Effect of high levels of dietary molybdenum and sulphate on SA Mutton Merino sheep. I. Mineral status and haematological parameters

F.E. van Niekerk * and C.H. van Niekerk

Department of Human and Animal Physiology, University of Stellenbosch, Stellenbosch 7600, Republic of South Africa

Received 30 May 1988; accepted 29 October 1988

Forty-five adult SA Mutton Merino ewes and 36 eight-month-old ram lambs were divided into three groups so that each group contained an equal number of animals of each haemoglobin type AA, AB and BB. Three diets were compiled. The control diet (C) contained 8,02 mg Cu/kg, 1,3 mg Mo/kg and 0,22% S; the molybdenum supplemented diet (M) contained 3,45 mg Cu/kg, 40,0 mg Mo/kg and 0,22% S; and the molybdenum and sulphate (MS) supplemented diet 3,55 mg Cu/kg, 38 mg Mo/kg and 0,34% S. After receiving these diets for a period of four months, the mean ± SD concentration of copper in the livers of ewes in group C was 234 ± 98 μg/g; of ewes in group M 28 ± 4 μg/g; and of ewes in group MS 26 ± 4 μg/g, whereas in the rams it was 153 ± 31 μg/g in group C, 56 ± 24 μg/g for group M and 26 ± 5 μg/g for group MS. The concentrations of plasma copper in groups M and MS were higher than that of the control group in both the ewes and the rams. In the case of group C, the concentration of TCA-soluble plasma copper represented about 95% of the total plasma copper compared to 65% in the case of groups M and MS. The induced copper deficiency in groups M and MS was characterized by a loss in wool crimp and wool production as well as loss of body mass. Total concentrations of plasma copper may not be a reliable diagnostic index of copper deficiency in sheep in the presence of high concentrations of molybdenum and sulphate in pastures or drinking water. Under these circumstances, liver copper concentration may be regarded as the most accurate index of the copper status of such animals. The supplementation of molybdenum and sulphate did not influence the concentrations of plasma zinc, calcium, magnesium or phosphorus. However, an effective systemic copper deficiency in both the rams and ewes in groups M and MS appeared to be induced by this treatment.

Vyf-en-veertig volwasse SA Vleismerino-ooie en 36 agt-maande-oue ramlammers is in drie gelyke groepe verdeel sodat daar ewe veel diere van elke hemoglobientipe AA, AB en BB per groep was. Drie rantsoene is saamgestel. Die kontrolerantsoen (C) het 8,02 mg Cu/kg, 1,3 mg Mo/kg en 0,22% S bevat. Die molibdeengesupplementeerde rantsoen (M) het 3,45 mg Cu/kg, 40,0 mg Mo/kg en 0,22% S bevat en die molibdeen- en sulfaatgesupplementeerde rantsoen (MS) 3,55 mg Cu/kg, 38,0 mg Mo/kg en 0,34% S. Nadat die diere die rantsoene vir 'n tydperk van vier maande ontvang het was die gemiddelde lewerkoperkonsentrasie van die ooie in groep C 234 ± 98 μg/g, van ooie in groep M 28 ± 4 μg/g en van dié in groep MS 26 ± 4 μg/g, teenoor 153 ± 31 μg/g van ramme in groep C, 56 ± 24 μg/g van ramme in groep M en 26 ± 5 μg/g van ramme in groep MS. Die plasmakoperkonsentrasies van groepe M en MS was egter hoër as dié van die kontrolegroep. TCA-oplosbare plasmakoperkonsentrasies van groep C het ongeveer 95% van die totale plasmakoper verteenwoordig in vergelyking met 65% by die gesupplementeerde groepe. Die kopertekort by groepe M en MS is verder bevestig deur die afname in wolproduksie en liggaamsmassa asook die verlies aan wolkarteling. Totale plasmakoperkonsentrasie is nie 'n betroubare diagnostiese maatstaf vir die identifisering van kopertekorte by skape indien die molibdeen- en sulfaatkonsentrasies in die weiding of drinkwater hoog is nie. Onder sulke toestande is die lewerkoperkonsentrasie die akkuraatste maatstaf vir die vasstelling van die dier se koperstatus. Die byvoeging van molibdeen en sulfaat het geen invloed op die plasmasink-, kalsium-, magnesium- of fosfaatkonsentrasies gehad nie, maar het wel 'n effektiewe sistemiese kopertekort in die ramme sowel as die ooie van groepe M en MS veroorsaak.

Keywords: Copper deficiency, molybdenum, sheep, sulphate.

* To whom correspondence should be addressed at present address: Elsenburg Agricultural Centre, Private Bag, Elsenburg 7607, Republic of South Africa.

Introduction

Copper deficiencies in soil and pastures prevail in large parts of the intensive sheep farming areas along the south and western coasts of South Africa (Van der Merwe & Perold, 1967). Induced copper deficiencies caused by high molybdenum and sulphate intakes cause serious losses in animal production in many parts of the world (Underwood, 1977). Excessive copper intake through natural grazing, as found in large parts of the Karoo, leads to copper poisoning, a condition also referred to as enzoötic icterus (Bath, 1979). Copper metabolism differs widely between animal species (Ward, 1978) as well as within sheep breeds (Wiener, 1979). Moreover, within sheep breeds, copper metabolism is affected by genetic aspects such as haemoglobin type (Wiener, 1979). Considerable care must therefore be taken not to use the results of one species or breed as a model for another to study copper metabolism.

The aim of the present investigation was to study the effect of high dietary molybdenum and molybdenum in combination with sulphate intakes on copper metabolism as well as other macro and micro minerals and certain haematological parameters in SA Mutton Merino sheep. Secondly, the effectiveness of molybdenum and sulphate in decreasing blood and liver copper reserves, which has a possible practical application in areas of endemic excess copper, was also evaluated. Ewes with an induced copper deficiency were used as experimental models to study the effect of a copper deficiency on certain aspects of reproduction in ewes as descibed in the subsequent paper.

Materials and Methods

Forty-five adult SA Mutton Merino ewes and 36 eight-month-old ram lambs were used. The ewes and rams were divided into three groups each, having an equal number of haemoglobin types AA, AB and BB in each group. The

animals were kept individually in a feeding shed during the experimental period.

The control group (C) of each sex was fed on the control diet consisting of 45,3% oat hay, 38% oats, 10% lucerne hay, 1,5% feed grade urea, 2,5% white sugar, 1,0% $CaCO_3$, 0,3% Na_2PO_4 and 0,7% NaCl. Copper sulphate (15 g) was added to ration C. Rations M and MS were each supplemented with 100 g $(NH_4)_6Mo_4.4H_2O$ per ton of feed whereas ration MS received an additional 4,5 kg Na_2SO_4 per ton. The rams as well as the ewes received 1,2 kg feed daily during the experimental period.

For the purpose of this study, the experimental period lasted 12 weeks for the ewes and 24 weeks for the rams. All the sheep were sheared after four weeks and again six months later. All the animals were weighed monthly.

During the experimental period, blood samples (20 ml) from the jugular veins of the experimental animals were collected fortnightly in heparinized vacuum sampling tubes (Vac U Test). After having been on the rations for 12 weeks, three ewes and three rams from each group were slaughtered and the livers and kidneys removed for trace mineral analysis.

Concentrations of copper, zinc, manganese and iron in the food, liver and kidney samples were determined by means of atomic absorption spectrophotometry after acid digestion. Total plasma copper and zinc concentrations were determined directly on the atomic absorption spectrophotometer after the samples had been diluted with distilled, deionized water in a concentration of 1:5 (Van Niekerk, Van Niekerk & Morgenthal, 1988). Trichloroacetic (TCA) acid-soluble plasma copper was determined by using the method of Smith & Wright (1975). Caeruloplasmin-associated copper was determined by the method described by Ravin (1956) using the equation:

$$Y = -0,07 + 0,94\ X \quad \text{(Bingley, 1974)}$$

where X is the non-direct-reacting copper and Y the absorbance.

Plasma inorganic phosphate concentrations were determined as described by Goldenberg & Fernandez (1974), plasma calcium concentrations as described by Grindler & King (1972) and magnesium concentrations according to the method of Varley, Gowenlock & Bell (1980).

The red (RBC) and white (WBC) blood-cell counts, the mean cell volume (MCV) and the haemoglobin concentration (Hb) were determined by using an automatic cell counter (Model ZF, Coulter Electronics, USA).

After acid digestion of samples, the molybdenum content of food, liver and kidney samples was determined spectrophotometrically, using the method of Quin & Brooks (1975). Phosphate content of the rations was determined spectrophotometrically (AOAC, 1980) and nitrogen content according to the macro-kjeldahl method as described by the AOAC (1980). Sulphur content was determined spectrophotometrically according to the method of Tabatabai & Bremner (1970). Using the *in vitro* technique as described by Engels & Van der Merwe (1967), digestible organic matter (DOM) of feed samples was determined.

Haemoglobin types were identified by the Genetics Section, ADSRI, Irene, by making use of gel electrophoresis (Van Niekerk, 1985).

Results were statistically evaluated according to a one-way variance and covariance analysis using the standard BMDP program, P1V (Dixon, 1981). In cases where only mean values are presented, the standard deviation ($\pm$ *SD*) of the mean is also indicated.

Results

Mineral, crude protein and digestable organic matter contents of the rations are given in Table 1.

Table 1 The macro and micro mineral content, percentage crude protein, and percentage digestable organic matter of the three rations

	%		mg/kg								
Diet	CP	DOM	Ca	P	Mg	S	Cu	Zn	Mn	Se	Mo
C	12,1	60,1	0,60	0,21	0,15	0,22	8,02	33,0	40,0	0,05	1,3
M	12,2	60,8	0,63	0,21	0,15	0,22	3,45	31,0	42,0	0,05	40,0
MS	11,9	61,0	0,59	0,21	0,15	0,34	3,55	34,0	45,0	0,05	38,0

The mean monthly body mass of the rams and ewes during the experimental period, as well as for a part of the period during which the reproduction trial was done, is given in Table 2. In group C the rams gained mass and the ewes maintained their initial mass. Body mass of animals in group M was not so severely affected by treatment as was the case in group MS.

Table 2 Mean monthly body mass (kg) of the rams and ewes in the three treatment groups

	Control (C)		Molybdenum (M)		Molybdenum-sulphate (MS)	
Months	Rams	Ewes	Rams	Ewes	Rams	Ewes
0	32,8[a]	51,8[a]	33,3[a]	50,7[a]	31,6[a]	53,7[a]
1	35,1[a]	51,4[a]	33,7[a]	49,2[a]	30,3[b]	50,3[a]
2	37,8[a]	52,2[a]	35,9[a]	49,3[a]	30,3[b]	47,7[a]
3	40,6[a]	52,6[a]	38,4[a]	51,1[a]	31,7[b]	48,8[a]
4	43,8[a]		41,5[a]		35,6[b]	
5	46,4[a]		44,8[a]		37,5[b]	
6	49,6[a]		47,4[a]		40,2[b]	
7	52,2[a]		49,8[a]		42,0[b]	
8	54,9[a]		51,0[b]		44,5[c]	

[a,b] Values in the same row, same sex, with different headings differ significantly ($P \leq 0,05$).

Wool production of both the ewes and rams is given in Table 3. A mild to severe loss of crimp in the wool produced from animals in groups M and MS was evident.

The mean concentrations of total plasma copper and zinc, TCA-soluble plasma copper and the caeruloplasmin-associated copper for both the ewes and rams

Table 3 Mean fleece mass (± *SD*) of the rams and ewes in the three groups

	Fleece mass (kg)		
	Group C	Group M	Group MS
Rams	3,455 ± 0,46	2,993 ± 0,23	2,077 ± 0,53
% Wool loss	0	14	40
n[a]	9	8	8
Ewes	3,779 ± 0,61	3,321 ± 0,54	2,467 ± 0,65
% Wool loss	0	12	35
n	11	11	11

[a] Number of animals.

are presented in Tables 4 & 5. Concentration of plasma copper remained constant in group C but increased in groups M and MS to concentrations higher than those found in group C. Simultaneously, concentrations of TCA-soluble plasma copper in group M, and particularly group MS, decreased when compared to group C. Concentrations of plasma zinc were not affected by treatment.

The mean concentrations of liver and kidney copper, iron, zinc, manganese and molybdenum of both the ewes and the rams are presented in Table 6. Concentrations of liver copper were drastically decreased by treatment in groups M and MS. Concentrations of iron, zinc and manganese were not affected by treatment. Concentrations of molybdenum in both the liver and kidneys of sheep in groups M and MS were increased.

The concentrations of plasma zinc, phosphorus, calcium and magnesium of the ewes during the experimental period are given in Table 7. According to the concentrations of these minerals in the plasma, treatment had no effect thereupon.

In Table 8 haematological parameters such as the red blood-cell count (RBC), white blood-cell count (WBC), haemoglobin content (Hb) and the mean cell volume (MCV) were recorded. The ewes of group MS became anaemic whereas ewes of group M were not affected by treatment. White blood-cell concentrations increased in group MS while it remained constant in group C and group M.

Table 4 Mean (± *SD*) concentrations of plasma copper and zinc, TCA-soluble copper, and caeruloplasmin-associated copper of the ewes

		Time in weeks on experimental diets				
Mineral	Group	1	3	5	7	9
Plasma copper	C	122 ± 14	111 ± 19	114 ± 10	121 ± 9	115 ± 10
(μg/dl)	M	199 ± 30	176 ± 21	170 ± 27	176 ± 30	135 ± 9
	MS	162 ± 28	159 ± 24	140 ± 21	137 ± 22	113 ± 15
TCA-soluble	C	111 ± 15	97 ± 14	111 ± 11	107 ± 8	106 ± 8
copper	M	118 ± 8	124 ± 12	98 ± 13	91 ± 14	87 ± 11
(μg/dl)	MS	105 ± 10	111 ± 14	84 ± 9	90 ± 12	78 ± 8
Caeruloplasmin	C	49 ± 5,8	46 ± 3,6	45 ± 5,0	43 ± 3,5	47 ± 3,9
copper	M	47 ± 8,7	38 ± 4,0	40 ± 4,9	36 ± 3,6	45 ± 2,9
(μg/dl)	MS	45 ± 6,3	33 ± 4,1	41 ± 3,1	40 ± 5,0	45 ± 3,1
Zinc	C	120 ± 12	131 ± 13	114 ± 13	91 ± 9	88 ± 20
(μg/dl)	M	119 ± 11	123 ± 12	122 ± 11	98 ± 9	84 ± 7
	MS	118 ± 8	127 ± 10	117 ± 9	91 ± 11	83 ± 9

Table 5 Mean (± *SD*) concentrations of plasma copper, TCA-soluble copper, plasma zinc and caeruloplasmin copper of the rams

		Time in weeks									
Component	Group	1	3	5	7	9	11	13	15	17	19
Plasma copper	C	105 ± 15	120 ± 6	118 ± 12	114 ± 8	120 ± 8	126 ± 10	126 ± 7	131 ± 7	122 ± 11	133 ± 11
(μg/dl)	M	148 ± 35	189 ± 18	166 ± 14	169 ± 18	160 ± 30	168 ± 29	164 ± 18	165 ± 21	165 ± 25	150 ± 19
	MS	145 ± 21	161 ± 24	161 ± 17	162 ± 23	138 ± 21	124 ± 26	145 ± 30	156 ± 20	144 ± 31	130 ± 14
TCA-soluble	C	99 ± 15	112 ± 7	115 ± 11	109 ± 9	113 ± 7	115 ± 13	114 ± 13	119 ± 8	117 ± 7	127 ± 15
copper	M	92 ± 23	114 ± 20	85 ± 11	82 ± 6	86 ± 21	83 ± 18	75 ± 11	71 ± 6	88 ± 14	92 ± 21
(μg/dl)	MS	95 ± 11	97 ± 12	83 ± 9	90 ± 12	84 ± 19	72 ± 19	73 ± 13	73 ± 14	83 ± 7	87 ± 8
Caeruloplasmin	C	43 ± 1,5	41 ± 2,8	42 ± 2,1	43 ± 2,7	44 ± 7,8	46 ± 2,7	36 ± 2,0	36 ± 4,2	36 ± 5,1	32 ± 4,2
copper	M	43 ± 1,2	37 ± 4,8	39 ± 2,3	43 ± 2,5	43 ± 8,3	49 ± 4,6	32 ± 4,2	22 ± 4,2	33 ± 5,7	33 ± 2,9
(μg/dl)	MS	14 ± 2,5	41 ± 3,2	40 ± 1,6	40 ± 1,4	41 ± 4,4	44 ± 2,2	40 ± 3,6	27 ± 4,2	23 ± 1,9	30 ± 5,0
Plasma	C	120 ± 7	118 ± 8	128 ± 8	115 ± 7	114 ± 9	120 ± 6	107 ± 12	116 ± 6	120 ± 8	131 ± 7
zinc	M	119 ± 9	124 ± 9	134 ± 8	124 ± 11	129 ± 9	125 ± 8	107 ± 9	114 ± 7	122 ± 8	144 ± 6
(μg/dl)	MS	111 ± 10	113 ± 11	114 ± 15	124 ± 10	121 ± 11	113 ± 6	100 ± 8	112 ± 9	113 ± 9	138 ± 12

Table 6 Mean (± *SD*) concentrations of liver and kidney trace elements of the rams and ewes after having received the diets for a period of 12 weeks

			Mineral in µg/g DM				
Sex	Group	Organ	Cu	Fe	Zn	Mn	Mo
Ewes	C	Liver	234 ± 98	446 ± 11	106 ± 11	11,3 ± 2,3	7,6 ± 1,3
	M	Liver	28 ± 4	505 ± 88	95 ± 18	10,7 ± 2,5	22,2 ± 2,7
	MS	Liver	26 ± 7	516 ± 165	170 ± 52	10,3 ± 2,1	18,9 ± 8,4
Ewes	C	Kidney	17 ± 9	405 ± 229	73 ± 40	3,3 ± 1,1	7,8 ± 1,9
	M	Kidney	70 ± 38	612 ± 242	93 ± 4	4,3 ± 0,5	64,7 ± 2,1
	MS	Kidney	76 ± 36	651 ± 256	145 ± 64	5,3 ± 2,1	47,2 ± 13,0
Rams	C	Liver	153 ± 31	374 ± 99	113 ± 11	8,3 ± 1,5	6,3 ± 0,3
	M	Liver	56 ± 24	294 ± 76	119 ± 5	8,7 ± 0,6	30,1 ± 7,2
	MS	Liver	26 ± 5	544 ± 99	122 ± 6	9,4 ± 1,5	19,2 ± 4,5
Rams	C	Kidney	39 ± 6	257 ± 95	97 ± 3	4,0 ± 1,0	5,5 ± 0,9
	M	Kidney	35 ± 23	398 ± 247	74 ± 50	2,0 ± 1,0	37,8 ± 11,1
	MS	Kidney	41 ± 8	513 ± 100	100 ± 39	2,3 ± 0,6	30,1 ± 8,5

Table 7 Mean (± *SD*) concentrations of plasma calcium, phosphorus and magnesium of the ewes

		Time in weeks on experimental data				
Mineral	Group	1	3	5	7	9
Calcium	C	1,82 ± 0,19	1,93 ± 0,06	1,88 ± 0,21	1,90 ± 0,09	1,83 ± 0,09
(mmol/l)	M	1,82 ± 0,27	1,86 ± 0,26	1,89 ± 0,21	1,86 ± 0,14	1,77 ± 0,11
	MS	1,76 ± 0,17	1,85 ± 0,16	1,84 ± 0,21	1,76 ± 0,11	1,76 ± 0,18
Phosphorus	C	1,50 ± 0,20	1,48 ± 0,24	1,43 ± 0,34	1,27 ± 0,23	1,82 ± 0,23
(mmol/l)	M	1,50 ± 0,29	1,46 ± 0,21	1,63 ± 0,39	1,40 ± 0,35	1,73 ± 0,29
	MS	1,36 ± 0,22	1,36 ± 0,24	1,46 ± 0,36	1,38 ± 0,34	1,58 ± 0,39
Magnesium	C	0,63 ± 0,04	0,67 ± 0,03	0,69 ± 0,06	0,64 ± 0,03	0,62 ± 0,03
(mmol/l)	M	0,64 ± 0,03	0,64 ± 0,04	0,65 ± 0,06	0,62 ± 0,03	0,59 ± 0,04
	MS	0,63 ± 0,02	0,63 ± 0,04	0,63 ± 0,07	0,57 ± 0,05	0,58 ± 0,04

Table 8 Mean (± *SD*) red blood-cell count (RBC), white blood-cell count (WBC), haemoglobin (Hb) concentration and mean cell volume (MCV) of whole blood of the ewes in the three experimental groups

		Time in weeks on experimental diets				
Component	Group	1	3	5	7	9
RBC	C	7,49 ± 1,05	7,17 ± 0,93	8,02 ± 1,11	8,38 ± 1,42	9,14 ± 0,68
($\times 10^6$/ml)	M	7,66 ± 0,89	7,38 ± 1,23	7,44 ± 1,25	7,98 ± 1,79	8,69 ± 1,25
	MS	7,07 ± 0,84	6,68 ± 1,16	5,94 ± 1,97	6,84 ± 2,18	6,18 ± 2,27
MCV	C	42 ± 1,9	42 ± 2,4	43 ± 1,7	45 ± 1,9	44 ± 2,6
(cu/µ)	M	42 ± 2,4	42 ± 2,4	44 ± 3,9	45 ± 3,4	45 ± 3,2
	MS	42 ± 3,1	41 ± 1,9	42 ± 2,2	46 ± 4,5	44 ± 3,3
WBC	C	5,45 ± 1,30	5,39 ± 1,75	5,59 ± 1,51	7,29 ± 3,43	6,72 ± 2,46
($\times 10^3$/ml)	M	5,33 ± 1,48	5,16 ± 2,36	6,56 ± 5,12	8,01 ± 5,12	6,48 ± 2,29
	MS	5,69 ± 1,42	6,31 ± 1,57	6,19 ± 1,99	9,90 ± 4,78	9,00 ± 4,10
Hb	C	10,7 ± 1,3	11,2 ± 1,2	11,9 ± 1,5	13,4 ± 1,4	13,6 ± 1,1
(g/dl)	M	11,1 ± 1,3	10,7 ± 1,6	11,4 ± 1,7	12,8 ± 2,0	12,7 ± 2,1
	MS	10,9 ± 1,4	10,0 ± 1,7	8,7 ± 2,9	9,9 ± 2,9	8,8 ± 3,5

Discussion

All constituents (Table 1), determined on a dry matter basis, were within normal ranges, except for copper and molybdenum (AOAC, 1980). The concentration of copper was increased in the diet of group C as copper concentrations of between 8 and 12 μg/kg are expected to meet the requirement of sheep (Underwood, 1977). Feed copper concentration of less than 4μg Cu/g DM is low, as was the case with the basic diet, and therefore diet C was supplemented with copper.

Adult animals (ewes) receiving molybdenum and sulphate (group MS) lost mass while the young rams in group MS gained mass at a slow rate. The animals of group C maintained their body mass and even gained mass in some cases, indicating that the control ration was sufficient for maintenance purposes. The fact that the animals of the two supplemented groups lost mass or gained mass more slowly, is a first indication that the induced copper deficiency was of such a nature that it might have affected body functions.

The rams as well as the ewes in the supplemented groups suffered a loss in wool production (Table 3). There was also a mild to severe loss of crimp in the wool from these groups which further suggests that a copper deficiency existed. According to Underwood (1977), a loss of crimp in the wool is one of the first symptoms shown by woolbearing sheep suffering from a copper deficiency.

During the first part of the experiment, concentrations of plasma copper of the ewes (Table 4) and rams (Table 5) in groups M and MS reached higher concentrations than those in group C. This is in agreement with results of Dick (1953), who also reported a rise in the concentrations of plasma copper after supplementation of rations with molybdenum and sulphate. Concentrations of plasma copper of 80—150 μg/dl are regarded as normal (Underwood, 1977). Therefore it is clear that, when sheep receive molybdenum and molybdenum in combination with sulphate, the concentration of total plasma copper rises initially to well above normal concentrations. This may be ascribed to the formation of inaccessible thiomolybdate complexes which bind copper (Suttle, 1980). These inactive complexes from which copper is not biologically available, continue to circulate in the blood stream for an indefinite period of time. Therefore, in spite of high concentrations of total plasma copper, a systemic deficiency of biologically active copper develops. As a result, more copper is withdrawn from the liver, only to be continuously converted into an inactive copper thiomolybdate complex in the blood. This is in agreement with the findings of Dick (1956) and Marcilese, Ammerman, Valsecchi, Dunavant & Davis (1969), who found that, when a molybdenum- and sulphate-induced copper deficiency arises, the concentration of liver copper usually decreases.

Under primary copper deficiency, a decrease in plasma copper concentration does occur, but in circumstances where a secondary copper deficiency arises as a result of high molybdenum and sulphate concentrations in the diet, a decrease in plasma copper does not take place as a primary event (Suttle, 1980).

Dick (1956) and Huisingh, Gomez & Matrone (1973) originally described the interaction of copper and molybdenum as two separate processes. According to this hypothesis, high concentrations of molybdenum and sulphate in the same ration lead to a combined antagonistic effect that is far greater than their individual effects at the same concentrations. It is, however, not clear how this effect is brought about (Suttle, 1980). Matrone's hypothesis (1973), however, allowed for a three-part interaction of copper, molybdenum and sulphate in ruminants. This reaction leads to the formation of thiomolybdate in the rumen. The presence of certain copper- and molybdenum-containing proteins in the plasma of ewes indicated that the formation of the thiomolybdate complex may possibly also occur systemically in the ruminant (Suttle, 1980). By dosing sheep with thiomolybdate, Dick, Dewey & Gawthorne (1975) found a rise in TCA-insoluble plasma copper which corresponded with that of sheep receiving molybdenum and sulphate in their rations.

Important in the evaluation of the thiomolybdate hypothesis, was the verification of thiomolybdate formation *in vivo* at low molybdenum concentrations, which are antagonistic to copper. Bremner & Young (1978) found indications that the formation of thiomolybdate does occur systemically. The distribution of copper between the different blood cells and plasma, and thus the biochemical reactions involved, differ markedly between animals suffering from a secondary (induced) copper deficiency and those suffering from a primary copper deficiency.

Dick *et al.* (1975) indicated that these thiomolybdate complexes in plasma may be precipitated by using TCA. According to the results in Tables 4 and 5, the mean concentration of TCA-soluble plasma copper in groups M and MS failed to indicate that a serious copper deficiency might have developed in these sheep. After some time, concentrations of TCA-soluble copper in these supplemented groups did, however, decline. The total as well as the TCA-soluble concentration of plasma copper of group MS (Tables 4 & 5) remained consistently lower than those of the sheep in group M. In group C the concentration of TCA-soluble plasma copper represented approximately 95% of the total plasma copper, compared to approximately 65% in groups M and MS. With this in mind, it appears that the total concentration of TCA-soluble plasma copper is not a true reflection of the biologically available copper. According to current results, it seems that the relation of the concentration of total plasma copper to the TCA-soluble plasma copper might serve as a possible indicator of secondary copper deficiency.

The mean concentration of liver copper in the ewes and rams of group C (Table 6) remained within the normal limits of 100—500 μg/g DM (Underwood, 1977), but the rams as well as the ewes in groups M and MS developed a serious copper deficiency. Supplementation of molybdenum and molybdenum in combination

with sulphate results in a drastic decline in liver copper reserves. This is in greement with the findings of Dick (1956) and Marcilese *et al.* (1969). The liver concentrations of iron, zinc and manganese were not affected by treatment.

In group C, the mean concentration of molybdenum in the livers of both the rams and ewes remained normal and was almost the same as that of the kidneys. In contrast, the mean liver molybdenum concentrations of groups M and MS increased by approximately 3—5 times the concentrations found in group C. Concentrations of kidney molybdenum in groups M and MS were approximately 1,5—3 times greater than in the liver. The kidney Cu:Mo ratio in groups M and MS varied between 1—1,6 :1 which is somewhat lower than the values reported by Van Ryssen & Stielau (1980). Grace & Suttle (1979) demonstrated that these copper-containing thiomolybdates are poorly excreted by both the urinary and faecal routes in sheep. Present results suggest that this copper-thiomolybdate complex may be accumulated by the kidneys, in agreement with the findings of Van Ryssen & Stielau (1981).

According to the results given in Tables 4 and 5, treatment had no effect on the plasma concentrations of zinc in both the rams and ewes, as the mean concentrations were constantly within normal limits (80—160 μg/dl; Underwood, 1977). Concentrations of plasma calcium, phosphorus and magnesium in the ewes (Table 7) were not affected by treatment and were within normal levels (Underwood, 1977).

As indicated in Table 8, the ewes in all three groups were slightly anaemic at the beginning of the experiment, based on normal values for RBC concentrations in sheep [8—13 ($\times 10^6$) RBC/ml; Swenson, 1977]. The mean RBC concentration of group C remained normal, while animals of group M developed a mild anaemia and those in group MS a severe degree of anaemia during the experimental period. According to Underwood (1977), a copper deficiency may affect haematopoiesis when the concentration of plasma copper drops below 30 μg/dl. These findings serve as further evidence that the concentration of TCA-soluble plasma copper is not a true reflection of biologically available copper in the plasma. The mean cell volume (MCV) was not affected by treatment. The mean concentration of white blood cells (WBC) of groups C and M remained within normal limits (4000—8000/ml; Swenson, 1977). The mean WBC concentration in group MS rose to abnormally high after the ewes had received the rations for seven weeks. Group MS, which appeared to suffer a serious systemic copper deficiency, experienced a sharp drop in the RBC count concurrent with a rise in the WBC concentration. Since differential white blood-cell counts were not carried out, the contribution of the various leucocyte groups remains uncertain. Van Rensburg (1961) made a similar observation and pointed out that a change in the ratio of the various white blood-cell types to one another may result in malfunction of the endocrine system. The haemoglobin values of groups C and M were within the normal range of 10—13 g/dl (Swenson, 1977), while the value of group MS was abnormally low.

Conclusion

A mild systemic (liver) copper deficiency may result from a high concentration of molybdenum in the ration, in natural grazing or in the drinking water. Should high concentrations of sulphate occur in conjunction with high molybdenum concentrations, a serious systemic copper deficiency may develop with all its harmful consequences, such as poor growth, poor wool production, anaemia, low fertility and poor postnatal growth. However, when the concentration of copper is naturally high in the pastures grazed, e.g. in parts of the Karoo, it may result in copper toxicity, but molybdenum and sulphate supplementation of the ration, the lick or the drinking water may ameliorate the problem to a large extent.

According to results obtained in this study, total concentration of plasma copper is by no means a reliable diagnostic index in identifying copper deficiencies, particularly in the presence of high concentrations of molybdenum and sulphate in the pastures grazed. Actual availability of the TCA-soluble copper to the animal may therefore be questioned. It would appear that the ratio of the total plasma copper to the TCA-soluble plasma copper might be of some value in this regard. A rise in the concentration of kidney molybdenum to concentrations of more than 8 μg/g DM might be indicative of an excess of molybdenum. From these results, it is clear that concentrations of liver copper may be regarded as the most accurate method in diagnosing the copper status of sheep, especially in the presence of high levels of molybdenum and sulphate in the soil or drinking water.

Acknowledgements

The authors thank Dr E. Meyer and Mr G. Reid, Genetics Section, ADSRI, for the haemoglobin typing of blood samples as well as Mr E.W.P. Heine, Regional Veterinary Laboratory, for his assistance.

References

AOAC, 1980. Official methods of analysis (9th edn.). Association of Official Analytical Chemists, Washington, DC.

BATH, G., 1979. Enzoötic icterus – A form of chronic copper poisoning. *J. S. Afr. Vet. Assoc.* 50 (1), 3.

BINGLEY, J.B., 1974. Effects of high doses of molybdenum and sulphate on the distribution of copper in plasma and in blood of sheep. *Aust. J. agric. Res.* 25, 467.

BREMNER, I. & YOUNG, B.W., 1978. Effects of dietary molybdenum and sulphur on the distribution of copper in plasma and kidneys of sheep. *Br. J. Nutr.* 39, 325.

DICK, A.T., 1953. The control of copper storage in the liver of sheep by inorganic sulphate and molybdenum. *Aust. Vet. J.* 29, 233.

DICK, A.T., 1956. In: Inorganic nitrogen metabolism. Eds. McElroy, W.D. & Glass, B. John Hopkins Press, Baltimore. p. 445.

DICK, A.T., DEWEY, D.W. & GAWTHORNE, J.M., 1975. Thiomolybdates and the copper-molybdenum-sulphur interaction in ruminant nutrition. *J. agric. Sci.* 85, 567.

DIXON, W.J., 1981. BMDP Statistical Software, University of California Press, Los Angeles.

ENGELS, E.A.N. & VAN DER MERWE, F.J., 1967. Application of an *in vitro* technique to South African forages with special reference to the effect of certain factors on the results. *S. Afr. J. agric. Sci.* 10, 783.

GOLDENBERG, H. & FERNANDEZ, A., 1966. In: Clinical Chemistry. Eds. Henry, R.J., Cannon, D.C. & Winkelman, J.W., Harper & Row Publishers, New York.

GRACE, N.D. & SUTTLE, N.F., 1979. Some effects of sulphur intake on molybdenum metabolism in sheep. *Br. J. Nutr.* 41, 125.

GRINDLER, E.M. & KING, J.D., 1972. Rapid calorimetric determination of calcium in biologic fluids with methylthymol blue. In: Practical Clinical Biochemistry (Vol 1, 5th Edn.). Eds. Varley, H., Gowenlock, A.H. & Bell, M., William Heineman Medical Books Ltd., London.

HUISINGH, J., GOMEZ, G.G. & MATRONE, G., 1973. Interactions of copper, molybdenum and sulphate in ruminant nutrition. *Fedn. Proc. Am. Soc. Exp. Biol.* 32, 1921.

MARCILESE, N.A., AMMERMAN, C.B., VALSECCHI, R.M., DUNAVANT, B.G. & DAVIS, G.K., 1969. Effect of dietary molybdenum and sulphate upon copper metabolism in sheep. *J. Nutr.* 99, 177.

MATRONE, 1973. In: Proceedings, first international symposium on trace element metabolism in animals. Ed. Mills, C.F., E & S Livingstone, Edinburgh, Scotland. p. 354.

QUIN, R.F. & BROOKS, R.R., 1975. The rapid calorimetric determination of molybdenum with dithiol in biological, geological and steel samples. *Clin. Chim. Acta* 74, 75.

RAVIN, H.A., 1956. Rapid test for hepatolenticular degeneration. *Lancet* 1, 726.

SMITH, B.S.W. & WRIGHT, H., 1975. Copper: molybdenum interaction. Effect of dietary molybdenum on the binding of copper to plasma proteins in sheep. *J. comp. Path.* 85, 299.

SUTTLE, N.F., 1980. The role of thiomolybdates in the nutritional interactions of copper, molybdenum and sulphur: Fact or fantasy? *An. NY Acad. Sci.* 355, 195.

SWENSON, M.J., 1977. Dukes Physiology of domestic animals (9th edn.). Cornell University Press Ltd., London.

TABATABAI, M.A. & BREMNER, J.M., 1970. A simple turbidimetric method of determining total sulphur in plant materials. *Agron. J.* 62, 805.

UNDERWOOD, E.J., 1977. Trace elements in human and animal nutrition (4th edn.). Academic Press, New York.

VAN DER MERWE, F.J. & PEROLD, I.S., 1967. Trace elements in natural pastures. *J. SA Vet. med. Assoc.* 38, 355.

VAN NIEKERK, F.E., 1985. Die effek van sekere spoorelemente op die vrugbaarheid van SA Vleismerino-ooie. MSc.-verhandeling, Universiteit van Stellenbosch.

VAN NIEKERK, F.E., VAN NIEKERK, C.H. & MORGENTHAL, J.C., 1988. Effect of an induced copper deficiency on the total plasma copper and unconjugated plasma progesterone concentrations during the oestrous cycle of the ewe. *S. Afr. J. Anim. Sci.* 18 (3), 83.

VAN RENSBURG, S.W.J., 1961. Kopertekorte beïnvloed vrugbaarheid by diere. *Boerdery in SA* 36 (12), 50.

VAN RYSSEN, J.B.J. & STIELAU, W.J., 1980. The influence of dietary sulphur on copper and molybdenum metabolism in sheep. *S. Afr. J. Anim. Sci.* 10, 49.

VAN RYSSEN, J.B.J. & STIELAU, W.J., 1981. Effect of different levels of dietary molybdenum on copper and Mo metabolism in sheep fed on high levels of Cu. *Br. J. Nutr.* 45, 203.

VARLEY, H., GOWENLOCK, A.H. & BELL, M., 1980. Practical Clinical Biochemistry (5th edn.). William Heineman Medical Books Ltd., London.

WARD, G.M., 1978. Molybdenum toxicity and hypocuprosis in ruminants. A review. *J. Anim. Sci.* 46 (4), 1078.

WIENER, G., 1979. Review of genetic aspects of mineral metabolism with particular reference to copper in sheep. *Livest. Prod. Sci.* 6, 223.