues.

MATERIALS AND METHODS

Ten clinical healthy male buffalo calves about one-year-old were randomly divided into five groups of two each. Each group was kept in a different pen. The basal diets fed to calves was composed of enough fresh grasses, hays and the proper amount (about 1–1.5 kg each calf) of concentrates (corn, grain or soybean). The average cadmium, copper and molybdenum concentrations of basal diets were 0.42, 5.9 and 0.76 ppm (DW), respectively. The experimental rations fed to each groups were:

Control group: basal diets; cadmium group: the daily oral dose of 0.2 mg cadmium (as cadmium sulfate) per kilogram body weight was fed to each calf, with basal diets; molybdenum group: the daily oral dose 1.2 mg molybdenum (as ammonium molybdate) per kilogram body weight; cadmium-molybdenum group: 0.2 mg plus 1.2 mg molybdenum per kilogram body weight; copper-cadmium-molybdenum group: 0.2 mg cadmium plus 1.2 mg molybdenum plus 3.6 mg copper (as copper sulfate) per kilogram body weight.

All animals were bled from the jugular vein into EDTA-Na heparinized glass bottles prior to and at an interval of ten days after the beginning of experimental administration. At the same time, the urine was collected. Blood and urine were kept frozen until analyzed. After the experimental period of 130 days, all animals were slaughtered, and liver, kidneys, testicles, spleen, heart, lung and brain were collected. All of the samples were digested by the nitric acid-perchloric acid method. Cadmium and copper concentrations of blood, urine and tissues were determined by atomic absorption spectrophotometer, and molybdenum levels of the above sample were determined by polarograph.

All data abtained were statistically analyzed for differences among groups by analysis of variance. Duncan's multiple range test for variable values was used as a post-test to check for differences among the groups.

RESULTS AND DISCUSSION

Blood

The mean blood copper concentrations had no marked difference ($P > 0.05$) among the groups of calves. This result was different from those reported by other researchers. There was a record that blood copper declined in animals with molybdenoses (Blood, 1979; Clark, 1979). Rotkiewiez *et al.* (1979) reported that animals exposed to cadmium had decreased blood copper. Blood molybdenum was significantly higher in the molybdenum group, cadmium-molybdenum group and copper-cadmium-molybdenum group than in control group (Table 1). It showed an increased intake of molybdenum in animals and offered a clue for the diagnosis of molybdenoses. In this study, the difference of blood molybdenum between calves of the

cadmium-molybdenum group and other groups was significant ($P > 0.01$). The result suggested that cadmium could increase the blood molybdenum concentration when they were given orally to the animals together. In addition, a comparison of the control blood with the blood of calves fed 0.2 mg cadmium per kilogram body weight showed an increase of cadmium after cadmium supplementation. The same trend has been reported by many other authors (Lamphere, 1984).

Table 1 Concentrations of cadmium, copper and molybdenum in blood[1]

Group	Cadmium, μg/ml	Copper, μg/ml	Molybdenum, μg/ml
Control	0.0909±0.0325	0.7795±0.5625	0.0777±0.0572
Cadmium	0.1070±0.0409[a]	0.6670±0.4191	0.0857±0.0527
Molybdenum	0.0877±0.0299	0.8269±0.4377	0.2574±0.1433[a]
Cadmium-molybdenum	0.1026±0.0380[a]	0.7750±0.4329	0.3247±0.2961[ab]
Copper-cadmium-molybdenum	0.0934±0.0341	0.8328±0.4254	0.2068±0.3021[a]

[1]Values are means±SD, number of sample is 28.

[a]The mean is significantly different($P < 0.01$) from the control group.

[ab]The mean is significantly different($P < 0.01$) from the molybdenum group.

Urine

The concentrations of cadmium and molybdenum in urine are presented in Table 2. Urinary copper concentration was not examined because it was too low in calves of all groups. There was not a marked difference in urinary cadmium among the groups. However, Bartik *et al.* (1981) considered that if much cadmium is stored in the kidneys, the elimination of cadmium in the urine may increase 50 to 100 times. The mean urinary molybdenum levels in calves fed molybdenum, cadmium-molybdenum, or copper-cadmium-molybdenum were significantly higher than in controls. Table 2 showed also that the urinary molybdenum concentration was not higher in the copper-cadmium-molybdenum group than in the cadmium-molybdenum group. This indicated that copper could not alleviate the toxicity of cadmium-molybdenum by increasing urinary molybdenum excretion.

Table 2 Concentrations of cadmium and molybdenum in urine[1]

Group	Cadmium, μg/ml	Molybdenum, μg/ml
Control	0.0160±0.0139	0.2294±0.1893
Cadmium	0.0191±0.0178	0.2478±0.2732
Molybdenum	0.0159±0.0069	0.5080±0.3453[a]
Cadmium-molybdenum	0.0188±0.0084	0.5752±0.4135[a]
Copper-cadmium-molybdenum	0.0208±0.0201	0.4698±0.2742[a]

[1]Values are mean±SD, number of samples is 28.

[a]The mean is significantly different ($P < 0.01$) from the control group.

Liver

The copper concentration in the liver was significantly greater in all treated groups than in the controls (Table 3). Most of the data considered that hepatic copper levels in the animals fed molybdenum was decreased (Ward, 1978; Underwood, 1977). However, it was recorded that the hepatic copper level was not decreased in cattle fed with molybdenum as in natural case with molybdenum toxication (Blood, 1979), and the case with serious diarrhoea and lesions did not show the hypocuprosis (Underwood, 1977). Kulwich *et al.* found that a diet containing a high level of molybdenum could cause a high copper accumulation in pigs' liver (Clarke, 1979). The phenomenon of increased liver copper was considered as the result of molybdenum entering into the body and combining with copper and/or sulfate in the diet, and forming a complex which stayed in the liver and inhibited copper (Chappell, 1976). In our experiment reported here, the result of liver copper in calves fed cadmium were different from those reported by some other workers(Lamphere, 1984; Doyle, 1975).

Table 3 The hepatic cadmium, copper and molybdenum accumulations in calves[1]

Group	Cadmium	Copper, ppm, dry weight	Molybdenum
Control	0.8583±0.3520	21.77±2.63	0.6665±0.2210
Cadmium	5.5991±1.3016[a]	34.55±17.19[a]	0.4666±0.0432
Molybdenum	0.9434±0.1373	28.23±7.54[a]	2.9862±1.3700[a]
Cadmium-molybdenum	8.5592±2.0232[c]	45.43±2.82[a]	3.5064±1.4047[ab]
Copper-cadmium-molybdenum	5.3335±1.2442[a]	379.67±166.98[b]	3.8581±3.8387[ab]

[1]Values are means±$SD, n = 2$

[a]The mean is significantly different from the control group.

[b]The mean is significantly different from the cadmium-molybdenum groups.

[c]The mean is significantly different from other groups.

The cadmium concentration in the liver was significantly greater in calves fed cadmium than in controls. The same trend has been reported by other researchers (Ashby, 1980; Doyle, 1975; Lamphere, 1984; Basile, 1982; Hill, 1979). The hepatic cadmium concentration of the cadmium-molybdenum group was higher than that of control, cadmium or copper-cadmium-molybdenum groups. This indicated that molybdenum could increase the accumulation of cadmium in the liver of buffalo calves, and copper could reduce the accumulation. However, the hepatic cadmium concentration in calves fed copper-cadmium-molybdenum was significantly higher than in controls. It means that copper could not reduce the hepatic cadmium concentration caused by cadmium-molybdenum entirely to normal.

The molybdenum concentrations in the liver of calves fed molybdenum, cadmium-molybdenum and copper-cadmium-molybdenum were significantly greater than that in the liver of controls, while hepatic molybdenum levels in the cadmium-molybdenum and copper-cadmium-

molybdenum groups were higher than in the molybdenum group. These results suggested that cadmium could increase the molybdenum accumulation in liver, and copper could not reduce this accumulation of molybdenum to normal.

Kidneys

The trace element composition of the kidneys is shown in Table 4. The copper concentration in the kidneys in all treatment groups was significantly greater than in controls. This coincided with the recorded data for animals fed cadmium or molybdenum (Sandstead, 1977; Doyle, 1975).

A comparison of cadmium accumulation in the liver of calves fed cadmium with that in kidneys showed a higher cadmium accumulation in the kidneys.

Table 4 Concentration of cadmium, copper and molybdenum in kidneys[1]

Group	Cadmium	Copper, ppm dry weight	Molybdenum,
Control	3.4459±2.4445	5.36±1.22	0.5299±0.1873
Cadmium	8.7120±3.3989[a]	31.33±38.01[a]	0.8738±0.4556
Molybdenum	1.7979±1.5071	20.06±3.46[a]	2.8388±0.1271[a]
Cadmium-molybdenum	11.2626±2.4113[c]	63.96±2.86[c]	9.2668±1.1015[c]
Copper-cadmium-molybdenum	8.8065±6.0587[a]	20.86±16.57[a]	3.1783±0.0112[a]

[1]Values are mean±SD, $n = 2$

[a]The mean is significantly different from the control group.

[c]The mean is significantly different from other groups.

This coincides with the theory suggested by Bartik(1981), which indicated that in chronic poisoning more cadmium was stored in the kidneys than in the liver. Frazier suggested that upon chronic exposure, kidneys accumulated greater levels of cadmium by both direct intake of cadmium as well as rapid intake of cadmium-thioneine released slowly from the liver. In our experiment, we found that the renal cadmium level in the cadmium-molybdenum group was significantly higher than in the cadmium group. This suggested that molybdenum could make the kidneys accumulate more cadmium. In addition, we also found that the renal cadmium concentration in the copper-cadmium-molybdenum group was significantly higher than in the control group and lower in the cadmium-molybdenum group, which indicated that copper could not reduce the renal cadmium accumulation caused by cadmium-molybdenum entirely to normal.

The renal molybdenum concentration in the molybdenum, cadmium-molybdenum and copper-cadmium-molybdenum groups was about 5-fold, 18-fold and 6-fold respectively as high as in the control group. The higher renal molybdenum level in the cadmium-molybdenum group than in the molybdenum group. That cadmium could increase the renal molybdenum concentration when cadmium and molybdenum were fed to calves together. Meanwhile, the

renal molybdenum level in the copper-cadmium-molybdenum group was greater than in the control group, and lower than in the cadmium-molybdenum group. It indicated that copper could only partly reduce the molybdenum accumulation caused by cadmium-molybdenum.

Other organs

The concentration of cadmium, copper and molybdenum in the heart, lungs, spleen and testes are present in Table 5. There were no marked differences in copper, molybdenum and cadmium concentrations in the heart among these groups. Doyle *et al.* (1975) reported similar copper results in lambs exposed to cadmium, but he considered that the cadmium level in the heart was elevated with increased cadmium content in the diet.

Table 5 Concentrations of cadmium, copper and molybdenum in heart, lungs, spleen and testes[1]

Organ	Group	Cadmium,	Copper,	Molybdenum,
			ppm, dry weight	
Heart	Control	0.4173	13.49	0.7511
	Cadmium	0.7420±0.4099	10.97±5.85	0.6102±0.1877
	Molybdenum	0.1686±0.2384	6.05±4.79	1.0566±0.3309
	Cadmium-molybdenum	0.4698±0.2085	8.71±3.20	0.9350±0.7567
	Copper-cadmium-molybdenum	0.3645±0.3548	7.90±4.32	0.4164±0.3458
Lungs	Control	1.7732±0.5801	4.12±2.53	1.5247±0.6889
	Cadmium	2.3567±1.5760	2.86±1.69[a]	5.1876±4.5530
	Molybdenum	1.5999±0.7037	4.04±1.54	3.9188±0.8653
	Cadmium-molybdenum	1.0264±0.6090	4.67±0.42	4.4647±4.8127
	Copper-cadmium-molybdenum	1.4607±0.5217	5.41±1.61	3.7901±1.6806
Spleen[2]	Control	0.8730±0.1744	2.77±0.95	0.6311±0.1220
	Molybdenum	0.8268±0.7476	2.04±0.06	0.4276±0.2494
	Cadmium-molybdenum	0.8686±0.4463	5.69±4.79[a]	4.5567±1.3624[a]
	Copper-cadmium-molybdenum	0.9516±0.2739	5.16±0.09[a]	5.9117±1.6070[a]
Testes	Control	0.7357±0.1268	10.66±10.34	0.4040±0.0450
	Cadmium	1.2037±0.0552[a]	6.89±7.13	1.0960±0.4009
	Molybdenum	0.7621±0.1189	2.71±0.42	1.8846±0.7669[a]
	Cadmium-molybdenum	0.7246±0.7131	3.40±1.69	3.2946±2.5072[a]
	Copper-cadmium-molybdenum	1.1041±0.7690	3.48±0.52	2.3819±0.0547[a]

[1] Values are means$\pm SD, n = 2$

[2] The spleen in cadmium-group was polluted.

[a] The mean is significantly different from the control group.

There were no significant differences in molybdenum and cadmium levels in lungs. The copper concentration in lungs of calves fed cadmium was significantly higher than that in controls.

The copper and molybdenum concentrations of the spleen in calves fed cadmium-molybdenum and copper-cadmium-molybdenum were significantly higher than that in controls. The cadmium concentration in the spleen did not vary greatly.

There were no marked differences in copper levels of testes among the groups. However, Doyle *et al.* (1975) found that the testicular copper concentration was lowered significantly in lambs fed cadmium. In the present study, the testicular molybdenum level was significantly increased in the molybdenum, cadmium-molybdenum and copper-cadmium-molybdenum groups. The cadmium concentration in testes of calves fed cadmium was significantly greater than that in controls.

CONCLUSION

The daily oral dose of 0.2 mg cadmium or/and 1.2 mg molybdenum per kilogram body weight to buffalo calves could cause the significant change in trace elements of blood, urine and tissues. This indicated high susceptibility of buffalo calves to cadmium and molybdenum.

The mean blood copper did not changed but the blood cadmium and molybdenum were increased when the calves were treated by cadmium and/or molybdenum.

Cadmium and molybdenum could increase the accumulations of molybdenum and cadmium in liver and kidneys of buffalo calves, and copper could not reduce to normal the levels of molybdenum and cadmium in these organs caused by cadmium-molybdenum.

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