

Copper, Sulfate and Molybdenum Interrelationships in Sheep

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ABSTRACT The experiment was designed to study the interrelationship of copper, molybdenum and sulfur in ruminant nutrition. Eighty lambs were used in the replicated, factorially arranged experiment, which involved 2 levels of copper (10 and 40 ppm), molybdenum (2 and 8 ppm), and sulfate-sulfur (0.10 and 0.40%). Response criteria were growth rate, hematology, and blood and liver mineral levels. Gain and efficiency were reduced by increasing the sulfur level in diets containing 2 ppm of molybdenum; however, this was not true when the diet contained 8 ppm of molybdenum, indicating that molybdenum alleviated the detrimental effects of sulfate. Hemoglobin concentration, erythrocyte counts and plasma protein, copper and calcium were not affected by treatments. Three-way interactions existed for hematocrit, plasma phosphorus and liver molybdenum. Hematocrit was lowered when sulfate-sulfur was increased from 0.10 to 0.40% except in the diet containing 10 ppm of copper and 8 ppm of molybdenum. Plasma phosphorus was also lowered by feeding 0.40% sulfate-sulfur except when 40 ppm of copper and 8 ppm of molybdenum were included in the diet. The three-way interaction for liver molybdenum was caused by decreases in liver molybdenum when 2 ppm of molybdenum and 10 or 40 ppm of copper were fed; feeding of 0.40% sulfate-sulfur, 8 ppm of molybdenum and 40 ppm of copper resulted in slightly higher final liver values, whereas high values were obtained for sheep fed 0.40% sulfur, 8 ppm of molybdenum and 10 ppm of copper. Liver copper was decreased by feeding 0.40% sulfate-sulfur or 8 ppm of molybdenum and increased by feeding 40 ppm of copper. Liver iron was increased as the sulfate-sulfur level was increased from 0.10 to 0.40%. Correlations among the plasma and liver values were also discussed.

Interrelationships among copper, sulfate and molybdenum have been demonstrated many times since Dick (1) reported that the limiting effect of molybdenum on the copper nutrition of sheep was dependent on the sulfate level of the ration. Other results have shown that excess levels of copper, molybdenum or sulfate also exert independent effects (2). As only a few experiments have been conducted for the specific purpose of studying the effects of improper ratios of the 3 minerals, the study reported herein was conducted to study the interrelationships of copper, sulfate and molybdenum when 2 levels of each in all possible combinations were fed to sheep.

EXPERIMENTAL PROCEDURE

A replicated, randomized block design with a 2³ factorial arrangement of treatments was used so that all possible combinations of 2 levels of copper, molybdenum and sulfur were fed to growing lambs. Levels were 10 and 40 ppm of copper, 2

and 8 ppm of molybdenum and 0.10 and 0.40% of sulfur. The first trial was initiated in the late autumn of 1964 with 40 lambs, which were grade Rambouillets and averaged 27.1 kg, and the second trial was initiated in the early spring of 1965 with 40 Rambouillet × Suffolk crossbred lambs averaging 33.9 kg. Lambs in the first trial were fed for 66 days and in the second for 60 days. Blocking on location in the barn was done in both treatments.

The lambs, which were wormed with a phenothiazine-lead arsenate bolus 14 days prior to the start of the experiments and placed in individual pens with slatted floors, were fed the basal ration during this adjustment period. At the end of the adjustment period, initial weights were taken after feed and water had been removed for 17 hours. All animals then were fed their

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TABLE 1
Composition of the basal purified diet

	g/100 g diet
Cornstarch ¹	34.40
Dextrose	24.40
Cellulose ²	30.00
Urea ³	4.20
Corn oil ⁴	1.00
Polyethylene resin ⁵	1.00
Choline chloride	0.10
Vitamins A and D ⁶	0.02
K ₂ CO ₃	2.22
CaHPO ₄	1.32
MgSO ₄	0.12
MgCO ₃ ·Mg(OH) ₂ ·3H ₂ O	0.27
Na ₂ SO ₄	0.25
NaCl	0.62
	mg/100 g diet
FeSO ₄	42.50
MnSO ₄ ·H ₂ O	11.50
Na ₂ B ₄ O ₇	12.50
ZnSO ₄ ·7H ₂ O	15.00
CuCO ₃ ·Cu(OH) ₂	1.75
Na ₂ MoO ₄ ·2H ₂ O	0.50
CaF ₂	0.20
	μg/100 g diet
KI	15.00
Cr ₂ (SO ₄) ₃	40.00
CoCl ₂ ·6H ₂ O	45.00
Na ₂ SeO ₄	25.00

¹ This diet contained 0.10% sulfur, 10 ppm of copper and 2 ppm of molybdenum. All modifications to obtain the experimental diets were made by reducing starch in accord with an increase in Na₂SO₄, CuCO₃·Cu(OH)₂ or Na₂MoO₄·2H₂O.

² Solka-Floc (B-W 20), Brown Company, Berlin, New Hampshire.

³ Crystalline urea, courtesy of John Deere Chemical Company, Pryor, Oklahoma.

⁴ Mazola, Corn Products Company, Santoquin (Monsanto Company, St. Louis) added to give 0.0125% in total ration.

⁵ Alathon, E. I. DuPont de Nemours, Inc., Wilmington, Delaware.

⁶ 20,000 IU and 2,500 USP units of vitamins/g.

appropriate experimental rations, compositions of which are shown in table 1. Feed and water were provided free-choice. Final weights were also preceded by a period of 17 hours without food and water.

Blood samples, taken by jugular puncture, were obtained at the beginning and end of the growth trial. Hemoglobin values were determined on the citrated blood by the method of Sheard and Sanford (3). Erythrocytes and the percentage of packed cells were measured by the method of Schalm (4) and the microhematocrit method, respectively. All analyses involving whole blood were completed soon after bleeding, but the plasma was frozen until analysis was performed. Plasma copper was determined by the method of Cart-

wright et al. (5) and plasma calcium by the method of Kramer and Tisdal (6) with modifications for citrated plasma as described by Harrison (7) and with a Perkin-Elmer Atomic Absorption Spectrophotometer, Model 303, using methods suggested by the manufacturer. Other procedures included plasma phosphorus (8), plasma protein (9) and plasma molybdenum and liver copper, iron and molybdenum (10). The data were subjected to analysis of variance.

RESULTS AND DISCUSSION

As the replication × treatment and block-within-replication × treatment interactions were insignificant ($P > 0.05$), the results of the 2 trials were combined and are shown in table 2. Main effects which are also a part of a significant interaction are presented in the footnotes to table 2, but are discussed only in relation to the second factor. Sulfur level × molybdenum level interactions were found for gains ($P < 0.05$) and feed efficiency ($P < 0.01$): When the sulfur level was increased from 0.10 to 0.40% in diets containing 2 ppm of molybdenum both of these response criteria were reduced ($P < 0.01$); however, no such reduction was obtained when the sulfur level was increased in diets containing 8 ppm of molybdenum, indicating that 8 ppm of molybdenum partly overcame the depression caused by feeding 0.40% sulfur. This interaction did not exist for feed consumption, however; the higher sulfur level reduced ($P < 0.05$) feed consumption regardless of level of molybdenum. Other workers (11, 12) have shown that sulfate exerts a protective effect against molybdenum toxicity because it reduced absorption and increased urinary excretion of this element. Results of the present experiment indicate that the poor performance caused by a high level of sulfate was partially alleviated by feeding additional molybdenum, a relationship not found by previous workers.

Hemoglobin concentration and erythrocyte counts were not significantly affected by treatments. A three-way interaction existed for hematocrit; the higher sulfur level lowered hematocrit values except in sheep receiving the diet containing 10 ppm of copper and 8 ppm of molybdenum.

TABLE 2
Effects of sulfur, molybdenum and copper levels on sheep

Sulfur level, %	0.10						0.40						SE
	2		8		40		2		8		40		
	10	40	10	40	10	40	10	40	10	40	10	40	
No. of lambs ¹	10	10	10	10	10	10	10	10	10	10	10	10	15.40
Avg daily gain, ² g	102.7	98.6	87.5	78.2	50.1	39.9	94.7	60.5	0.08	0.92	1.12	0.92	0.08
Avg daily feed, ³ kg	1.12	1.19	1.15	1.09	0.94	0.86	1.12	0.92	1.20	1.20	1.26	0.34	1.20
Gain/100 g feed, ⁴ g	8.86	7.61	6.53	7.01	4.34	3.58	8.03	5.99	1.21	1.21	1.21	2.91	1.21
Hemoglobin, g/100 ml blood	13.1	13.5	12.4	13.0	12.2	12.9	13.2	38.7	1.21	1.21	1.21	2.91	1.21
Hematocrit, ⁵ %	41.3	41.4	38.9	40.4	37.7	40.7	41.2	38.7	1.21	1.21	1.21	2.91	1.21
Erythrocytes, 10 ⁶ /mm ³	12.8	12.1	12.7	12.2	11.7	12.3	11.9	12.1	0.09	0.09	0.09	7.44	0.09
Plasma protein, g/100 ml	6.47	6.66	6.61	6.92	6.47	6.22	6.34	6.30	0.18	0.18	0.18	7.44	0.18
Plasma copper, µg/100 ml	111.1	112.2	104.4	107.2	99.8	105.4	110.9	116.2	0.10	0.10	0.10	7.44	0.10
Plasma calcium, mg/100 ml	10.9	10.9	11.8	11.5	11.5	11.6	11.8	11.6	0.18	0.18	0.18	7.44	0.18
Plasma phosphorus, mg/100 ml	7.27	8.66	7.28	7.14	6.54	6.65	6.66	7.20	0.10	0.10	0.10	7.44	0.10
Plasma molybdenum, ⁷ µg/100 ml	10.7	10.5	29.2	20.3	10.2	7.9	41.0	30.4	0.10	0.10	0.10	7.44	0.10
Liver copper, ⁸ µg/g dry liver	153.9	267.7	118.4	179.0	117.0	120.1	67.2	113.8	0.17	0.17	0.17	7.44	0.17
Liver iron, ⁹ µg/g dry liver	497	456	431	466	502	551	576	659	0.17	0.17	0.17	7.44	0.17
Liver molybdenum, ¹⁰ µg/g dry liver	3.83	3.78	4.57	4.33	3.65	3.70	9.38	5.72	0.17	0.17	0.17	7.44	0.17

Sulfur level, %	0.10% S		0.40% S	
	10 ppm Cu	40 ppm Cu	10 ppm Mo	40 ppm Mo
5) S × Cu level interaction (*)	4.20	**	6.52	**
6) Mo × Cu interaction (*)	4.06		4.71	
7) S × Mo × Cu level interaction (*)	2 ppm Mo	8 ppm Mo	3.74	**
	10 ppm Cu	40 ppm Cu	3.74	*
	10 ppm Mo	40 ppm Mo	5.02	

Sulfur level, %	0.10% S		0.40% S	
	2 ppm Mo	8 ppm Mo	2 ppm Mo	8 ppm Mo
7) Hematocrit: S × Mo × Cu level interaction (*)	7.96	**	6.60	
** P < 0.05.				
** P < 0.01.				
8) Plasma phosphorus, mg/100 ml:				
1) 0.10% S(7.59) > 0.40% S(6.76)(**)				
2) 40 ppm Cu(7.41) > 10 ppm Cu(6.94)(*)				
3) S × Mo level interaction (*)				
9) Liver copper, µg/g dry liver:				
1) 0.10% S(176.8) > 0.40% S(104.5)(**)				
2) 2 ppm Mo(164.7) > 8 ppm Mo(119.6)(**)				
3) 40 ppm Cu(170.2) > 10 ppm Cu(114.1)(**)				
10) Liver molybdenum, µg/100 ml:				
1) 8 ppm Mo(30.2) > 2 ppm Mo(9.8)(**)				
4) S × Mo × Cu level interaction (*)				
9) Plasma molybdenum, µg/100 ml:				
1) 8 ppm Mo(30.2) > 2 ppm Mo(9.8)(**)				

¹ Five lambs per treatment for erythrocyte counts; all other values are an average obtained from 10 lambs.
² Gain, g/day:
 1) 0.10% S(91.8) > 0.40% S(61.3)(**). Main effects which were found to be different and the level of significance are enclosed in parentheses.
 2) Significant S × Mo level interaction (*).
³ Feed, kg/day: 0.10% S(1.14) > 0.40% S(0.96)(**).
⁴ Efficiency, g gain/100 g feed:
 1) 0.10% S(7.50) > 0.40% S(5.48)(*).
 2) S × Mo level interaction (**).
⁵ Liver iron, µg/g dry liver:
 1) 0.40% S(572) > 0.10% S(462)(*).
⁶ Liver molybdenum, µg/g dry liver:
 1) 0.40% S(5.61) > 0.10% S(4.13)(**).
 2) 8 ppm Mo(6.00) > 2 ppm Mo(3.74)(**).
 3) 10 ppm Cu(5.36) > 40 ppm Cu(4.38)(**).
 4) S × Mo level interaction (**).
⁷ Liver molybdenum, µg/g dry liver:
 1) 0.10% S(176.8) > 0.40% S(104.5)(**).
 2) 2 ppm Mo(164.7) > 8 ppm Mo(119.6)(**).
 3) 40 ppm Cu(170.2) > 10 ppm Cu(114.1)(**).

Protein, copper and calcium levels in blood plasma were not affected by treatments. Plasma phosphorus was affected ($P < 0.05$) by both copper and sulfur levels; the higher level of sulfur decreased ($P < 0.05$), whereas the higher level of copper increased ($P < 0.05$) plasma phosphorus levels. Also, there was a significant interaction between sulfur and molybdenum levels on plasma phosphorus level: Increasing the molybdenum level from 2 to 8 ppm caused reduced ($P < 0.05$) plasma phosphorus levels when 0.10% sulfur was fed but had no effect when the diet contained 0.40% sulfur. Also, when the level of sulfur was increased from 0.10 to 0.40% in diets containing 2 ppm of molybdenum plasma phosphorus was reduced ($P < 0.01$) but the addition had no effect when the diet contained 8 ppm of molybdenum.

There was a copper \times sulfur \times molybdenum level interaction ($P < 0.05$) in plasma phosphorus level: Sheep fed 0.40% sulfur had a lower level of plasma phosphorus except when the diet contained 40 ppm of copper and 8 ppm of molybdenum. Increased dietary levels of both copper and molybdenum were required to return plasma phosphorus to normal. Shirley et al. (13, 14) reported losses of phosphorus from the bodies of steers or rats to be two to three times normal when the diet contained high levels of molybdenum and low levels of copper. As increased levels of sulfate lowers the retention of copper in sheep (15) and adequate copper levels are required for proper phosphorus metabolism (14), it appears that molybdenum acts to correct the effect of a high level of sulfate and that additional copper was required because the molybdenum level did not completely counteract the effect of sulfate. Growth results of the present experiment, in which sheep fed 0.40% sulfur and 8 ppm of molybdenum gained much faster than those fed 0.40% sulfur and 2 ppm of molybdenum, support this idea.

Plasma molybdenum levels increased as the dietary levels increased and these results agree with those of Cox and Harris (16) and Gray and Daniel (17).

The higher level of sulfur or molybdenum reduced ($P < 0.01$) copper storage in the liver and increased dietary copper caused increased storage of the element.

Goodrich and Tillman (15) observed that dietary sulfate in comparison with elemental sulfur lowered liver copper even in the presence of low levels of molybdenum. As Dick (18) reported that ferrous sulfide reduced copper absorption, it appears that insoluble copper sulfide may be formed from either sulfate or sulfide, resulting in reduced absorption of copper from the intestinal tract of ruminants. Molybdenum also reduces copper stores (19, 20). Dick (18) suggested that high intakes of molybdenum reduce copper absorption and increase copper excretion only in the presence of adequate endogenous or exogenous sulfate. Results of the present experiment indicate that 0.10% sulfur, as sulfate, was adequate for molybdenum to exert this effect. As the effect of elemental sulfur upon copper storage appears to be different (15), further work in this area is indicated.

Liver iron levels were significantly increased as the sulfur level of the ration was increased and are interpreted to be a reflection of the influence of sulfate on copper metabolism since Matrone (21) observed that during copper deficiency iron can enter liver tissue.

Liver molybdenum levels were affected ($P < 0.05$) by all treatments, and all interactions were significant. The copper \times sulfur \times molybdenum interaction was caused by liver molybdenum being lower when the diet contained 0.40% sulfur plus 2 ppm of molybdenum and 10 or 40 ppm of copper, in contrast with the small increase obtained when the diet contained 0.40% sulfur, 8 ppm of molybdenum and 40 ppm of copper and a large increase when the diet contained 0.40% sulfur, 8 ppm of molybdenum and 10 ppm of copper. These data are in agreement with results of other workers (2), but the reason for the increase in liver molybdenum when 0.40% sulfur, 8 ppm of molybdenum and 10 or 40 ppm of copper were fed remains obscure.

Correlations of various plasma and liver mineral levels are shown in table 3. The correlations represent within-treatment and within-replication calculations and thus are unbiased by treatment means. Only the plasma molybdenum level and liver molybdenum coefficient was significant ($P < 0.05$); however, the data tend to sup-

TABLE 3
Within treatment, within replication correlation coefficients among some plasma and liver values

Variables	Correlation Coefficients ¹
Liver Cu and liver Fe	0.11
Liver Cu and liver Mo	-0.19
Liver Cu and plasma Cu	0.04
Liver Cu and plasma Mo	-0.03
Liver Fe and liver Mo	-0.08
Liver Mo and plasma Mo	0.49 ²
Plasma Cu and plasma Mo	0.26

¹ Degrees of freedom are 48.

² P < 0.01.

port the idea (2) that lambs with high molybdenum levels have low liver copper and high plasma molybdenum levels and those with high plasma copper levels also tend to have high plasma molybdenum levels.

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