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THE INFLUENCE OF MOLYBDENUM AND SULPHUR ON SHEEP RECEIVING HIGH LEVELS OF COPPER AND BROILER LITTER IN THEIR RATIONS

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OPSOMMING: DIE INVLOED VAN MOLIBDEEN EN SWAWEL OP SKAPE WAT 'N HOË PEIL KOPER EN BRAAIKUIKENMIS IN HULLE RANTSOENE ONTVANG.

'n Hoë konsentrasie koper (Cu) is in die lewers van skape wat slegs Cu en geen addisionele molibdeen (Mo) en swawel (S) ontvang het nie, neergelê. Oor die tydperk van 157 dae was die spoed van Cu-akkumulasie in die lewer egter laer as in ander soortelyke proewe en is afgelei dat minerale in hoendermis, antagonisties tot Cu-opname, 'n rol kon speel. Weens die hoë S-inhoud van hoendermis was addisionele S ook nie nodig om die negatiewe uitwerking van Mo op lewer-Cu te verkry nie. Mo het 'n dramatiese verhoging in nierkoperinhoud tot gevolg gehad asook stygings in die Cu-peile van bloedserum en die milt. Geen aanduiding van Mo-vergiftiging is waargeneem nie.

SUMMARY:

Coper (Cu) accumulated in the livers of sheep receiving high levels of dietary Cu but low levels of molybdenum (Mo) and sulphur (S). However, the liver Cu accumulation was much lower than levels found in similar trials in the literature. It is suggested that the minerals present in broiler litter and antagonistic to Cu absorption could have been responsible for this lower Cu accumulation. No additional S was required to obtain the depressing effect of Mo on liver Cu accumulation because of the high S content of broiler litter. The feeding of additional Mo produced a dramatic increase in kidney Cu levels as well as a rise in blood serum Cu and spleen Cu levels. No indication of Mo toxicity was observed.

Introduction

Cases of copper (Cu) toxicity have been reported in Natal in sheep fed rations containing broiler litter as a protein source. In a survey to determine the mineral composition of poultry manure in South Africa Van Ryssen, Channon and Stielau (1977) found that some of the broiler litter samples contained very high levels of Cu, viz. between 296 and 570 mg Cu/kg dry matter (DM).

Molybdenum (Mo) plus sulphur (S) has long been used to control the accumulation of Cu in sheep livers (Dick, 1954). However, the danger of Mo toxicity exists, usually expressed in the form of an induced Cu deficiency, if Mo plus S is given without caution (Harker, 1976).

A trial was conducted to determine the influence of Mo and S on sheep receiving high levels of Cu in rations containing broiler litter.

Procedure

Animals, treatments and experimental routine

Forty South African Mutton Merino wethers, approximately two years of age were randomly allocated to five treatments, viz. a pre-experimental slaughter treatment, a control, a high Cu (as $CuSO_4$), a high Cu plus high Mo (as ammoniummolybdate) and a high Cu, high Mo and high S (as Na_2SO_4) group. The groups were called pre-experimental, control, Cu, Cu + Mo and Cu + Mo + S respectively. The trial lasted 157 days.

The groups were group-fed in concrete-floored pens. They received long *Eragrostis curvula* hay and a basic concentrate mixture consisting of 57 per cent broiler litter and 43 per cent maize meal. The broiler litter with wood shavings as litter material was obtained from a commercial source and contained 23,5 mg Cu/kg DM. The broiler litter was sieved and mixed with the maize meal. Three weeks before the onset of the trial the sheep were inoculated against botulism. Tap water was available *ad libitum.*

The sheep were fed once a day and were weighed every fortnight after 18 hours' starvation. Jugular blood samples were taken at the onset and once a month after the first two months of the trial.

The sheep were slaughtered at the Pietermaritzburg abattoir. The liver, kidneys and spleen as well as a 5 x 5 cm muscle sample from the *m longissimus dorsi*

were collected and weighed. Samples were taken and dried at 80°C for dry matter determination. These dried samples were kept for further analyses.

The sheep were shorn prior to the trial and wool samples were taken from their side for chemical analyses at the end of the trial. The wool samples were washed and the fat extracted.

Analytical methods

The Cu, Fe, Zn and Mn contents of feeds, tissues and wool were obtained through atomic absorption spectrophotometry after wet acid digestion. Serum Cu was determined directly on diluted serum. The Ca, P and crude protein determinations were done on an auto-analyser and S according to the method of Blanchar, Rehm and Caldwell (1965). A molybdenum-iron-thiocyanate method as modified by Blamey (1971) was used for the Mo determinations after a wet acid digestion.

Packed cell volume (PCV) was determined by a micro-haematocrit method.

The F- and t- tests as described by Rayner (1967) were used in the statistical analyses.

Results

Clinical condition

No clinical signs of copper toxicity were observed during the trial, nor did any other problems related to the feeding of the minerals or broiler litter become apparent. All sheep increased in body mass without any differences among the groups.

Intakes and composition of rations

The average hay intake was 607 g DM and the concentrate intake 654 g DM per sheep per day. Broiler litter intake was 373 g or 29,6 per cent of the total ration. The concentration of minerals in the total ration and the mineral intake per sheep per day are given in Table 1. Minerals potentially antagonistic to Cu in the body are included in Table 1.

Minerals in organs and wool

The accumulation of Cu in the body due to the feeding of high levels of Cu, Mo and S is given in Table 2.

The addition of Cu to the ration resulted in a highly significant increase in the Cu content of the liver. Within this group liver Cu levels ranged from 505 to 1 388 mg/kg DM. The addition of Mo to the Cu supplemented ration reduced liver Cu levels significantly and the combination of Mo + S added to Cu supplemented rations decreased liver Cu levels to values not statistically different from those of the control.

The addition of Mo with or without S dramatically increased kidney Cu levels. Significantly higher Cu levels were also measured in the spleens of those groups receiving Mo or Mo + S as compared to the other treatments.

Table 1

Average concentration of minerals in experimental rations (DM basis) and the mineral intakes per sheep per day

	Concentration of minerals							
Treatments	Cu mg/kg	Mo mg/kg	Zn mg/kg	Fe mg/kg	Mn mg/kg	S %	Ca %	P %
Control	13,5	0,38	66	348	192	0,26	0,84	0,63
Cu	59,8	0,42	64	344	202	0,27	0,85	0,65
Cu + Mo	58,2	45,72	76	362	215	0,27	0,92	0,69
Cu + Mo + S	55,6	48,31	65	324	192	0,48	0,86	0,63
	Daily intake of minerals							
	mg	mg	mg	mg	mg	g	g	g
Control	17,0	0,48	83	438	242	3,3	10,6	7,9
Cu	75,4	0,53	80	434	254	3,4	10,8	8,2
Cu + Mo	73,4	57,6	96	457	271	3,4	11,7	8,7
Cu + Mo + S	70,1	60,9	82	410	243	6,1	10,9	8,0

Table 2

The influence of Cu, Mo and S intake on the accumulation of Cu in the body tissues of sheep

	Pre-exp.*	Treatments** Control	Cu	Cu + Mo	Cu + Mo + S
Liver:					
Concentration (mg/kg DM)	176	282^{a}	878^{b}	517^{c}	401^{ac}
Total Cu content (mg)		43^{a}	124^{b}	74^{d}	62^{ad}
Cu as % of Cu intake		1,68	1,09	0,66	0,59
Kidneys:					
Concentration (mg/kg DM)	18,9	22^{a}	21^{a}	161^{b}	157^{b}
Total Cu content (mg)		$0,48^{a}$	$0,44^{a}$	$3,36^{b}$	$3,27^{b}$
Spleen:					
Concentration (mg/kg DM)	9,8	$4,3^{a}$	$5,4^{a}$	$11,2^{b}$	$11,5^{b}$
Total Cu content (mg)		$0,064^{a}$	$0,080^{a}$	$0,174^{b}$	$0,167^{b}$
Muscle (L. dorsi):					
Concentration (mg/kg DM)	7,3	6,7	5,3	7,7	7,3

* Minerals in tissues of pre-experimental slaughter group calculated using estimated DM values. Data not included in statistical analyses.

** Different superscripts designate differences between treatment averages:
a – b and a – c at P = 0,01; a – d at P = 0,05

Table 3

The influence of Cu, Mo and S intakes on the concentration of Mo, Fe and Zn in the liver and Cu and Mo in clean, fat-free wool

	Treatments* Control	Cu	Cu + Mo	Cu + Mo + S
Liver:				
Mo (mg/kg DM)	$4,0^{a}$	$4,3^{a}$	$35,6^{b}$	$39,5^{b}$
Zn (mg/kg DM)	115	112	114	111
Fe (mg/kg DM)	207	219	195	208
Wool:				
Cu (mg/kg DM)	5,89	6,13	4,90	5,11
Mo (mg/kg DM)	0,87	0,65	2,05	1,38

* Different superscripts designate differences between treatment averages at P = 0,01 level of significance.

The concentrations of Mo, Zn and Fe in the liver and Cu and Mo in clean, fat-free wool are given in Table 3. The addition of Mo to the rations resulted in significant increases in the liver Mo concentration above those treatments without Mo. Cu, Mo or S had no influence on the Zn and Fe levels of the liver. None of the differences in wool Cu or Mo levels between treatments were statistically significant, mainly because of big variations in the Cu and Mo levels within treatments.

Blood analyses

No significant differences in PCV were observed between treatments, though the PCV values increased in all groups from an average of 28,7 per cent at the onset of the trial to an average of 30,6 per cent at the end of the trial. In Table 4 serum Cu levels for the pre-experimental collection are presented. The average serum Cu and Mo levels did not change over the last four months

Table 4

The influence of Cu, Mo and S intake on the Cu and Mo levels in blood serum

	Treatments*			
	Control	Cu	Cu + Mo	Cu + Mo + S
Pre-experimental collection				
Cu (mg/L)	1,00	0,83	0,74	0,85
Averages of last four months				
Cu (mg/L)	$1,00^a$	$1,04^a$	$2,04^b$	$1,94^b$
Mo (mg/L)	$0,04^a$	$0,27^a$	$2,00^b$	$1,30^c$

* Different superscripts designate differences between treatment averages at P = 0,01 level of significance.

of the trial and only the average serum Cu and Mo concentrations for this period are, therefore, given in Table 4. The two Mo treatments caused a significant increase in serum Cu and Mo levels above the treatments without Mo. Additional S at the high Mo intake reduced serum Mo levels significantly below the Mo levels where Mo was given without S.

Discussion

In adult sheep, liver Cu levels ranging from 100 to 600 mg/kg DM are considered as normal, while levels above 600 mg/kg DM might indicate the risk of hypercuprosis (Harker, 1976). The acute stage of Cu toxicity, the haemolytic crisis, has been experienced at Cu levels in the liver as low as 1 400 mg/kg DM (Ross, 1964) up to Cu levels of 6 530 mg/kg DM (MacPherson & Hemingway, 1965). Breed differences and stress are important factors determining the onset of the haemolytic crisis (Todd, 1969). In the present experiment the group receiving Cu only was therefore well within the dangerous levels of hepatic Cu with an upper range of 1 388 mg/kg DM. When comparing the accumulation of Cu in the liver over the 157 days of this treatment with rates of accumulation reported by others the accumulation of Cu in this trial was much lower than values such as 2 340 mg Cu/kg liver DM over 177 days at intakes of 33,6 mg Cu per sheep per day (Dick, 1954), 890 mg Cu/kg DM within 49 days at an intake of 62 mg Cu per day (MacPherson & Hemingway, 1965) or 1 740 mg Cu/kg DM over 77 to 98 days at 60 mg Cu per day (Ross, 1966). However, Suttle, Munro and Field (1977) found a hepatic Cu accumulation of 874 mg Cu/kg liver DM over 70 to 105 days at an intake of 57,5 mg Cu per sheep per day in sheep receiving poultry manure in their rations. This lower than expected accumulation they ascribed to either the high levels of Cu antagonists present in manure or to some physiological resistance to Cu absorption at higher levels of Cu intake. The present results tend to substantiate the observations by Suttle, *et al.* (1977) that other factors, probably the antagonistic action of other minerals in the ration, reduce the rate of accumulation of Cu in the liver.

The depressing effect of Mo on the retention of Cu in the sheep liver is in agreement with other reports including those of Dick (1954), Wynne and McClymont (1956), Van der Berg and Van der Schee (1973), Harker (1976) and many others. Additional S (usually given as sulphate) was found to be essential for the expression of the Mo effect (Dick, 1956). The high S content of the basal ration, probably due to the high S content in broiler litter (Van Ryssen, *et al.*, 1977) in the present trial apparently had a similar effect to that of added sulphate on liver Cu levels. The Mo content of serum and wool decreased when additional S was given as compared to the Mo only treatment. This observation is in agreement with the results of Dick (1956) and Bremner (1976). However, the Mo levels in the liver in this experiment did not show a reduction due to added S.

Todd, Gracey and Thompson (1962), Ross (1964) and MacPherson and Hemingway (1969) found kidney damage and dramatic increases in renal Cu levels during the haemolytic crisis stage of Cu toxicity. Even though Van Adrichem (1965) and Ross (1966) treated sheep with Mo and S at the first signs of an approaching crisis, viz. elevated GOT levels in plasma, the kidney Cu levels of the sheep remained well above normal and there were signs of kidney damage. Van Adrichem (1965) ascribed this kidney damage to the Mo + S treatment and warned against the use of Mo + S for prolonged periods. Whether this is in fact a danger has not been proved. Ross (1966) found no increase in kidney Cu levels in sheep receiving Mo + S for the duration of his trial, while Marcilese, Ammerman, Valsecchi, Dunavant and Davis (1970) observed an accumulation of Cu in the kidneys on their Mo + S treatment similar to the observations in the present trial.

The Mo and Zn content of the liver corresponded well with levels observed by Dick (1956) and Bremner, Young and Mills (1976) respectively at similar intakes of these minerals. Lesperance and Bohman (1961) suggested liver Mo levels as a long run criterion of Mo toxicity in cattle. The most common symptoms of Mo toxicity in sheep are those of induced Cu deficiency, eg. sub-normal blood PCV, haemoglobin, caeruloplasmin levels, reduced wool Cu levels and loss of wool crimp (Wynne & McClymont, 1956; Bingley, 1974; Ward, 1978). None of these symptoms were observed in the present trial. Bingley (1974) suggested that sheep tolerate high doses of Mo + S for protracted periods. The results from the present trial seem to support this statement, provided sufficient Cu is supplied to prevent the risk of a Cu deficiency.

References

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, G. & CALDWELL, A.C., 1965. Sulfur in plant materials by digestion with nitric and perchloric acid. *Proc. Soil Sci. Soc. Am.* 29,71.

BREMNER, I., 1976. The effect of dietary molybdenum and sulphate on plasma copper distribution in sheep. *Proc. Nutr. Soc.* 35, 21A.

BREMNER, I., YOUNG, B.W. & MILLS, C.F., 1976. Protective effect of zinc supplementation against copper toxicosis in sheep. *Br. J. Nutr.* 36, 551.

DICK, A.T., 1954. Studies on 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.* Ed. McElroy, W.D. & Glass, B., Baltimore. 455: John Hopkins Press.

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

HILL, R. & WILLIAMS, H.L., 1965. The effects on intensively reared lambs of diets containing excess copper. *Vet. Rec.* 77, 1043.

LESPERANCE, A.L. & BOHMAN, V.R., 1961. Criteria for measuring molybdenum toxicity. *J. Anim. Sci.* 20,28.

MACPHERSON, A. & HEMINGWAY, R.G., 1965. Effects of protein intake on the storage of copper in the liver of sheep. *J. Sci. Fd. Agric.* 16, 220.

MACPHERSON, A. & HEMINGWAY, R.G., 1969. The relative merit of various blood analyses and liver function tests in giving an early diagnosis of chronic copper poisoning in sheep. *Br. vet. J.* 125, 213.

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

RAYNER, A.A., 1967. Biometry for agriculture students. Pietermaritzburg: Univ. of Natal Press.

ROSS, D.B., 1964. Chronic copper poisoning in lambs. *Vet. Rec.* 76, 875.

ROSS, D.B., 1966. The diagnosis, prevention and treatment of chronic copper poisoning in housed lambs. *Br. vet. J.* 122, 279.

SUTTLE, N.F., MUNRO, C.S. & FIELD, A.C., 1978. The accumulation of copper in the liver of lambs on diets containing dried poultry waste. *Anim. Prod.* 26, 39.

TODD, J.R., 1969. Chronic copper toxicity of ruminants. *Proc. Nutr. Soc.* 28, 189.

TODD, J.R., GRACEY, J.F. & THOMPSON, R.H., 1962. Studies on chronic copper poisoning. 1. Toxicity of copper sulphate and copper acetate in sheep. *Br. vet. J.* 118,482.

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 DER BERG, R. & VAN DER SCHEE, W., 1973. Mogenlijkheden ter preventie van chronische kopervergiftiging bij schapen: toevoeging van molybdeen en sulfaat aan het krachtvoeder. *Tijdschr. Diergeneesk.* 98, 328.

VAN RYSSEN, J.B.J., CHANNON, P. & STIELAU, W.J., 1977. Minerals and nitrogen in poultry manure, *S. Afr. J. Anim. Sci.* 7, 195.

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

WYNNE, K.N. & MCCLYMONT, G.L., 1956. Copper-molybdenum-sulphate interaction in induction of ovine hypocupraemia and hypocuprosis. *Aust. J. agric. Res.* 7, 45.