ELSEVIER Animal Feed Science and Technology 79 (1999) 107–117

ANIMAL FEED SCIENCE AND TECHNOLOGY

Estimated relative bioavailability of supplemental inorganic molybdenum sources and their effect on tissue molybdenum and copper concentrations in lambs

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Received 19 August 1998; accepted 23 December 1998

Abstract

Forty crossbred wether lambs, 44 kg initial body weight, were used to study relative bioavailability of Mo in supplemental Mo sources and their effects on body Cu storage. The basal maize-soya-bean meal-cottonseed hulls diet contained 11.5 mg kg^{-1} Cu, 1.1 mg kg^{-1} Mo and 2.1 g kg^{-1} S (DM basis) and was supplemented with 0, 15, 30, or 45 mg kg^{-1} (as-fed basis) added Mo as reagent grade sodium molybdate as the standard, or 30 mg kg^{-1} added Mo as ammonium molybdate, molybdenum trioxide, or molybdenum metal. Diets were fed for 28 days and feces and urine were collected for the last 5 days and composited by lamb. At the end of the trial, serum, liver, kidney, muscle, and bile were collected and analyzed for Mo and Cu. Based on multiple regression slope ratios of tissue concentration or daily excretion (including that from bile) of Mo on added dietary Mo concentration, the average relative bioavailability values were 1.00, 1.04, 1.10 and 0.18 for sodium molybdate, ammonium molybdate, molybdenum trioxide, and molybdenum metal. Serum and muscle Mo concentrations had the best fits to a linear model. Kidney Cu, serum total Cu, serum TCA-insoluble Cu, and fecal Cu concentrations increased with increasing dietary intakes of Mo.

Keywords: Molybdenum; Copper; Lambs; Bioavailability

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0377-8401/99/$ – see front matter
PII: S0377-8401(99)00019-X

1. Introduction

Chronic toxicosis in intensively reared sheep fed 10–35 mg kg^{-1} Cu in conjunction with low Mo has been reported worldwide (Tait et al., 1971; Niederman et al., 1987). Oral administration of tetrathiomolybdate has been shown to control outbreaks of Cu toxicosis (Kincaid and White, 1988), however, this compound is not readily available commercially.

Tissue uptake of various trace elements in chicks or lambs fed high dietary concentrations of those elements in natural diets has been reported as a method to estimate relative bioavailability of supplemental sources (Ammerman et al., 1995). Pott et al. (1999) reported that tissue Mo concentrations following the feeding of 0, 15, 30, or 45 mg kg^{-1} Mo for 28 days to sheep may be useful in estimating the bioavailability of supplemental Mo sources.

The objective of the present experiment was to estimate relative bioavailability values for ammonium molybdate, molybdenum trioxide and molybdenum metal, compared to sodium molybdate as the standard source, and study effects of added dietary concentrations of Mo on tissue concentrations of Mo and Cu in lambs.

2. Materials and methods

Forty Texas crossbred wether lambs, 44 kg body weight initially, were assigned randomly to one of seven dietary treatments. The basal diet (Table 1) contained 11.5 mg kg^{-1} Cu, 1.1 mg kg^{-1} Mo, and 2.1 g kg^{-1} S on a dry matter (DM) basis, by analysis, and was formulated to meet requirements for growing lambs (National Research Council, 1985). Treatments were the basal diet alone (control) or supplemented with 15, 30, or 45 mg kg^{-1} Mo as reagent grade sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) or 30 mg kg^{-1} Mo as either reagent grade ammonium molybdate [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$], reagent grade molybdenum trioxide (MoO_3), or molybdenum metal. There were six lambs per treatment group except for 15 and 45 mg kg^{-1} Mo as sodium molybdate which had five lambs each. One animal fed the control diet was removed on day 6 of the experiment due to inappetence.

Animals were housed in individual metabolic cages and allowed a 6-days adjustment period prior to the 28-days experiment. Lambs were fed 1.0 kg of diet once daily. Tap water containing 0.02 mg l^{-1} Mo and no detectable Cu was available ad libitum.

Feces and urine were collected daily during the last 5 days of the experiment. Feces were collected in cotton bags. Urine was collected individually into plastic buckets containing 100 ml of 3N HCl. Total daily fecal and urinary outputs were measured and composited on a 10% basis for each lamb. Feces were kept frozen in plastic bags until grinding and mixing in a blender with stainless steel blades and subsampling for laboratory analyses. Urine samples were refrigerated in plastic bottles, then filtered with No. 40 Whatman paper into acid washed plastic bottles and frozen until analyzed.

Blood samples were taken on day 27 of the experiment by jugular vein puncture. Blood was centrifuged for 30 min at 2500 × g. An equal volume (2 ml) of trichloroacetic acid (TCA, 10 g in 100 ml of water) was added to serum samples, mixed and centrifuged for

Table 1
Composition (unless otherwise marked, g kg^{-1}, as-fed basis) of basal diet for lambs

Ingredients	Amount
Ground maize	569.5
Cottonseed hulls	210.0
Soya-bean meal (480 g kg^{-1} CP)	120.0
Alfalfa meal	30.0
Maize oil[a]	30.0
Maize starch + Mo[b]	22.8
Ground limestone	5.5
Trace mineralized salts[c]	10.0
Sodium sulfate	2.2
Vitamins[d]	+
Dry matter (g kg^{-1})	889.0
Calcium[e] (g kg^{-1})	4.3
Magnesium[e] (g kg^{-1})	1.5
Phosphorus[e] (g kg^{-1})	2.8
Sulfur[e] (g kg^{-1})	2.1
Copper[e] (mg kg^{-1})	11.5
Molybdenum[e] (mg kg^{-1})	1.1
Iron[e] (mg kg^{-1})	115.0
Manganese[e] (mg kg^{-1})	42.0
Zinc[e] (mg kg^{-1})	65.0

[a] Ethyoxyquin added at 125 mg kg^{-1} as an antioxidant (Novus, St. Louis, MO).
[b] Molybdenum supplements added in place of equivalent weight of maize starch.
[c] Contained in g kg^{-1}: NaCl, 930; Zn, 3.5; Mn, 2.8; Fe 1.75; Cu, 0.35; I, 0.07; Co, 0.07; Se, 0.02.
[d] Supplied in (kg^{-1} diet): retinyl palmitate, 2200 IU; cholecalciferol, 440 IU; DL-α-tocopheryl acetate, 15 IU.
[e] Dry matter basis by analysis.

25 min at 2500 × *g*. Supernatant was saved and precipitate was resuspended into TCA (5 g in 100 ml of water), and mixing and separation procedure repeated. Supernatants removed from both extractions were combined for TCA-soluble Cu analysis. Animals were stunned with a captive bolt shot and killed by exsanguination for collection of bile, kidney, liver and muscle (*sterno mandibularis*) which were frozen immediately for subsequent mineral analysis.

Samples of kidney, liver, and muscle were predigested in HNO_3, dry ashed at 550°C overnight, then solubilized in HNO_3. Feces and feed were treated the same way except they were not predigested. Bile was evaporated on a hot plate and the residue predigested and then ashed and solubilized. Urine was also evaporated and the residue ashed and solubilized. The Mo sources were refluxed in 25 ml concentrated HNO_3 : HCl (1 : 1; vol : vol) for 4 h. Copper concentrations in water, tissues, excretions and TCA-supernatant; Cu, Fe, Mn, Zn, Ca, and Mg in Mo sources and in basal diet were determined by flame atomic absorption spectrophotometry on a Model 5000 spectrophotometer with an AS-50 autosampler (Perkin-Elmer, Norwalk, CT; Anonymous, 1982). Serum TCA-insoluble Cu was calculated from the difference between total Cu in serum and TCA-soluble Cu. Molybdenum concentrations in feed, water, tissues, excretions, and Mo sources were determined by graphite furnace with a Perkin-Elmer Zeeman/3030 atomic absorption spectrophotometer with an AS-60 autosampler (Anonymous, 1984).

Three firings with deionized water were made in between Mo samples or after the Mo standards to decrease carryover effects. For Mo analysis, serum was digested with HNO_3 plus $HClO_4$ and diluted to 25 ml followed by a complexation and coprecipitation procedure. Precipitate was dissolved with HNO_3 and diluted to 3 ml, which was run by inductively coupled plasma spectrometry. Serum samples of unsupplemented lambs had to be combined to get readings greater than detection limits. This was done on an equal volume basis. Phosphorus in Mo sources and basal diet was determined colorimetrically (Harris and Popat, 1954). Sulfur concentration in basal diet was determined with a Model S-132 S analyzer (Leco, Warrendale, PA). Standards were matched for macroelement and acid concentrations as appropriate and standard reference material from the National Institute of Standards and Technology (Gaithersburg, MD) was run with samples.

Solubility in water, 0.12N HCl, 2% (wt : vol) citric acid and neutral ammonium citrate of Mo sources was determined (Watson et al., 1970). X-ray diffraction patterns were obtained and interpreted.

Multiple linear regression was done by least squares using the general linear models procedure of SAS Institute (1988). Slope ratios and their standard errors were estimated using the method of error propagation as described by Kempthorne and Allmaras (1965). A $\log_{10}$ transformation was used to reduce variance heterogeneity if a Burr-Foster Q test indicated that it was necessary (Anderson and McLean, 1974). In the statistical analysis of Mo in serum, the results of combined samples were assigned to each of the animals of the group fed the basal diet.

3. Results and discussion

3.1. Molybdenum sources

Chemical and physical characteristics of Mo sources are shown in Table 2. Sodium molybdate contained 396 g kg^{-1} Mo; ammonium molybdate, 545 g kg^{-1} Mo; molybdenum trioxide, 663 g kg^{-1} Mo; and molybdenum metal, 982 g kg^{-1} Mo. No major mineral contaminants were found in chemical compounds, as should be expected from reagent grade sources, except for some Mg and higher quantities of P. Sodium molybdate, ammonium molybdate and molybdenum trioxide particles were predominantly between 150 and 850 μm. All molybdenum metal particles were smaller than 75 μm.

Solubility of Mo sources was quite variable. The most soluble source in solvents used was ammonium molybdate, which was completely soluble in water and 0.12N HCl, almost completely soluble in neutral ammonium citrate but somewhat less soluble in 2% citric acid. In contrast, molybdenum metal was almost completely insoluble in all four solvents. Sodium molybdate was quite soluble in water and HCl, but much less soluble in neutral ammonium citrate and 2% citric acid solutions. The molybdenum trioxide was most soluble in neutral ammonium citrate, but was less soluble in 2% citric acid and water and much less in HCl. Mills and Davis (1987) referred to molybdenum trioxide as an insoluble compound. Based on results from the present study, this was certainly true when the solvent was HCl, but not so with water, neutral ammonium citrate or citric acid, at least not so when close to physiological temperature, as used in this study. In another

Table 2
Chemical and physical characteristics of molybdenum sources

Item	Molybdenum sources			
	Sodium molybdate, reagent grade	Ammonium molybdate, reagent grade	Molybdenum trioxide, reagent grade	Molybdenum metal, technical grade
Chemical constituents, as-fed basis[a]				
Molybdenum (g kg^{-1})	396	545	663	982
Magnesium (mg kg^{-1})	22	12	25	12
Phosphorus (mg kg^{-1})	2350	2079	3468	538
Particle size (g kg^{-1})				
>850 μm	45	0	51	0
850–150 μm	847	951	931	0
150–75 μm	100	41	17	0
<75 μm	8	8	1	1000
Physical appearance	White, fine crystals	White, fine crystals	Light gray, fine powder	Black, fine powder
Mo solubility[b]				
Water	0.899	1.000	0.470	0.004
Neutral ammonium citrate	0.356	0.855	0.807	0.002
0.12N HCl	0.913	0.990	0.199	0.001
2% Citric acid	0.376	0.738	0.549	0.002
Interpretation of X-ray patterns	$Na_2MoO_4 \cdot 2H_2O$	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	MoO_3	Mo metal

[a] Cu, Fe, Mn, Zn, and Ca not detected.
[b] Solubility of 0.1 g in 100 ml solvent at 37°C for 1 h with constant stirring, expressed as a portion of total (1.000) analyzed Mo concentration.

study, molybdenum trioxide from industrial emission of a Mo-containing catalyst, was 28.6% soluble in water (Gardner and Hall-Patch, 1962). Interpretation of X-ray patterns was consistent with the labels as provided by suppliers.

3.2. Feed consumption

Feed offered (1 kg per day) in general was totally consumed shortly after feeding. Three lambs, however, one fed the basal diet, one fed 15 mg kg^{-1} Mo as sodium molybdate and one fed 30 mg kg^{-1} Mo as molybdenum metal had average daily intakes of 824, 754, and 943 g, respectively.

3.3. Tissue minerals

Liver, kidney and muscle Mo concentrations were influenced ($P < 0.0001$) by dietary Mo treatments (Table 3). Tissue Mo accumulation was greatest in lambs fed molybdenum trioxide and was lowest in lambs fed molybdenum metal compared with similar amounts from other sources.

The increase in liver Mo concentration in lambs fed 45 mg kg^{-1} Mo as sodium molybdate deviated from linearity, and thus, data from this treatment group were not included in the multiple regression to estimate bioavailability. A similar plateau in liver Mo concentration was observed in a previous experiment (Pott et al., 1999). The fit to a linear model in the present experiment was not as good as observed for sheep fed 28 days in the previous experiment for kidney ($R^2 = 0.61$ versus 0.75) or muscle ($R^2 = 0.77$ versus 0.81; Table 4); however, only one source of supplemental Mo was used in the previous study. In the present experiment, as in the previous study, it appeared that elevated muscle Mo would not be a burden in the human food chain. For equations describing liver, kidney, and muscle Mo uptake, the slope representing molybdenum

Table 3
Effect of source and dietary concentration of molybdenum (as-fed basis) on tissue molybdenum concentrations in lambs[a]

Mo source	Added Mo[b], mg kg^{-1}	Mo, mg kg^{-1} DM basis			Mo, mg l^{-1}
		Liver	Kidney	Muscle	Serum
Control	0	3.34	1.64	0.11	0.007
Sodium molybdate	15	6	2.8	0.28	0.28
Sodium molybdate	30	7.52	4.3	0.44	0.48
Sodium molybdate	45	7.77	10.64	0.67	1.2
Ammonium molybdate	30	8.38	6.05	0.49	0.85
Molybdenum trioxide	30	9.24	6.9	0.56	0.81
Molybdenum metal	30	5.51	1.7	0.27	0.01
Pooled SE		0.21	0.34	0.02	0.03
ANOVA treatment (significance)		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

[a] Mean of five lambs for 0, 15, and 45 mg kg^{-1} Mo and six lambs for other treatments.
[b] Basal diet contained 11.5 mg kg^{-1} Cu, 1.1 mg kg^{-1} Mo and 2.1 g kg^{-1} S (DM basis).

Table 4
Estimated relative bioavailability of molybdenum sources based on multiple linear regression slope ratios

Mo source	Slope ± SE[a]	Relative value ± SE	95% confidence limits
Liver[b]			
Sodium molybdate	0.138 ± 0.027[c]	1	
Ammonium molybdate	0.161 ± 0.025[c]	1.17 ± 0.20	0.77–1.56
Molybdenum trioxide	0.190 ± 0.025[c]	1.38 ± 0.23	0.92–1.83
Molybdenum metal	0.066 ± 0.025[d]	0.48 ± 0.15	0.17–0.78
Kidney			
Sodium molybdate	0.188 ± 0.031[c]	1	
Ammonium molybdate	0.184 ± 0.043[c]	0.98 ± 0.19	0.59–1.37
Molybdenum trioxide	0.212 ± 0.043[c]	1.13 ± 0.20	0.72–1.53
Molybdenum metal	0.034 ± 0.043[d]	0.18 ± 0.21	−0.25–0.61
Muscle			
Sodium molybdate	0.0639 ± 0.0013[c]	1	
Ammonium molybdate	0.0647 ± 0.0019[c]	1.01 ± 0.025	0.96–1.06
Molybdenum trioxide	0.0670 ± 0.0019[c]	1.05 ± 0.025	1.00–1.10
Molybdenum metal	0.0057 ± 0.0019[d]	0.09 ± 0.029	0.03–0.15
Serum			
Sodium molybdate	0.0249 ± 0.0025[d]	1	
Ammonium molybdate	0.0309 ± 0.0035[c]	1.24 ± 0.12	0.99–1.49
Molybdenum trioxide	0.0295 ± 0.0035[c]	1.18 ± 0.12	0.94–1.43
Molybdenum metal	0.0029 ± 0.0035[e]	0.12 ± 0.13	−0.15–0.39
Urine[f]			
Sodium molybdate	0.355 ± 0.036[c]	1	
Ammonium molybdate	0.230 ± 0.050[d]	0.65 ± 0.12	0.41–0.89
Molybdenum trioxide	0.279 ± 0.050[d]	0.79 ± 0.12	0.55–1.02
Molybdenum metal	0.029 ± 0.050[e]	0.08 ± 0.13	−0.19–0.36
Bile			
Sodium molybdate	0.00129 ± 0.00026[c]	1	
Ammonium molybdate	0.00152 ± 0.00036[c]	1.18 ± 0.25	0.67–1.68
Molybdenum trioxide	0.00138 ± 0.00036[c]	1.07 ± 0.24	0.57–1.56
Molybdenum metal	0.00021 ± 0.00036[c]	0.16 ± 0.26	−0.37–0.69

[a] Multiple linear regression, where x = added dietary Mo mg kg^{-1} (as-fed basis) and y = mg kg^{-1} DM for liver, kidney and muscle; mg l^{-1} for serum and bile and mg per day for urine. Intercepts were 3.54, 0.531, 0.941, −0.0782, −0.812, and 0.00476 and R^2 values were 0.71, 0.61, 0.77, 0.83, 0.79, and 0.54 for liver, kidney, muscle, serum, urine, and bile, respectively.
[b] Data from the 45 mg kg^{-1} group omitted from regression due to lack of linearity.
[c,d,e] Slopes differ ($P < 0.05$).
[f] $\log_{10}$ transformed data.

metal was lower ($P < 0.05$) than those for sodium molybdate and ammonium molybdate or molybdenum trioxide. When the response for the standard (sodium molybdate) was set at 1.00, the average bioavailability estimates based on Mo concentrations in liver, kidney, and muscle were 1.05, 1.19, and 0.25 for ammonium molybdate, molybdenum trioxide, and molybdenum metal, respectively. No studies designed specifically to study bioavailability of Mo sources were found in the literature. In a study of Mo toxicosis

Table 5
Effect of source and dietary concentration of molybdenum (as-fed basis) on tissue copper concentrations (mg kg^{-1} DM basis) in lambs[a]

Mo source	Added Mo[b], mg kg^{-1}	Tissue Cu concentration		
		Liver	Kidney	Muscle
Control	0	390	21.9	8.3
Sodium molybdate	15	350	23.3	6.8
Sodium molybdate	30	373	25.3	7.6
Sodium molybdate	45	300	39.3	7.7
Ammonium molybdate	30	280	28.9	6.7
Molybdenum trioxide	30	366	32.9	7.3
Molybdenum metal	30	369	23.3	8.3
Pooled SE		23	0.56	0.19
ANOVA treatment (significance)		$P > 0.10$	$P < 0.0001$	$P > 0.10$

[a] Mean of five lambs for 0, 15, and 45 mg kg^{-1} Mo and six lambs for other treatments.
[b] Basal diet contained 11.5 mg kg^{-1} Cu, 1.1 mg kg^{-1} Mo and 2.1 g kg^{-1} S (DM basis).

in rats fed 100 mg Mo daily, liver and kidney Mo concentrations were 0.024 and 0.014 mg g^{-1} in fresh tissue in those fed molybdenum trioxide for 14 days and 0.016 and 0.016 mg g^{-1} in fresh tissue in those fed ammonium molybdate for 13 days (Fairhall et al., 1945). In their study, rats fed molybdenum trioxide, ammonium molybdate, and calcium molybdate ($CaMoO_4$) all showed signs of toxicosis while those fed molybdenite (MoS_2) did not.

Kidney Cu concentrations were increased ($P < 0.0001$) by dietary Mo treatments (Table 5). There was a decreasing trend in liver and muscle Cu concentrations, but treatment means did not differ significantly ($P > 0.10$). The increase in kidney Cu was observed by Pott et al. (1999) in lambs fed similar Mo concentrations for 28 days. In the present experiment, muscle Cu concentrations were numerically lower in lambs fed an available form of supplemental Mo but this trend failed to reach statistical significance as observed by Pott et al. (1999).

3.4. Serum minerals

Serum Mo increased ($P < 0.0001$) in response to dietary treatment (Table 3) and had the best fit to a linear model (Table 4) as observed in the previous experiment (Pott et al., 1999). The slopes for molybdenum trioxide and ammonium molybdate were greater ($P < 0.05$) than that for sodium molybdate which was greater ($P < 0.05$) than for molybdenum metal (Table 4). Relative Mo bioavailability values compared with 1.00 for sodium molybdate were 1.24, 1.18, and 0.12 for ammonium molybdate, molybdenum trioxide and molybdenum metal, respectively.

Serum total Cu ($P < 0.01$) and TCA-insoluble Cu concentrations ($P < 0.0001$) were increased by dietary Mo (Table 6) and that for TCA-soluble Cu was decreased ($P < 0.05$) with greater dietary Mo. Concentrations of serum Cu, as well as trends in responses, were similar to those reported previously (Pott et al., 1999).

Table 6
Effect of source and dietary concentration of molybdenum (as-fed basis) on serum copper concentrations (mg l^{-1}) in lambs[a]

Mo source	Added Mo[b], mg kg^{-1}	Serum Cu concentration		
		Total	TCA-soluble	TCA-insoluble
Control	0	0.86	0.78	0.08
Sodium molybdate	15	1.09	0.87	0.22
Sodium molybdate	30	1.07	0.79	0.28
Sodium molybdate	45	1.34	0.68	0.66
Ammonium molybdate	30	1.30	0.73	0.57
Molybdenum trioxide	30	1.19	0.73	0.46
Molybdenum metal	30	1.04	0.94	0.10
Pooled SE		0.03	0.02	0.03
ANOVA treatment (significance)		$P < 0.01$	$P < 0.05$	$P < 0.0001$

[a] Mean of five lambs for 0, 15, and 45 mg kg^{-1} Mo and six lambs for other treatments.
[b] Basal diet contained 11.5 mg kg^{-1} Cu, 1.1 mg kg^{-1} Mo and 2.1 g kg^{-1} S (DM basis).

3.5. Mineral excretion

Concentrations of Mo in bile and urine increased ($P < 0.0001$) with dietary Mo supplementation (Table 7). Total urine volume averaged 9633 $\pm$ 1362 ml per day and did not differ ($P > 0.10$) among treatments. Daily bile output was not measured. Excretion of Mo in bile or urine of lambs fed molybdenum metal did not differ from those fed the control diet. The slope for urine representing lambs fed sodium molybdate was greater ($P < 0.05$) than those for animals fed ammonium molybdate or molybdenum trioxide (Table 4). The molybdenum metal had the lowest ($P < 0.05$) slope. Urinary Mo was the only sample measured in which sodium molybdate was greater than all other forms. Values relative to sodium molybdate as 1.00 were 0.65, 0.79 and 0.08 for ammonium molybdate, molybdenum trioxide, and molybdenum metal, respectively. Relative values based on biliary excretion of Mo were 1.00, 1.18, 1.07, and 0.16 for sodium molybdate, ammonium molybdate, molybdenum trioxide, and molybdenum metal, respectively. Biliary and urinary Cu excretion (Table 7) were not influenced ($P > 0.10$) by dietary treatment, but daily fecal Cu output increased ($P < 0.05$) with increasing dietary Mo.

Ammonium molybdate and sodium molybdate have been found to be well absorbed by ruminants in numerous experiments as cited in Pott et al. (1999) and thus, a discussion will not be repeated herein. The overall average relative values based on liver, kidney, muscle, serum, biliary, and urinary Mo concentrations were 1.00, 1.04, 1.10 and 0.18 for sodium molybdate, ammonium molybdate, molybdenum trioxide, and molybdenum metal, respectively. At the present time, sodium molybdate is the only compound listed in the official publication of the Association of American Feed Control Officials (1998) as an acceptable form of supplemental Mo for livestock. In the present studies, however, ammonium molybdate and molybdenum trioxide were equally as available as sodium molybdate as sources of supplemental Mo. All of the above inorganic Mo compounds are more readily available in the marketplace than tetrathiomolybdate as possible preventatives for Cu oversupplementation. Although molybdenum trioxide was less

Table 7
Effect of source and dietary concentration of molybdenum (as-fed basis) on molybdenum and copper excretion in lambs[a]

Mo source	Added Mo[b], mg kg^{-1}	Molybdenum		Copper		
		Bile, mg l^{-1}	Urine, mg per day	Bile, mg l^{-1}	Urine, mg per day	Feces, mg per day
Control	0	0.01	0.06	0.73	0.37	5.5
Sodium molybdate	15	0.021	3.93	0.55	0.22	6.0
Sodium molybdate	30	0.037	8.61	0.92	0.49	6.3
Sodium molybdate	45	0.069	16.31	0.63	0.47	6.7
Ammonium molybdate	30	0.050	6.09	0.67	0.32	6.9
Molybdenum trioxide	30	0.046	7.56	0.76	0.41	7.3
Molybdenum metal	30	0.011	0.05	0.47	0.41	6.6
Pooled SE		0.003	0.23	0.04	0.04	0.13
ANOVA treatment (significance)		$P < 0.0001$	$P < 0.0001$[c]	$P > 0.10$	$P > 0.10$	$P < 0.05$

[a] Mean of five lambs for 0, 15, and 45 mg kg^{-1} Mo and six lambs for other treatments.
[b] Basal diet contained 11.5 mg kg^{-1} Cu, 1.1 mg kg^{-1} Mo and 2.1 g kg^{-1} S (DM basis).
[c] $\log_{10}$ transformation.

soluble than either sodium molybdate or ammonium molybdate, it was equally available. The totally insoluble molybdenum metal was included as a negative control to evaluate the various tissues and excretory products for their capacity to identify an unavailable source of the element.

Urinary Mo excretion provided the lowest availability estimate for molybdenum metal, but also yielded lower utilization values for ammonium molybdate and molybdenum trioxide forms. In the present experiment, biliary Mo provided relative values similar to those obtained with the four tissues examined, however, this measurement had the poorest fit to a linear model. In a previous experiment, biliary Mo concentrations were so erratic that data were not reported (Pott et al., 1999). Unless total daily excretion of bile can be measured, the analysis of Mo concentration as an indicator of dietary Mo absorption is probably not worthwhile. Serum may be the best indicator of Mo bioavailability, but does require use of more expensive inductively coupled plasma atomic emissions methodology rather than atomic absorption spectroscopy for analysis. Analysis of Mo in a serum matrix by graphite furnace was not deemed suitably accurate or precise, although results of tissue, feed or urine in an acid matrix were acceptable provided that carryover effects were controlled through use of three water blank firings between samples. Of the three soft tissues analyzed, results with muscle had the best fit to a linear model and gave the lowest estimate of utilization for molybdenum metal. This tissue could also be sampled by biopsy with relative ease if animals could not be slaughtered at the end of a trial. Liver gave a higher value for molybdenum metal and for other sources as well, and may be more subject to effects of dietary Cu and S than other tissues. In an earlier experiment, liver appeared to have two Mo pools, one with slow and the other with rapid turnover, but this was not confirmed by Pott et al. (1999) with a kinetic study.

Bioavailability of Mo from sodium molybdate and ammonium molybdate and molybdenum trioxide was similar based on serum and muscle Mo concentrations in

lambs after 28 days of supplementation at 15–45 mg kg^{-1} added Mo. Increasing dietary Mo increased concentrations of kidney Cu, total serum Cu and TCA-insoluble Cu, as well as increasing daily fecal excretion of Cu.

Acknowledgements

The authors wish to acknowledge Moorman Manufacturing Co., Quincy IL for funds in support of this research; Pfizer Inc., New York, NY for providing vitamins and Novus, St. Louis, MO for providing ethoxyquin. This paper is Florida Agricultural Experiment Station Journal Series Number R-02400.

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