S. Afr. J. Anim. Sci. 10, 49–57 (1980)

THE INFLUENCE OF DIETARY SULPHUR ON COPPER AND MOLYBDENUM METABOLISM IN SHEEP

Receipt of MS 15-05-1979

J.B.J. van Ryssen and W.J. Stielau
Department of Animal Science, University of Natal, Pietermaritzburg, 3200

(Key words: *Sulphur, copper, molybdenum, sheep)*
(Sleutelwoorde: *Swawel, koper, molibdeen, skape)*

OPSOMMING: DIE INVLOED VAN SWAWEL IN DIE DIEËT OP KOPER- EN MOLIBDEENMETABOLISME IN DIE SKAAP SKAAP

Verskillende hoeveelhede S is aan skape verskaf wat hoë peile Cu (55 mg/skaap/dag) en Mo (34 mg/skaap/dag) in hulle rantsoene ontvang het. Teen S-innames van 2,9, 4,0 en 5,3 g/skaap/dag was die retensies van Cu in die lewer 0,24%, 0,16% en —0,04% van totale Cu-inname respektiewelik. 'n Verhoging van S in die rantsoen van 2,9 tot 4,0 g het gelei tot betekenisvolle verlagings in die Mo-konsentrasies in die plasma, milt, longe, spierweefsel en testes. Geen verdere verandering in Mo-peile in hierdie weefsels is waargeneem met 'n verdere verhoging in die S-inname van 4,0 tot 5,3 g/skaap/dag nie. 'n Hoë positiewe verwantskap (r = 0,916) tussen Cu- en Mo-konsentrasies van die nierkorteks is waargeneem in die hoë S-groepe (4,0 en 5,3 g S). Dit is voorgestel dat hierdie korrelasie die gevolg is van die onttrekking van Mo uit die niere deur S, soos waargeneem in die ander organe, met 'n gelyktydige opbou van Cu- Mo- verbindings in die niere wat onbeskikbaar vir die liggaam is.

SUMMARY:

Different levels of dietary S were supplied to sheep receiving high levels of Cu (55 mg/sheep/day) and Mo (34 mg/sheep/day) in their rations. At S intakes of 2,9, 4,0 and 5,3 g/sheep/day the hepatic Cu retentions expressed as percentages of total Cu intakes, were 0,24%, 0,11% and —0,04% respectively. An increase in S in the ration from 2,9 to 4,0 g resulted in significant decreases in Mo concentrations of the plasma, spleen, lungs, muscle and testes of the sheep. No further changes in Mo concentration of these organs were observed with a further increase of S from 4,0 to 5,3 g/sheep/day.

At the S intakes of 4,0 and 5,3 g/sheep/day, a positive correlation (r = 0,916) was obtained between the Cu and Mo concentrations of the kidney cortex. Such a high correlation may arise because of the depletion of Mo from the kidneys due to the additional S, as observed in the other tissues, while at the same time compounds containing Cu and Mo (unavailable to the body) accumulated in the kidneys.

From the literature it appears to be the accepted practice to supply additional S whenever Mo is used in the treatment or prevention of Cu toxicosis in sheep (Pierson & Aanes, 1958; Van Adrichem, 1965; Ross, 1970; Harker, 1976). Garner (1963), for instance, recommended the daily addition of between 0,3 and 1,0 g sodium thiosulphate with 50 to 500 mg ammonium molybdate to control Cu toxicity in sheep. Suttle (1975) pointed out that natural variations in S and Mo content of grazing could influence the Cu status of the grazing animal, while Ward (1978) suggested the use of the protein content of a ration as an indication of the S content of the diet. The importance of the S content in the basic diet was demonstrated by Van Ryssen (1979).

A trial was carried out in which different levels of S were supplied after a period of Cu accumulation in the body, to determine the effect of level of S intake on the Cu and Mo metabolism of sheep receiving high levels of these minerals.

Procedure

Experimental animals, treatments and procedure

Thirty S.A. Mutton Merinos, age 1½ to 2 years, were divided into 5 groups consisting of 5 rams and 1 wether per group. All groups received a diet high in Cu and Mo, but low in S for the first 42 days of the trial. One group was slaughtered after 42 days (Initial slaughter), 1 group (Low S) was kept on the low S ration to the end of the trial, while the remaining 3 groups received the medium level of additional S for a further 42 days. At this stage 1 of the latter groups was slaughtered (Mid-slaughter), 1 group was left on the medium S ration (Medium S), while the last group received an even higher level of additional S (High S). These 3 remaining groups were slaughtered after a further 70 days on their respective treatments. The treatments applied and the actual Cu, Mo and S intakes are given in Table 1.

Veld hay and a concentrate mixture consisting of 86% maize meal and 4,6% each of urea, salt and monocalcium phosphate were fed to the sheep. Additional Cu in the form of cupric sulphate, Mo as ammonium molybdate and S as sodium sulphate were mixed into the concentrates fed. The sheep were group-fed once a day in concrete floored pens. Tap water was available *ad libitum*. The body masses of the sheep were determined once a month after 18 hours of starvation. Jugular blood samples were taken at regular intervals for the determination of haemoglobin and packed cell volume (PCV) in whole blood and Cu and Mo in the plasma. Serum glutamic oxaloacetic transaminase (GOT) concentrations were determined towards the end of the trial to monitor tissue damage in the body (Van Adrichem, 1965).

The sheep were slaughtered at the Pietermaritzburg abattoir. Livers, kidneys, spleens, lungs, testes and 5 × 5 cm muscle samples from the *M. longissimus dorsi* were collected from each sheep. Samples were dried at 80°C for dry matter determinations and kept for further analyses.

Analytical methods

Cu, Fe, Zn and Mn contents of feeds and tissues were obtained using atomic absorption spectrophotometry after wet acid digestion. Plasma Cu was determined directly on diluted plasma. Ca, P and crude protein determinations were done on an auto-analyser and S according to the method of Blanchar, Rehm & Caldwell (1965). A molybdenum-iron-thiocyanate method as modified by Blamey (1971) was used for the Mo determination after a wet acid digestion. However, a 50 : 50 mixture of iso-amyl alcohol and chloroform was used as the extractant instead of carbon tetrachloride and iso-amyl alcohol. PCV was determined by a micro-haematocrit method and haemoglobin and GOT with Boehringer standard kits.

The F- and Student's t- tests as described by Rayner (1967) were used in the statistical analyses.

Results

Feed intake and composition of rations

An average intake per sheep of 893 g DM in the form of veld hay and 176 g of the concentrate mixture on a dry basis, was recorded. Treatments and average daily Cu, Mo and S intakes for the various stages of the trial are presented in Table 1. The average ratio of Cu : Mo in the ration was 1,63 : 1. The average daily intake per sheep of other nutrients during the trial were: 96 g crude protein, 9,5 g Ca, 2,9 g P, 29 mg Zn, 256 mg Mn and 493 mg Fe.

Table 1

Treatments, average Cu and Mo intake/sheep/day during the experiment and average S intakes/sheep/day during the different stages of the trial

		Copper		Molybdenum		Sulphur					
						First 42 days		Second 42 days		Last 70 days	
Treatments	Duration days	per day mg	Total mg	per day mg	Total mg	per day g	Total g	per day g	Total g	per day g	Total g
Initial-slaughter	42	53,1	2 230	33,9	1 424	2,88	121	—	—	—	—
Mid-slaughter	84	55,9	4 696	34,7	2 915	2,88	121	3,86	162	—	—
Low-S	154	58,2	8 963	35,1	5 405	2,88	121	2,98	125	2,90	203
Medium-S	154	56,3	8 670	33,1	5 097	2,88	121	3,86	162	4,01	281
High-S	154	53,6	8 254	32,9	5 067	2,88	121	3,86	162	5,30	371

Clinical condition and body mass

No clinical signs of abnormality due to any treatment were observed. One sheep from the Mid-slaughter group was lost due to theft. A drop in body mass was observed during the pre-experimental 42 days, but the mass of all groups increased slightly for the rest of the experimental period (Table 2). No statistically significant differences in body mass were observed between treatments.

Table 2

The average body mass of the sheep and changes in mass between the various stages of the trial

Treatment	Initial mass kg	Mass at 42 days Mass kg	Mass at 42 days Gain* kg	Mass at 84 days Mass kg	Mass at 84 days Gain* kg	Mass at 154 days Mass kg	Mass at 154 days Gain* kg
Initial-slaughter	49,3	44,5	—4,8	—	—	—	—
Mid-slaughter	52,4	46,5	—5,9	47,0	0,5	—	—
Low-S	52,4	47,8	—4,6	49,3	1,5	50,7	1,4
Medium-S	51,8	47,2	—4,6	49,3	2,1	51,1	1,8
High-S	52,0	47,2	—4,8	49,5	2,3	50,8	1,3

*Difference from previous period.

Mineral content of body tissues

(a) *Liver*

Very little accumulation of Cu or Mo took place in the liver after the first 42 days of the trial (Table 3). The addition of S to the diet resulted in negative Cu retentions in two treatment groups, the Mid-slaughter and High-S groups. These Cu levels were significantly ($P < 0,01$) lower than those found in the low S group. Mo concentrations in the liver decreased during the period when dietary S intakes were increased to below the level of the Initial-slaughter group. Negative liver Mo retentions were observed in all groups as compared to the Initial-slaughter group, though differences between groups were insignificant.

Table 3

The levels and accumulation of Cu and Mo in the livers of sheep receiving different levels of S (± SE of means)

Treatment	Copper*** Intake	Copper*** Liver mg kg DM	Copper*** Liver mg	Copper*** Retention* %	Molybdenum*** Intake mg	Molybdenum*** Liver mg kg DM	Molybdenum*** Liver mg	Molybdenum*** Retention* %
Initial-slaughter	0**	379 ± 59	53,7	0	0**	$10,7^a$ ± 1,39	1,49	0
Mid-slaughter	2 466	317^c ± 52	49,6	0,17	1 491	$6,4^b$ ± 0,52	0,98	0,034
Low-S	6 733	515^a ± 26	69,7	0,24	3 981	$8,6^a$ ± 0,76	1,15	0,009
Medium-S	6 440	458 ± 43	64,1	0,16	3 673	7,6 ± 0,81	1,06	0,012
High-S	6 024	342^c ± 36	51,3	0,04	3 643	7,7 ± 0,83	1,14	0,010

* Pre-experimental level subtracted; % of total Cu intake

** Cu and Mo intakes during first 42 days deducted

***Values within columns per trial with different superscripts denote significance:
a b at $P < 0,05$; a c at $P < 0,01$ levels of significance

(b) *Kidney cortex*

Significant increases in cortex Cu concentration were observed when additional dietary S was supplied above the Low-S treatment (Table 4). The Cu concentrations in the cortices of the Mid-slaughter and Medium-S groups (which received the same level of S supplementation/day) were similar, even though the treatment period for the latter was 70 days longer than for the Mid-slaughter group. A combined regression equation with a correlation of r = 0,916 was observed between the Cu and Mo concentrations in the kidney cortices of the three treatments receiving additional S above that of the Low-S level, viz. the Mid-slaughter, Medium-S and High-S treatments (Fig. 1). An average atomic ratio of 2,15 was observed between the Cu and Mo concentrations in these groups. For the Initial-slaughter and Low-S treatment groups, the average correlation between Cu and Mo was r = —0,02.

Table 4

The influence of different levels of dietary S on the concentration of Cu and Mo and the Cu : Mo ratio in the kidney cortices (± SE of means)

Treatment	Kidney Cortex Cu mg/kg DM	Kidney Cortex Mo mg/kg DM	Kidney Cortex Cu : Mo** ratio
Initial-slaughter	$16{,}0^{b} \pm 0{,}89$	$13{,}9 \pm 2{,}24$	$1{,}15 \pm 0{,}33$
Mid-slaughter	$28{,}3^{a} \pm 2{,}94$	$14{,}9 \pm 2{,}41$	$1{,}90 \pm 0{,}12$
Low-S	$18{,}0^{be} \pm 0{,}93$	$17{,}1 \pm 2{,}27$	$1{,}05 \pm 0{,}18$
Medium-S	$26{,}6^{ac} \pm 2{,}50$	$11{,}8^{c} \pm 1{,}67$	$2{,}25 \pm 0{,}10$
High-S	$40{,}3^{ad} \pm 4{,}70$	$18{,}8^{d} \pm 1{,}83$	$2{,}14 \pm 0{,}10$

* Different superscripts within columns designate differences between treatment averages:

a — b at $P < 0{,}01$ and c — d and c — e at $P < 0{,}05$ levels of significance

**Atomic ratio

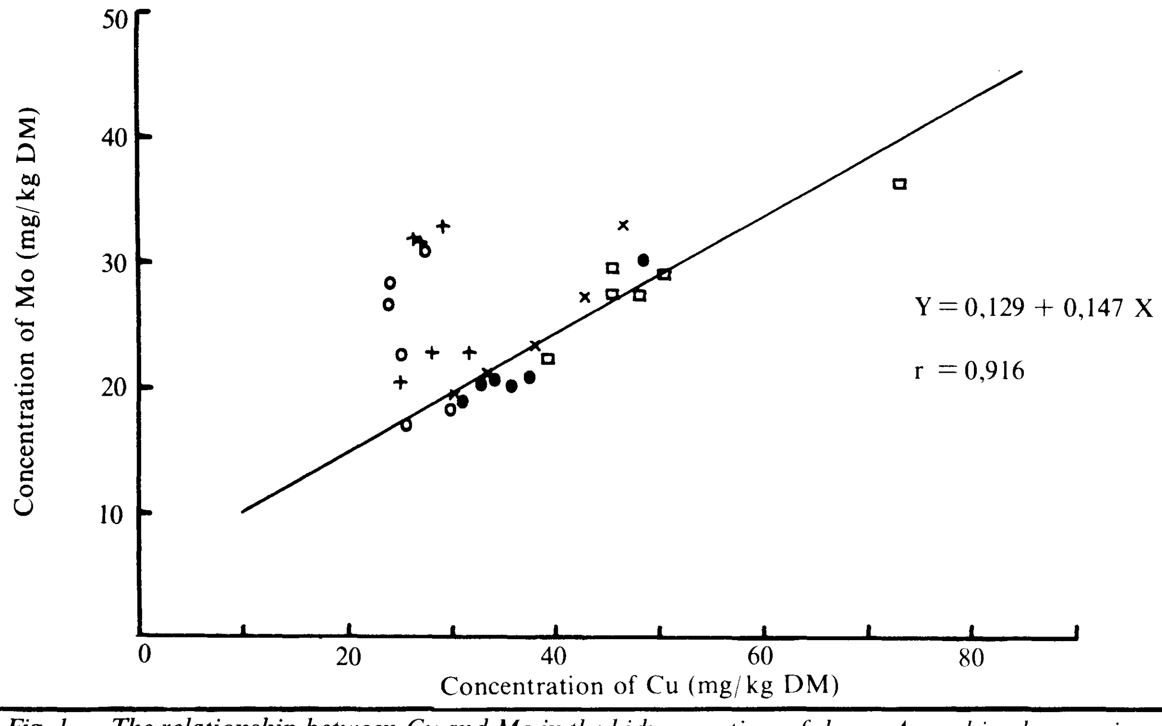

Fig. 1 *The relationship between Cu and Mo in the kidney cortices of sheep. A combined regression equation for the groups receiving additional S, viz.* □ *high-S,* ● *med-S and* x *mid-slaughter. The correlation between the groups without addition S,* o *initial-slaughter and* + *low-S was R = —0,02.*

(c) *Spleen, lungs, muscle and testes*

The effect of dietary S supplementation during the trial on Cu and Mo concentrations in the spleen, lungs, muscle and testes is given in Table 5 and Fig. 2. Statistically significant differences (P < 0,05) in Cu concentration were observed in the spleen between the Initial-slaughter and Medium-S groups and in the testes (P < 0,01) between the earlier-slaughter groups and those kept in the trial for 154 days. The feeding of additional S was associated with significant reductions in the Mo concentrations in all the tissues. The effect of additional S on Mo concentrations in these tissues showed a non-linear pattern with a marked reduction in Mo concentration between the Low S and Medium S treatments, but without any additional reduction due to further S supplementation.

Table 5

The effect of S supplementation of the Cu and Mo levels (DM basis) in organs and tissue (± SE of means)

Treatment	Copper*				Molybdenum*			
	Spleen mg/kg	Lungs mg/kg	Muscle mg/kg	Testes mg/kg	Spleen mg/kg	Lungs mg/kg	Muscle mg/kg	Testes mg/kg
Initial-slaughter	6,4^{a} ± 0,17	13,5 ± 0,64	3,22 ± 0,49	6,2^{a} ± 0,42	7,1^{a} ± 0,50	7,4^{a} ± 0,94	2,07^{a} ± 0,47	5,1^{a} ± 0,90
Mid-slaughter	5,3 ± 0,26	14,5 ± 0,52	3,18 ± 0,35	6,8^{a} ± 0,36	1,7^{c} ± 0,65	1,6^{c} ± 0,07	0,52^{b} ± 0,10	1,3^{c} ± 0,09
Low-S	4,4 ± 0,25	10,3 ± 0,43	3,35 ± 0,17	8,7^{c} ± 0,25	5,1^{a} ± 0,79	6,2^{a} ± 0,72	1,10 ± 0,24	3,5^{a} ± 0,51
Medium-S	4,2^{b} ± 0,13	9,0 ± 0,55	3,08 ± 0,11	9,2^{c} ± 0,77	1,3^{c} ± 0,14	1,5^{c} ± 0,19	0,50^{b} ± 0,14	1,4^{c} ± 0,11
High-S	5,6 ± 0,38	8,3 ± 0,63	3,73 ± 0,18	9,0^{c} ± 0,19	1,4^{c} ± 0,10	1,7^{c} ± 0,40	0,42^{c} ± 0,13	1,5^{c} ± 0,47

*Different superscripts within columns designate differences between treatment averages:
a — b at P < 0,05; a — c at P < 0,01 levels of significance.

No statistically significant differences in the Zn and Fe concentrations of the livers, kidneys, spleens, lungs, muscles or testicles were observed which could be related to any treatment effect.

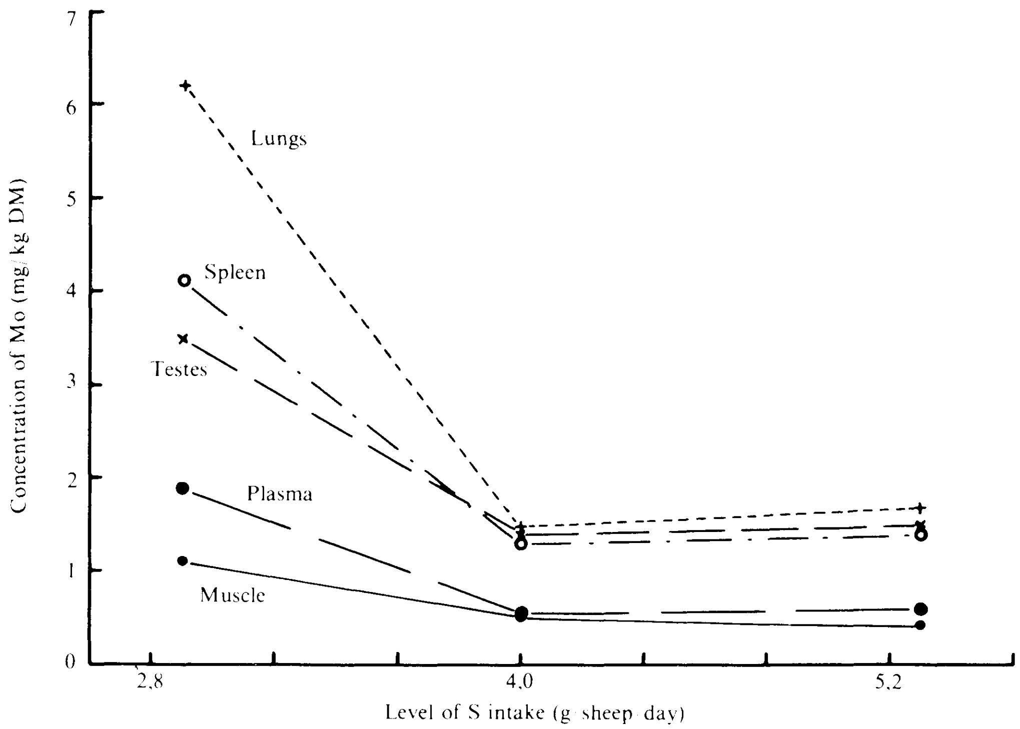

Fig. 2 Influence of level of S intake on the Mo concentrations of the spleen, lungs, muscle, testes and plasma of sheep (Plasma concentration in mg Mo/ℓ)

(d) *Plasma*

Average plasma Cu and Mo concentrations for the different stages of the trial are presented in Table 6. Changes in plasma Mo concentrations are depicted in Fig. 2 and followed the same trend as Mo concentrations in the other tissues. The addition of S, i.e. during the second and third stages, resulted in increased plasma Cu and decreased plasma Mo concentrations. The difference in plasma Cu levels between the control and the other groups receiving additional S, was not statistically significant during the second 42 day period, but was so during the last 70 days of the trial.

Table 6

The effect of S supplementation on the plasma Cu and Mo concentrations during various stages of the trial

Treatments	Plasma Cu (mg/ℓ) *			Plasma Mo (mg/ℓ) *		
	First 42 days	Second 42 days	Last 70 days	First 42 days	Second 42 days	Last 70 days
Initial-slaughter	1,28	—	—	2,43	—	—
Mid-slaughter	1,25	1,39	—	2,39	0,54[c]	—
Low-S	1,34	1,25	0,96[a]	2,26	1,88[a]	1,92[a]
Medium-S	1,36	1,40	1,22[c]	2,39	0,53[c]	0,48[c]
High-S	1,29	1,36	1,18[c]	2,34	0,60[c]	0,49[c]

*Different superscripts a — b within columns designate differences at $P < 0,01$ level of significance

Haematological parameters and serum enzymes

No changes or significant differences were observed between treatments in the haemoglobin and PCV values, during the various stages of the trial. Haemoglobin levels varied between 11 and 12,7 g/100 ml blood and PCV between 27,2 and 31,6%. The GOT levels in serum were determined towards the end of the trial. These values varied between 42 and 56 IU/ℓ with no significant differences between treatments. Only one sheep, in the High S group, had a GOT value of 104 IU/ℓ shortly before it was slaughtered.

Discussion

Hepatic copper retention

A relatively low rate of hepatic Cu retention (0,24%) was observed at a S intake of 2,9 g/sheep/day. Above this S level negative hepatic Cu retentions were obtained. Reduced liver Cu levels have been recorded at S and Mo levels similar to those used in the Low-S treatment of this trial (Dick, 1954; Harker, 1976). At the levels of Cu, Mo and S used it may be concluded that the low hepatic Cu retentions were to a great extent due to the Cu-Mo-S interaction.

Sulphur in the Cu-Mo interaction

It is evident from the results that liver Cu retentions can be restricted, or can be even reduced to negative values at a dietary Cu : Mo ratio of approximately 1,63. These results substantiate the suggestion by Miltimore & Mason (1971) that the danger of Cu deficiencies due to dietary Mo existed at Cu : Mo ratios of less than 2,0. However, it is clear that the level of S intake has an important influence on the degree of response to be expected, and cannot be ignored. Differences in S levels used may explain why different ratios of Cu : Mo, viz. 2 : 1 (Miltimore & Mason, 1971), 5 : 1 (Alloway, 1973; Pope, 1975) and 7 : 1 (Case, 1974) have been suggested as safe limits against molybdenosis. Suttle (1974b) suggested the formula "product log (Mo concentration) × log (S concentration)" for predicting the effects of dietary Mo and S on the availability of Cu in feeds, thus taking into account the S content of the food.

Although the importance of a knowledge of dietary S levels seems obvious, different factors can influence the amount and availability of S in the body. Suttle (1975) mentioned the possibility that some S may escape ruminal degradation and not participate in the interactions with Mo. Hume & Bird (1970) reported substantial endogenous additions of S through the saliva to the S pool in the rumen. Scaife (1956) suggested that protein catabolism in the body may contribute to the S pool involved in the Cu-Mo-S interaction. Although Marcilese, Ammerman, Valsecchi & Dunavant (1969) proved that intravenously injected SO_4^{2-} did not contribute to the Cu-Mo interaction in the body, it seems possible that S from protein catabolism could recycle into the rumen where it can influence the Cu-Mo interaction. The form in which S is ingested by the animal, eg. as amino acid S or SO_4^{2-}

(Huisingh & Matrone, 1976) and the proportion of amino acids directly incorporated in the microbial protein (Gawthorne & Nader, 1976) may also influence the availability of S to the Cu-Mo-S interaction. Suttle (1974a, 1975) proved with the use of the depletion-repletion technique and semi-purified diets that S in both the organic and inorganic forms was equally effective in the Cu-S and the Cu-Mo-S interactions respectively. Contrary to Suttle's results, Goodrich & Tillman (1966) and Huisingh & Matrone (1976) observed different responses in the interactions of S with Cu and Cu-Mo depending on the form in which S was applied. Since so many factors can influence the availability of S, it seems unlikely that fixed Cu : Mo ratios or formulae can be devised for use in animal nutrition to suit all circumstances.

Systemic effects

The low hepatic Cu retention observed at the low level of S intake is probably best explained by the Cu-Mo-S interaction in the rumen. The negative hepatic Cu retentions observed at the S intakes of 4,0 and 5,3 g/sheep/day indicate that the Cu-Mo-S interaction may occur also at the body tissue level. Elevated plasma Cu levels, mainly due to an increase in the direct reacting Cu fraction of plasma, were considered by Suttle (1974b) to be a true systemic effect of the Cu-Mo-S interaction. High kidney Cu levels and increased rates of Cu excretion through the urine were considered to be the result of the high concentration of the direct reacting Cu fraction in plasma (Suttle, 1974b). They may be also considered as systemic expressions of the Cu-Mo-S interaction. It is clear, therefore, that the reactions taking place between Cu, Mo and S can occur both in the digestive tract of the sheep and at cellular level, as suggested by Dick, Dewey & Gawthorne (1975). The supplementation of S during the present trial resulted in increased plasma and kidney cortex Cu levels typical of the so-called systemic effects of Cu-Mo-S interaction. The very high positive correlation between Cu and Mo concentrations in the kidney cortex for all treatments which received additional S may be, therefore, also considered as a true systemic effect of this interaction.

The relatively high plasma Cu levels during the first 42 days of the trial, even before any additional S was provided, might be considered as an expression of this systemic effect of the Cu-Mo-S interaction. These high Cu levels were observed in all sheep at the onset of the trial. Furthermore, none of the other systemic effects, viz. elevated kidney Cu levels or a high correlation between kidney Cu and Mo was observed in the Initial-slaughter treatment group. It may be concluded, therefore, that these systemic effects were not detectable at the S intake level of 2,88 g/sheep/day (2,7 g S/kg DM) during the first 42 days of the trial nor in the case of the Low S treatment during the remainder of the trial. Smith & Wright (1975a) suggested that changes in plasma Cu levels are elicited only above a critical dietary Mo concentration. From the present trial it appears that critical dietary S levels may also be necessary before systemic effects due to the Cu-Mo interaction can be expected. At a daily intake of 58 mg Cu and 35 mg Mo per sheep, the systemic effects were observed when the S intake increased from 2,88 g (0,27%) to 3,86 g (0,38%)/sheep/day. At concentrations of 58 mg Cu and 2,7 g S/kg DM, elevated plasma and kidney Cu levels were observed by Van Ryssen (1979) at Mo levels of 45 mg/kg DM.

Elevated plasma Cu and direct reacting Cu levels were reported by Smith, Field & Suttle (1968) at levels of 25 mg Mo and 1,8 g S/kg diet, by Suttle & Field (1968) at Mo and S concentrations of 50 mg and 3,6 g/kg feed respectively, and by Bingley (1974) at 120 mg Mo and 2,5 g S/sheep/day. At a Mo intake of 12 mg/day Bingley (1974) still observed slightly elevated plasma Cu levels. Smith & Wright (1975a) observed increased plasma Cu levels at Mo intakes of between 8 and 16 mg/day. Marcilese, Ammerman, Valsecchi, Dunavant & Davis (1970) reported increased kidney and urine Cu levels on diets containing 50 mg Mo and 1,3 g S/kg feed. Dick (1956) found an increase in plasma Cu levels at different stages after the onset of his trials, depending on Mo and S intakes. At the high Mo, high S intakes, plasma Cu increased immediately, while at lower levels of Mo and/or S these increases appeared later in the trial.

Widely different levels of both Mo and S are therefore apparently effective in promoting the systemic Cu-Mo-S interaction. An important contributing factor may be that semi-purified diets were used in most of the reported experiments where all the S was supplied as sulphate, while natural feeds were used in the present trial. Differences in S metabolism in the rumen have been reported to depend on the source of S (Huisingh & Matrone, 1976). Relatively high dietary levels of Mo and S are required to reduce the Cu content of the liver (Harker, 1976). It was observed that hepatic Cu concentrations can be maintained (Ross, 1970) and the sheep can die from Cu toxicity months after the withdrawal of all additional Cu from their diets (Bracewell, 1958; Barden & Robertson, 1962). The reduction of the hepatic Cu content is therefore essential if Cu accumulation has already taken place in the liver. This could be achieved by exploiting the systemic interaction between Cu, Mo and S.

Ratio of Cu : Mo in tissue

An atomic ratio between Cu and Mo of 2 : 1 was observed by Bremner & Young (1978) in the kidneys of sheep receiving additional S above a low S intake. Under similar conditions a ratio of 2,15 : 1 was observed in the present trial. Smith & Wright (1975b) observed an average Cu : Mo ratio of 1,7 : 1 (varying between 2 : 1 and 3 : 2) in the TCA insoluble fraction of plasma of sheep receiving additional dietary Mo and S. These ratios were considered as evidence for the presence of compounds containing Cu and Mo at fixed ratios and unavailable to the body. However, to observe such fixed ratios, the presence of Cu and Mo in other forms in the kidneys should be low.

The inclusion of S in the ration in this trial resulted not only in the high positive correlation between Cu and Mo in the kidney cortex, but also in a significant reduction in the Mo levels of the plasma, spleen, muscle and testes. This finding is in accord with the results of Dick (1956), Huisingh, Gomez & Matrone (1973) and Suttle (1975). It is suggested that S could have exerted a similar depleting effect on the Mo in the kidneys, while simultaneous deposition of Mo, in conjunction with Cu and in a form unavailable to the body, was occurring in the kidneys. Evidence of this is the fact that even though the cortex Mo levels during the trial were more or less the same for all treatments, the high correlation between Mo and Cu existed only when the S intakes were high, i.e. when the other so-called systemic effects were observed. Dick (1956) reported decreased Mo concentrations in all organs after the addition of dietary S, with the exception of a slight increase in the kidneys. Bremner & Young (1978), on the other hand, observed a drop in kidney Mo concentration with the feeding of extra S. These differences may be explained by the extent to which the Cu-Mo complex accumulated in the kidneys relative to the original renal Mo concentration.

Sulphur on molybdenum metabolism

The first increment of additional S resulted in a significant reduction in Mo levels in the tissues during the trial, while the second increment did not cause further reductions in tissue Mo levels. A similar non-linear effect of S supplementation on Mo absorption and Mo concentrations was observed in blood (Dick, 1956) and in plasma and urine (Grace & Suttle, 1979). During the present study the second increment of S exerted no further effect on Mo concentration in the tissues, though the Cu and Mo levels in the kidneys increased significantly above the levels measured at the medium S treatment. This might lend support to the suggestion that the effect of S on Mo concentration in tissues functions independently of the so-called systemic effect, resulting therefore, in a correlation between the Cu and Mo in the kidneys.

Acknowledgements

The authors wish to thank Mr B.D. Thring for attending to the sheep during the trial and Mrs J.A. Holmes for laboratory assistance.

References

ALLOWAY, B.J., 1973. Copper and molybdenum in swayback pasture. *J. agric. Sci., Camb.*, 80, 521.

BARDEN, P.J. & ROBERTSON, A., 1962. Experimental copper poisoning in sheep. *Vet. Rec.*, 74, 252.

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.

BLAMEY, F.P.C., 1971. Molybdenum in maize *(Zea Mays L.)* production in Northern Natal. M.Sc. Agric. Thesis, University of Natal.

BLANCHAR, R.W., REHM, J. & CALDWELL, A.C., 1965. Sulphur in plant material by digestion with nitric and perchloric acid. *Proc. Soil Sci. Soc. Am.*, 29, 71.

BRACEWELL, C.D., 1958. A note on jaundice in housed sheep. *Vet. Rec.*, 70, 342.

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.

CASE, A.A., 1974. Toxicity of various chemical agents to sheep. *J. Am. vet. med. Assoc.*, 164, 277.

DICK, A.T., 1954. Studies of the assimilation and storage of copper in crossbred sheep. *Aust. J. agric. Res.*, 5, 511.

DICK, A.T., 1956. Molybdenum and copper relationships in animal nutrition. In *Inorganic Nitrogen Metabolism*, p. 445. Ed. W.D. McElroy & B. Glass. Baltimore : John Hopkins Press.

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

GARNER, R.J., 1963. *Veterinary Toxicology*. 2nd ed. London : Tindall & Cox.

GAWTHORNE, J.M. & NADER, C.J., 1976. The effect of molybdenum on the conversion of sulphate to sulphide and microbial-protein-sulphur in the rumen of sheep. *Br. J. Nutr.*, 35, 11.

GOODRICH, R.D. & TILLMAN, A.D., 1966. Effects of sulphur and nitrogen sources and copper levels in the metabolism of certain minerals by sheep. *J. Anim. Sci.*, 25, 484.

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

HARKER, D.B., 1976. The use of molybdenum for the prevention of nutritional copper poisoning in housed sheep. *Vet. Rec.*, 99, 78.

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

HUISINGH, J. & MATRONE, G., 1976. Interactions of molybdenum in animal nutrition. In: *Molybdenum in the Environment*, Vol. 1, p. 125. Ed. W.R. Chappell & K.K. Petersen. New York : Marcel Dekker, Inc.

HUME, I.D. & BIRD, P.R., 1970. Synthesis of microbial protein in the rumen. IV. The influence of the level and form of dietary sulphur. *Aust. J. agric. Res.*, 21, 315.

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.

MARCILESE, N.A., AMMERMAN, C.B., VALSECCHI, R.M., DUNAVANT, B.G. & DAVIS, G.K., 1970. Effect of dietary molybdenum and sulphate upon urinary excretion of copper in sheep. *J. Nutr.*, 100, 1399.

MILTIMORE, J.E. & MASON, J.L., 1971. Copper to molybdenum ratio and molybdenum and copper concentrations in ruminant feeds. *Can. J. Anim. Sci.*, 51, 193.

PIERSON, R.E. & AANES, W.A., 1958. Treatment of chronic copper poisoning in sheep. *J. Am. vet. med. Assoc.*, 133, 307.

POPE, A.L., 1975. Mineral interrelationships in ovine nutrition. *J. Am. vet. med. Assoc.*, 166, 264.

RAYNER, A.A., 1967. *Biometry for Agriculture Students*. Pietermaritzburg, Univ. Natal Press.

ROSS, D.B., 1970. The effect of oral ammonium molybdate and sodium sulphate given to lambs with high liver copper concentrations. *Res. vet Sci.*, 11, 295.

SCAIFE, J.F., 1956. Molybdenum excretion and retention in the sheep. *N.Z.J. Sci. Techn.*, 38, 293.

SMITH, B.S.W., FIELD, A.C. & SUTTLE, N.F., 1968. Effect of intake of copper, molybdenum and sulphate on copper metabolism in the sheep. III Studies with radioactive copper in male castrated sheep. *J. Comp. Path.*, 78, 449.

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

SMITH, B.S.W. & WRIGHT, H., 1975b. Effect of dietary Mo on Cu metabolism. Evidence for the involvement of Mo in abnormal binding of Cu to plasma protein. *Clin. Chim. Acta.*, 62, 55.

SUTTLE, N.F., 1974a. Effects of organic and inorganic sulphur on the availability of diet copper to sheep. *Br. J. Nutr.*, 32, 559.

SUTTLE, N.F., 1974b. Recent studies of the copper-molybdenum antagonism. *Proc. Nutr. Soc.*, 33, 299.

SUTTLE, N.F., 1975. The role of organic sulphur in the copper-molybdenum-S interrelationship in ruminant nutrition. *Br. J. Nutr.*, 34, 411.

SUTTLE, N.F. & FIELD, A.C., 1968. Effect of intake of copper, molybdenum and sulphate on copper metabolism in sheep. I. Clinical condition and distribution of copper in blood of the pregnant ewe. *J. Comp. Path.*, 78, 351.

VAN ADRICHEM, P.W.M., 1965. Wijzigingen in de activiteit van serumenzymen en in het LDH iso-enzympatroon bij chronische koperintoxicatie van schapen. *Tijdschr. Diergeneesk.*, 90, 1371.

VAN RYSSEN, J.B.J., 1979. The influence of molybdenum and sulphur on sheep receiving high levels of copper and broiler litter in their rations. *S. Afr. J. Anim. Sci.*, 9, 21.

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