Ram fertility unaffected by high molybdenum intakes

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Two trials were conducted to determine the effect of dietary molybdenum (Mo) on the fertility of young rams, when fed for *ca* 14 weeks to prevent copper (Cu) toxicity. In the first trial, the sexual development of young weaner rams receiving 38 mg Mo/kg DM (Mo group) was compared to that of a control group receiving no Mo. Both groups reached puberty at the same body mass. Although the mass of the testes of the rams in the control group was lower ($P < 0,05$) than that of the Mo group at slaughter, testicular mass, expressed as a percentage of warm carcass mass, did not differ between treatments. The control group showed a lower degree of spermatogenesis, expressed as the percentage tubules in the testes with spermatozoa, than rams fed Mo. This was assumed to be due to the lower body and testicular mass of the control group at slaughter. No histological evidence of testicular degeneration was observed in either group. In the second trial, young sexually-mature rams were fed a diet in which Mo concentration increased from 20 to 65 mg/kg DM during the experimental period. Sulphur (5,4 g/kg DM, Mo + S group) was included in the diet of one group, while the other group received no additional sulphur (Mo group). Although the Mo concentration in the testes of the Mo group was higher ($P < 0,01$) than in that of the Mo + S group, no differences between treatments were observed in testicular mass or in any of the measurements of fertility in the rams.

Twee proewe is uitgevoer om die invloed van molibdeen (Mo) in die dieet op die vrugbaarheid van jong ramme vas te stel wanneer hoë vlakke Mo vir ongeveer 14 weke gevoer is om kopervergiftiging te voorkom. In die eerste proef is die geslagsontwikkeling van jong, speenoud-ramme wat 38 mg Mo/kg DM in hulle rantsoene ontvang het (Mo-groep), vergelyk met 'n kontrolegroep wat geen addisionele Mo ontvang het nie. Albei groepe het puberteit op ongeveer dieselfde liggaamsmassa bereik. Alhoewel die testes van die kontrolegroep met slagting ligter ($P < 0,05$) as dié van die Mo-groep was, het testesmassa, bereken as 'n persentasie van warm karkasmassa, nie tussen behandelings verskil nie. Die kontrolegroep het 'n laer voorkoms van spermatogenese, uitgedruk as persentasie spermbuise in die testes met sperms, as die Mo-groep gehad. Dit kan moontlik toegeskryf word aan die ligter liggaams- en testesmassas van die kontrolegroep met slagting. In geen groep is histologiese aanduidings van enige degenerasie van die testes waargeneem nie. In die tweede proef het jong, seksueel-volwasse ramme 'n rantsoen hoog in Mo ontvang. Die Mo-vlak is tydens die proef vanaf 20 tot 65 mg/kg DM verhoog. By die rantsoen van een groep is 5,4 g swael (Mo + S-groep) per kg DM gevoeg, terwyl die ander groep (Mo-groep) geen ekstra swael ontvang het nie. Alhoewel die Mo-konsentrasie van die testes van die Mo-groep hoër ($P < 0,01$) as dié in die Mo + S-groep was, is geen verskil tussen behandelings in testesmassa of in enige gemete maatstaf van vrugbaarheid by die ramme waargeneem nie.

Keywords: Copper, fertility, molybdenum, rams, sexual development, sheep.

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Enzootic icterus ('geelsiekte'), which is prevalent in many areas of the Karoo region in South Africa and causes between 1 000 and 10 000 sheep deaths annually, is a manifestation of copper (Cu) toxicity (Bath, 1979). Cu toxicity also occurs elsewhere in South Africa among stud and other intensively-fed sheep (Belonje, Hunter & Heine, 1971; Versfeld and Kitley, 1982). Usually, a small proportion of a flock will undergo the haemolytic crisis stage of the disease (Todd, 1969). The rest of the flock may appear to be normal, although their hepatic Cu levels may be high. Any stress such as a change in diet, transportation, shearing and drought may result in further mortalities due to Cu toxicity (Todd, 1969; Bath, 1979).

Outbreaks of copper toxicity have been effectively controlled through the intravenous administration of ammonium tetrathiomolybdate (Gooneratne, Howell & Gawthorne, 1981; Humphries, Mills, Greig, Roberts, Inglis & Halliday, 1986), whereas Van Ryssen, Van Malsen & Barrowman (1986) recommended the feeding of high levels of molybdenum (Mo) for short periods of time (two to three months) to reduce the liver Cu levels of sheep loaded with Cu.

Gooneratne *et al.* (1981) and Van Ryssen, Botha & Stielau (1982) did not observe any deleterious effects such as morphological damage or impaired kidney function in sheep which received high levels of tetrathiomolybdate or molybdenum, respectively. However, Thomas & Moss (1951) reported serious testicular degeneration in bull calves fed high levels of Mo. In a series of trials, Phillipo, Humphries, Bremner & Young (1982) and Phillipo, Humphries, Atkinson, Henderson & Garthwaite (1987b) demonstrated that dietary Mo had a deleterious effect on the fertility of heifers and cows. This was manifested as delayed onset of puberty, reduced conception and disrupted oestrous activity. Phillipo *et al.* (1987b) mentioned similar observations in sheep. Although the cattle used by Thomas & Moss (1951) and Phillipo *et al.* (1982; 1987b) were Cu deficient, it was demonstrated that the effect of Mo on

fertility was due to dietary Mo and not to Cu deficiency. However, Phillipo *et al.* (1987b) indicated that their experiments do not exclude the possibility that a low Cu status is a prerequisite for Mo to affect fertility.

Where Mo is supplemented to overcome Cu toxicity, the issue of Cu deficiency is obviously redundant. However, if high dietary levels of Mo can reduce fertility, the use of Mo supplements cannot be recommended for breeding animals. Trials were conducted to investigate: (1) whether high Mo intakes would impair the fertility of young rams that received adequate levels of Cu and (2) the influence of a reduction in dietary sulphur (S) levels to promote absorption of Mo without noticeably influencing Cu metabolism (Grace & Suttle, 1979). The latter procedure should permit the effect of Mo *per se* to be observed without the consequences of a Cu deficiency confusing the results.

Procedure

Trial 1

Twenty-eight SA Mutton Merino rams, 5 months of age, with a mean body mass of *ca* 24 kg were randomly allocated to two groups. The control group received a concentrated diet containing 1,6 mg Mo, 13 mg Cu, 397 mg iron (Fe), 70 mg manganese (Mn), 38 mg zinc (Zn) and 4,5 g S per kg DM. Thirty-eight mg Mo (as ammonium molybdate) per kg DM were added to the diet of the second group (Mo group). The rams were fed individually with feed intakes and mass changes recorded. Blood samples were collected every third week to monitor Cu status. Measurements of the scrota and testes were conducted every two weeks, *viz.* length and width of both testes and scrota circumference. A semen sample was obtained through electro-ejaculation and was evaluated microscopically for mass motility on a scale of 0 = no motility to 5+ = very high motility. A semen smear was stained with nigrosin/eosin and the percentage of live sperms was estimated. Each ram was slaughtered when the scrotal circumference reached *ca* 27 cm, provided a high spermatozoa count was observed. If the spermatozoa count was low at a scrotal circumference of 27 cm, the ram was kept for another two weeks before it was slaughtered. At slaughter, the masses of the testes, livers and kidneys were determined and tissue samples were taken for mineral analyses. A tissue sample taken from one testis per ram was preserved in buffered formalin for histological evaluation.

Trial 2

Fourteen Blackhead Persian rams, 12 months of age, with a body mass of *ca* 29 kg and which had a good mass motility of the spermatozoa in their semen, were allocated at random into two groups. The rams were fed individually. Each received 350 g concentrate and 500 g veld hay per day. The total ration contained 16 mg Cu, 277 mg Fe, 136 mg Mn, 45 mg Zn and 2,1 g S per kg DM. Molybdenum was included in the concentrate to supply 20 mg per ram per day for the first 25 days. This was increased to supply 48 mg Mo per ram per day for 37 days and when no deleterious effect was observed, each ram received 65 mg Mo per day for a further 28 days. Sulphur (as sodium sulphate) was included in one ration (Mo + S group) to supply a total of 5,4 g per kg DM, while the other group constituted the high Mo, low S treatment (Mo group). After three months all the rams were slaughtered. Further procedures were the same as described for Trial 1, except that testicular measurements were taken only at the onset of the trial as criteria for sexual maturity.

Analytical methods

Dietary Mo, Cu, Fe, Mn, and Zn; Mo and Cu in the organs and plasma, and packed cell volume (PCV) and haemoglobin (Hb) in whole blood were determined according to Van Ryssen & Stielau (1981). Dietary S was measured by the magnesium nitrate method for plants (AOAC, 1980). The procedure of Smith & Wright (1975) was followed for the determination of trichloroacetic acid (TCA) soluble Cu in plasma. Histological evaluation of testes was based on interstitial cell degeneration, seminiferous tubule epithelium degeneration, fibrosis and cell infiltration, while spermatogenesis was expressed as percentage tubules with spermatozoa.

Table 1 Mean body and carcass mass and performance of rams during the trials

Trial	Treatment[a]	Mass: Final body (kg)	Mass: Carcass (warm) (kg)	Duration (d)	Average daily gain (g)	Efficiency of feed[b] conversion (kg/kg)	Feed[b] intake/day (kg)
1	Mo	48,9	24,5	100,6	250	6,1	1,51
	Control	46,7	22,7	101,3	231	6,4	1,46
	SED	1,79	0,94	5,4	16,1	0,30	0,07
2	Mo	38,5	20,2	97	104	8,8	1,25
	Mo + S	39,0	20,8	97	105	8,9	1,26
	SED	1,4	0,78	–	9,4	0,86	0,02

[a] Difference between treatments within a trial not significant.

[b] DM content 90%.

Statistical analyses

Student's *t* test was employed to compare results. In the case of mineral levels in the liver and kidneys, logarithmic transformations were used before analyses.

Results

Mass and mass gains

During Trial 1 the control group, when compared to the Mo group, showed a slightly, though non-significantly poorer performance in terms of body and carcass mass, average daily gain and feed conversion (see Table 1). Similarly, the liver mass of the control group was lower, though not significantly, than that of the Mo group (Table 2). The mean mass of the kidneys of both groups was 125 g at the end of the trial. Although the attainment of sexual maturity was evaluated subjectively, the rams in both groups reached this stage on average after a period of 101 days. The rams in both treatments performed similarly during Trial 2 with no significant differences in body and carcass masses, average daily gains, efficiencies of feed conversion (Table 1), and liver and kidney masses (Table 2).

Table 2 Mean mass of fresh livers and kidneys, and mass of the organs as a percentage of warm carcass mass

		Liver		Kidney mass	
Trial	Treatment [a]	Fresh (g)	as % warm carcass	Fresh (g)	as % warm carcass
1	Mo	899	3,68	125	0,511
	Control	830	3,64	125	0,547
	SED	40	0,10	6,77	0,017
2	Mo	387	1,92	75	0,374
	Mo + S	374	1,80	79	0,381
	SED	13,1	0,10	2,5	0,013

[a] Differences between treatments within a trial not significant.

Testicular and semen evaluations

At the end of Trial 1, the mass of the testes of the rams in the control group was lower ($P < 0,05$) than that of the Mo group. However, the control group commenced the experiment at a slightly lower body mass and smaller scrotal circumference (Table 3). When testicular mass was expressed as a percentage of warm carcass mass, there was no difference between the treatments. The first viable spermatozoa were observed in the ejaculate of both groups at a body mass of *ca* 40 kg. At the last semen collection before slaughter, mean semen motility of the groups was similar and there was no difference in percentage live spermatozoa in the ejaculation. When spermatogenesis was expressed as percentage tubules with spermatozoa, the Mo group showed a higher percentage than the control group. The control group showed a slightly higher proportion of testes with mild tubule degeneration (54%) as compared to the Mo group (46%). In both treatments no interstitial cell degeneration, fibrosis or cell infiltration of the testicles was observed.

In Trial 2, no significant difference was observed between treatments in either testicular mass or testicular mass as a percentage of carcass mass (Table 3). In both treatments 58% of the rams showed live spermatozoa in 75—95%, and the rest in 50—74% of their testicular tubules. No interstitial cell degeneration, fibrosis, cell infiltration or seminiferous tubule epithelium degeneration was observed in any of the rams in either treatment. For the duration of Trial 2, motility of the spermatozoa in the ejaculate of all the rams was rated as 5+, except during the last two collections when one ram in the Mo group exhibited a low spermatozoan motility. However, 50—74% of the tubules in the testes of this ram contained spermatozoa.

Copper and molybdenum in organs

Feeding of Mo in Trial 1 resulted in higher ($P < 0,01$) concentrations of Mo in the testes, livers and kidney cortices as compared to those in the control rams (see Table 4). No difference in Cu levels between treatments

Table 3 Testicular measurements during trials

		Scrotal circumference		Mean [a] mass motility of spermatozoa on last collection	Testicular mass at slaughter – without epididymis	
Trial	Treatment	Initial (cm)	At slaughter (cm)		g	% of warm carcass
1	Mo	14,6	28,5	3,9	260	1,06
	Control	13,6	27,3	4,2	220	0,94
	SED	0,87	0,62	0,55	17,4 *	0,09
2	Mo	25,3	–	4,3	251	1,25
	Mo + S	25,4	–	5,5	238	1,14
	SED	0,99	–	0,82	14,8	0,07

[a] Mass motility of spermatozoa, rated as 0 = no motility to 5+ = very high motility.
* $P < 0,05$.

Table 4 Mean concentrations (mg/kg DM) of copper and molybdenum in the testis, liver and kidney cortex of the rams at termination of the trials

		Testis		Liver		Kidney cortex	
Trial	Treatment	Cu	Mo	Cu	Mo	Cu	Mo
1	Mo	5,5	3,7	62	18,3	66	51,0
	Control	4,7	0,9	400	4,4	16	2,5
	SED	0,37*	0,31**	$^{+}$1,15**	$^{+}$1,10**	$^{+}$1,13**	$^{+}$1,17**
2	Mo	7,6	9,7	378	20,0	19,4	32,4
	Mo + S	8,8	3,5	249	19,8	104,3	90,7
	SED	0,6	1,5**	$^{+}$1,22*	2,8	$^{+}$1,09**	$^{+}$1,15**

* $P < 0,05$.
** $P < 0,01$.
$^{+}$ Antilogarithms.

was observed in the testes. Dietary Mo supplementation significantly ($P < 0,01$) reduced the Cu levels in the livers and increased ($P < 0,01$) Cu levels in the kidney cortices. Liver Cu concentrations in the Mo group ranged from 29 to 128 mg/kg DM with 5 out of the 14 rams with values of below 50 mg/kg DM. In Trial 2 the Mo concentration in the testes of the group receiving Mo only was more than twice ($P < 0,01$) as high as that of the Mo + S group (Table 4). Molybdenum levels in the livers did not differ significantly between treatments, but in the kidney cortices mean Mo levels in the Mo + S group were higher ($P < 0,01$) than in the Mo treatment. When Mo and S were included in the diets, the liver Cu concentration was lower ($P < 0,05$) than when Mo only was included, while kidney Cu levels were higher ($P < 0,01$; Table 4).

Blood parameters

Total plasma Cu and Mo values in the group receiving Mo in Trial 1 were higher ($P < 0,05$ and $P < 0,01$, respectively) than in the control group (Table 5), while TCA soluble Cu was lower ($P < 0,01$). In Trial 2, the mean total plasma Cu of the Mo + S group was higher ($P < 0,01$) than in the Mo group, but the difference in plasma Mo levels between treatments was not significant. During Trial 1, PCV showed gradual increases from the initial 26 and 27% to 34 and 36% for the Mo and control groups, respectively. The difference between treatments at the end of the trial was significant ($P < 0,05$; Table 5) although, when compared with the use of covariance based on initial PCV values, this difference was not significant. Haemoglobin levels in Trial 1 and PCV and Hb levels in Trial 2 did not differ significantly between treatments (Table 5).

Table 5 Final haematological results, plasma copper (Cu) and plasma molybdenum (Mo) concentrations

		Plasma			Whole blood	
Trial	Treatment	Cu (mg/l)	TCA Cu [a] (mg/l)	Mo (mg/l)	PCV [b] (%)	Hb [c] (g/100 ml)
1	Mo	1,11	0,48	1,14	34	8,9
	Control	0,84	0,73	0,09	36	9,1
	SED	0,06**	0,04**	0,11**	0,90*	0,30
2	Mo	0,86	–	0,88	35	13,5
	Mo + S	1,21	–	0,94	37	13,4
	SED	0,11**	–	0,19	1,70	0,60

* $P < 0,05$.
** $P < 0,01$.
[a] Trichloro acetic acid soluble Cu.
[b] Packed cell volume.
[c] Haemoglobin.

Discussion

Sheep are considered to be in a state of marginal Cu deficiency if their plasma Cu levels range from 0,3 to 0,5 mg/l and liver Cu levels from 10 to 50 mg/kg DM (Caple & McDonald, 1983). In the present investigation, rams fed the Mo in Trial 1 became marginally deficient, but the other groups in both trials did not. A reduction in the PCV of blood can develop when animals are Cu deficient (Underwood, 1977). The fact that the Mo group in Trial 1 started off at a lower PCV, may preclude the possibility that its lower PCV at the end of the trial indicated a state of Cu deficiency.

In the trials, dietary Mo supplementation with high dietary S reduced liver Cu and elevated kidney Cu and Mo levels. These are well-documented consequences of Mo plus S feeding in sheep (Dick, 1956; Underwood, 1977; Bremner & Young, 1978; Van Ryssen & Stielau, 1981). The level of Mo in the body of ruminants was found to depend on dietary Mo (Lesperance & Bohman, 1963; Van Ryssen & Stielau, 1981) and dietary S (Grace & Suttle, 1979; Van Ryssen & Stielau, 1981). The increase in concentrations of Cu and Mo in plasma and kidneys, when high levels of Mo and S are fed, is attributed to the formation of compounds such as copper thiomolybdates which are apparently unavailable to the tissues; the so-called systemic effects (Dick, 1956; Suttle, 1974; Van Ryssen *et al.*, 1986), as observed in the present trials.

An interesting deviation from this pattern is the significantly higher concentrations of Mo in the testes of the low S group in Trial 2, a phenomenon not reported before. In Trial 1, testicular Mo levels followed the expected trend where tissue Mo concentrations were related to the level of dietary Mo (Lesperance & Bohman, 1963). Dietary S can reduce Mo levels in tissues (Huisingh, Gomez & Matrone, 1973) if systemic effects due to the Cu–Mo–S interactions do not elevate these levels. It is suggested that, in Trial 2, these systemic effects were responsible for the high Mo and Cu levels in the kidneys (Suttle, 1974) and contributed to the elevated liver Mo levels in the Mo + S group (Van Ryssen & Stielau, 1981). However, it would appear as if systemic effects did not have an influence on the Mo and Cu levels in the testes of this treatment. Molybdenum therefore showed a higher rate of accumulation in the testes when dietary S levels were low.

Phillipo *et al.* (1982, 1987b) argued that reduced fertility in cattle when Mo was fed, was not directly related to their Cu deficient status. In the trial by Thomas & Moss (1951) the bulls which developed testicular degeneration were clearly Cu deficient. However, Phillipo *et al.* (1987b) mentioned delayed onset of first oestrus in sheep before their liver and plasma Cu concentrations had declined below 30 mg/kg DM and 0,6 mg/ml, respectively. In the present trials, no effect on the fertility of the young rams due to dietary Mo could be observed. In fact, in Trial 1 the control group showed slightly slower sexual development than rams receiving Mo. However, this could be due to the fact that the rams in the control group were slaughtered at a slightly lighter body mass. The result was that their testes were smaller and lighter at slaughter.

As early as 11 weeks after onset of their trial, Phillipo *et al.* (1987b) observed changes in plasma luteinizing hormone (LH) release due to excess Mo. At this stage, no Cu deficiency symptoms were evident in the heifers. Symptoms such as delayed onset of puberty, reduced conception rates and disrupted oestrous activity in cattle were detectable only much later in the trial. In accordance with the recommended procedure to control Cu toxicity (Van Ryssen *et al.*, 1986), the present trials were terminated after about 14 weeks, at which stage a clear manifestation of the Mo effect on fertility had not occurred. Adverse effects of longer duration of exposure to diets high in Mo cannot be ruled out.

Phillipo, Humphries & Garthwaite (1987a) reported a reduced growth rate in heifers which started 16 weeks after the onset of Mo feeding; a response not observed when Cu deficiency was created by high Fe intakes. An inhibitory effect of dietary Mo on growth has also been reported in sheep by Bremner & Young (1978) and Ivan & Veira (1985); in cattle by Lesperance & Bohman (1963), Phillipo *et al.* (1982) and Humphries, Phillipo, Young & Bremner (1983); and in rats (Van Reen & Williams, 1956). Phillipo *et al.* (1987a) suggested that reduced feed intake could partially explain the slower growth, but does not account for the entire response observed in cattle. These trials in which reduced gains were recorded, usually lasted for extended periods, e.g. about 30 weeks (Bremner & Young, 1978; Ivan & Veira, 1985), although no Cu deficiency symptoms were observed in these trials with sheep. However, supplementation of dietary $CuSO_4$ cancelled out the harmful effect of Mo on the growth of sheep (Ivan & Veira, 1985). In the present trials of shorter duration, no effect on growth rate or feed intake due to Mo feeding was observed.

The level of dietary Mo does not seem to be a major factor in impairing fertility or growth rate, because the dietary inclusion rates of Mo in most of the reported studies were very low: 5 mg/kg feed (Phillipo *et al.*, 1982; 1987a, 1987b) or at the highest 25 mg/kg (Bremner & Young, 1978) relative to that in the present trials (38—65 mg Mo/kg). The time required for the detrimental effect of Mo on fertility and growth to be manifested, does not appear to be reduced by high inclusion rates of dietary Mo, except perhaps where such a response depends on a state of Cu deficiency in the animal. Many factors, such as interactions between Mo and S (Huisingh *et al.*, 1973; Grace & Suttle, 1979) or between Cu and Mo plus S in the digestive tract and body of sheep (Suttle, 1974; Smith & Wright, 1975), can determine the level and form of Mo present in blood and tissues. Therefore, a direct comparison between different studies on effects of Mo toxicity may not be meaningful.

From the present investigation it can be concluded that a high Mo intake did not have a deleterious effect on the fertility or growth of young rams when fed for the short durations of up to 14 weeks. This is recommended by Van Ryssen *et al.* (1986) to reduce the danger of a haemolytic crisis due to Cu toxicity when high levels of Cu have accumulated in the livers. The feeding of Mo for longer periods cannot be recommended because of the risk of an induced Cu deficiency. These results do not exclude the possibility that Mo may directly impair growth and fertility if supplied for longer periods.

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