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Effect of Molybdenum and Sulphur on Copper Status and Mohair Quality in Merghoze Goat

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Abstract: This study was made on the effects of a normal diet containing 12.8 mg Cu kg^{-1} DM which added gradually molybdenum and sulphur on the copper status and fibre quality in eight 1-year Merghoze goat. One group (n = 4 mean weight 31±2.0 kg) was treated with Mo and S supplements for 20 weeks, the second group (n = 4 mean weight 32±2.1 kg) served as controls. In addition of blood sampling for measuring copper status in plasma, the copper content and quality of fleeces were measured every 6 weeks. Mohair measurements were carried out by taking patch samples (10×10 cm^2) from the mid-side area of the goat. The analytical set consists of plasma copper concentrations (Pl Cu), Trichloroacetic acid soluble copper concentrations (TCA-Cu), and fleece copper content. The results indicated that the addition of 20-30 mg Mo and 2-2.5 g S kg^{-1} DM to the normal diet did produce sub clinical copper deficiency in treated goats. One such visual symptom was the loss of fleeces pigmentation and poorer crimp being observed. The Pl Cu minus TCA-Cu plasma became more than 2 μM in the blood of treated goat, indicating that there was a significant thiomolybdate formation in the body. The results showed that there was a significant decrease in Pl Cu ($p<0.05$) along with a significant increase in thiomolybdate (MoS) production after 4 months. The sub clinical signs of copper deficiency and mohair quality are likely to be from high molybdenum intake and thiomolybdate formation in the body.

Key words: Copper deficiency, molybdenum, sulphur, fiber, goat

INTRODUCTION

Clinical copper deficiency has been described in ruminant animals with various visual symptoms such as poor wool quality. There are several studies about the relationship between copper status and the amount of Mo, S and Fe in diet and it has long been known that molybdenum is involved in copper deficiency (Phillipoo *et al*., 1987; Mackenzie *et al*., 1999; Moeini, 1997; Williams, 2004). These studies indicated that the occurrence of Cu deficiency depends on the presence of the minerals, especially molybdenum and sulphur. The response of the animals to the dietary alteration also depends on the liver copper concentration, species and breed (Woolliams *et al.,* 1986; Kendall *et al*., 2001; Hansen *et al*., 2008). The cause of clinical copper deficiency has been defined as a lack of copper that is available for absorption into the animal's body. However, research by Kendall *et al*. (2001), Spears (2003) and Moeini and Souri (2004) has shown that clinical copper deficiency is due a toxic effect of molybdenum rather than a lack of copper for metabolic function. These studies indicated that the specific effect of molybdenum in producing clinical copper deficiency symptoms can be detected. Molybdenum combined with sulphur (MoS_2) and passes through into blood, then deactivated the copper related enzymes such as caeruloplasmin, diamine oxidase and cytocrom oxidase (Mackenzie *et al*., 1997; Moeini *et al*., 2003; Kendall *et al*., 2006).

The difference between total plasma copper and TCA soluble copper gives the amount of thiomolybdate in the blood. The TCA precipitates out any copper thiomolybdate (as $CuMoS_4$) already present in the blood. Our previous studies showed that the addition of 5 mg Mo, 2.5 g S kg^{-1} DM to diet caused clinical copper deficiency when sheep fed deficient copper dietary (≤ 1.5 mg Cu kg^{-1} DM) (Zervas, 1983; Moeini, 1997; Sanjabi *et al*., 2003). This experiment was made on the effect of a normal diet (containing 12.8 mg Cu kg^{-1} DM) which added molybdenum and sulphur supplements on the copper status sub clinical copper deficiency and mohair quality in Merghoze goat.

MATERIALS AND METHODS

Merghoze goat is a native breed scattered in west of Iran and has been known for its mohair production. Eight 1 year Merghoze goat were housed in individual crates in animal house of Razi University. One group (n = 4, mean weight 31± 2.0 kg) was treated with different levels of Mo and S supplements for 20 weeks, the second group (n = 4, mean weight 32±2.1 kg) served as controls. Animals fed a

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basal diet (2 kg alfalfa hay + 0.2 kg barley) according their live weight. The amount of copper (mg kg^{-1}) in this diet on dry matter basis was 12.8±2.3 which added Mo as (ammonium molybdate) and S as ($FeSO_4$) supplements during 4 months experiment (Table 1). In addition of blood sampling for measuring Cu status in plasma, the copper content and quality of fleeces were measured every 6 weeks. Mohair measurements were carried out by taking patch samples (10×10 cm^2) from the mid-side area of the animals.

The analytical set consists of plasma copper concentrations (PlCu), Trichloroacetic acid soluble copper concentrations (TCA-Cu) and fleece copper content using the methods described by Mackenzie *et al.* (1997) and Mulryan and Mason (1992). Fleece samples were clipped from the same area (lumber area behind the ribs) of each goat and these were then washed according to the procedure described by Sorenson and Melby (1973). Blood samples were taken by jugular vein and transfer to laboratory. Plasma was diluted 1 part plasma to 9 part double distilled water to measure total plasma copper on Atomic Absorption spectrophotometer (model Unicom 939 Germany) at 324.8 nm. Protein in the plasma samples was precipitated using 10% trichloroacetic acid (TCA) at a ratio of 1:1 TCA: sample/and then centrifuged for 15 min at (3000 rpm, 1500 g). The addition of TCA precipitates out any copper thiomolybdate ($CuMoS_4$) already present in the blood and the difference between total plasma copper and TCA soluble copper gives the amount of thiomolybdate formation.

The Student's t-test was used to detect significant differences between means of PlCu and TCA-Cu, copper concentration in fleece and fleece diameter were averaged between goats and analyzed using fit model procedure of proc ANOVA (SAS, 1999 V.8.1) at the level of $p<0.01$ in completely randomized design, and Duncan's mean test, according to the model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

y_{ij} = Dependent variable

μ = Overall mean

T_j = Fixed effect of the ith treatment

e_{ij} = Random residual

Table 1: Mineral supplements adding to daily diet during the experiment

	Weeks			
Minerals	1	6	12	18
S (g kg^{-1} DM)	1.5	2	2	2.5
Mo (mg kg^{-1} DM)	10	10	20	30

RESULTS AND DISCUSSION

The plasma copper concentrations were 8.5-16 μmol during the experiment, which was mostly 9.5-10.5 μmol (Table 2). According to literature, animal with blood copper concentrations less than 8 μmol Cu L^{-1} are deficient and 12 μmol are normal (Underwood and Suttle, 1999; Sanjabi *et al.*, 2003; Mackenzie *et al.*, 1999). The Plasma copper concentrations in all animals were 14-15 μmol at the start of experiment which decreased significantly to 11 μmol at the end of experiment. At the first week of the experiment, the differences between the pl Cu and TCA-Cu in all animals were less than 1 μmol L^{-1} compared to the end of experiment, where these differences in treated goats reached to 2 μmol L^{-1} (Table 2). This result indicated that the high molybdenum and sulphur increased the production of thiomolybdate that subsequently reacted with copper which was in agreement with Sanjabi *et al.* (2003), Williams (2004) and Kendall *et al.* (2006).

The plasma copper and TCA copper plasma concentrations of the treated goat decreased significantly at the end of experiment compare to the start of experiment ($p<0.05$). Goats in treated group indicated the signs of deficiency at the end of experiment and reached a minimum plasma copper concentration of 8-9 μmol L^{-1} with a distinct band of poorly crimped mohair. The results showed that there was a significant decrease in Cu plasma ($p<0.05$) with increase in thiomolybdate production toward to end of the experiment (Table 2).

Treated goats after 15 weeks showed an obvious de-pigmentation of the fleeces and poorer crimp which indicated that these goats were sub clinically deficient in copper (Table 2). This conclusion was supported by the analyses of other copper dependent parameters; plasma copper, TCA-Cu and fleece copper content. In this experiment the treated goat were fed a normal diet containing 12.8 mg Cu kg^{-1} DM, which supplemented with additional levels of molybdenum and sulphur. Clinical copper deficiency was not observed during 12 weeks of experiment except in goat No. 2, where there was some indication of depigmentation of mohair. The effect of the high molybdenum and sulphur in the diet of

Table 2: Changes in Pl Cu and TCA concentrations (μ mol L^{-1}) during the experiment

	Treated goat		Control	
Weeks	Pl Cu	TCA-Cu	Pl Cu	TCA-Cu
1	14.9±0.9	14.6±1.1	14.6±1.0	14.3±1.1
3	14.6±1.0	14.3±1.2	-	-
6	14.7±0.9	13.9±0.8	14.8±0.9	14.6±1.0
9	13.7±1.1	12.7±0.9	14.6±0.8	14.7±0.9
12	13.1±0.7	11.9±0.9	14.6±0.7	14.4±0.8
15	12.4±0.7^a	10.7±10^b	14.8±0.8^c	14.4±0.9^c
18	11.3±0.7^a	9.03±0.9^b	14.7±0.9^c	14.3±0.7^c

Different superscript letter(s) within rows were significant ($p<0.05$)

Table 3: Copper concentrations (Mean±SD) in fleeces towards end of experiment (mg Cu kg^{-1} DM)

	Animal	
Week	Treated goat	Control
1	7.8 ± 0.05^{a}	8.1 ± 0.85^{a}
9	6.5 ± 0.65^{a}	7.9 ± 0.73^{a}
18	4.2 ± 0.93^{b}	8.2 ± 0.85^{a}

Different superscript letter(s) within rows and columns were significant ($p<0.05$)

treated goat reduced the TCA soluble copper plasma, this being indicative of copper thiomolybdate production. Treated goat showed an obvious de-pigmentation of the fleece as well as steely wool with reduced fleece crimp at the end of experiment.

The clinical symptoms that were found here were from high dietary molybdenum and sulphur rather than purely a decreased copper status of blood. This is in agreement with previous studies which showed that the clinical symptoms are due to the presence of molybdenum and sulphur decreasing copper availability as a result of thiomolybdate production (Moeini, 1997; Spears, 2003; Hansen *et al*., 2008). Therefore the clinical symptoms of copper deficiency seen in the presence of molybdenum as described here is likely to be due to the effects of thiomolybdates on the function of the copper enzymes.

The copper content of the fleece can be also used as an indicator of copper deficiency (Kellaway *et al*., 1978; Suttle, 1981, 1991; Lee and Grace, 1988). The treated Merghoze goat showed an obvious de- pigmentation of the fleece as well as steely fleece with reduced fleece crimp. The copper concentration of the fleeces in goat was lower (3.8-4.3 mg kg^{-1} DM) than that of the control which was 7.8-8.6 mg kg^{-1} DM (Table 3). In the present work changes in the crimp and de-pigmentation of the fleece, with a decrease in its copper content, indicate the correlation between fleece copper concentration and copper deficiency.

Suttle (1981, 1991), Lee and Grace (1988) and Moeini (1997) showed that in a prolonged copper deficiency the copper level of the fleece decreased to 2-3 mg kg^{-1} DM. These result of our study indicated that high mineral supplement was effective in reducing copper content of the fleece, but the total reduction during this period of time was not to a level indicative of Cu deficiency. It is known for a long time that the silvering of the fleece will affect some lambs in a flock and not others. This could be due to genetic variation (some animals absorbing thiomolybdate with more ease than others), a variation in the rumen flora (it is the activity of rumen bacteria that produces the thiomolybdates or a behavioural difference and Some breeders have noted a familial tendency to show silvering in the fleece, which gives support to a genetic factor. It will be less easy to identify animals carrying this factor if they are treated with copper.

Table 4: Changes in fleece diameter (μ) during the experiment

Weeks/Treat	Control	Treated goat
3	30.9^{a}	29.0^{a}
6	31.1^{a}	30.8^{a}
9	31.4^{a}	31.3^{a}
12	31.7^{a}	32.4^{a}
15	30.8^{a}	34.1^{b}
18	30.7^{a}	34.0^{b}

Different superscript letter(s) within rows and columns were significant ($p<0.05$)

Table 5: Changes in mean live weight (kg) of animals during the experiment

	Weeks/Treat						
Animal	1	3	6	9	12	15	18
Control	32±2.1	34±2.2	34.2±1.8	33.7±1.9	33.5±2.4	33.5±2.1	34.0±2.2
Goat	31±2.0	33±2.1	33.6±2.0	32.5±2.4	32.4±2.6	31.6±2.3	31.1±1.7

There was a different response to the high molybdenum, sulphur in treated goat with the fleeces diameter significantly changed during the experiment (Table 4). The genetic variation between breeds suggests that difference responses arise from a variation in the efficiency of absorption of copper (Woolliams *et al*., 1986; Williams, 2004). Gooneratne *et al*. (1994) also has found a breed differences in biliary copper excretion by adding excess dietary levels of molybdenum and sulphur. When ruminal sulfide concentrations are low, molybdenum may have little effect on copper bioavailability. In sheep fed diets that contained only 1.0 g of sulfur kg^{-1} of diet, increasing the dietary molybdenum from 0.5 to 4.5 mg of molybdenum kg^{-1} of diet did not affect copper bioavailability. However, the addition of 3.0 g of sulfur and 4.0 mg of molybdenum kg^{-1} of diet to a basal diet that contained 1.0 g of sulfur and 0.5 mg of molybdenum kg^{-1} of diet reduced copper availability by 40-70% (Telfer *et al*., 1984; Gengelbach *et al*., 1994; Spears *et al*.,1997). With moderately high concentrations (2.7 g of sulfur kg^{-1} of diet) of sulfur in the diet, increasing dietary molybdenum from 5 to 10 mg of molybdenum kg^{-1} of diet did not further reduce copper status during a 196 days study with steers. This suggests that the synthesis of thiomolybdates may plateau with relatively low concentrations of molybdenum (Hansen *et al*., 2008).

Decreases in live weight were observed after 15 weeks of feeding the treated goat, high molybdenum but was not statically difference (Table 5). Similar responses were seen by Phillippo *et al*. (1987) who found a depression of live weight gain in molybdenum-supplemented cattle. A reduction in food intake and food utilization was probably the cause of the depressed weight gains found in treated animal with more than 20 mg Mo kg^{-1} added to diet. Gengelbach *et al*. (1994) also found that there was a depression in growth rate when more than 5 mg Mo kg^{-1} DM was given to calves.

Liver copper and total blood copper are still used alone as indicators of copper status, these do not take any account of the correction of the symptoms of sub clinical copper deficiency. Kendall *et al.* (2001), Moeini (1997) and Moeini and Souri (2004) showed that although the blood copper concentration decreased in sheep fed a low copper diet, no clinical signs of deficiency were observed over 17 week period but the addition of molybdenum, iron and sulphur to this diet did produce copper deficiency after 5 months. This was in agreement with Phillippo *et al.* (1987), Spears *et al.* (1997), Mackenzie *et al.* (1999) and Williams (2004) showed that additional Mo+S can act as a potent antagonist of copper and can also severely reduce the copper concentrations. The symptoms of copper deficiency are the symptoms of Mo toxicity. In the presence of Mo and S the rumen bacteria produce a chemical that is toxic to the copper-containing molecules in the body that are vital for fertility, growth and productivity (Kendall *et al.*, 2006).

CONCLUSION

The Merghoz goat fed a common diet containing 12.8 mg Cu kg^{-1} DM showing sub clinical copper deficiency (losses of fleeces pigmentation, increased fleece diameter and poorer crimp) by adding 25-30 Mo and 2-2.5 g S kg^{-1} DM/day after four months. The Pl Cu minus TCA-Cu plasma concentrations were greater than 2 μM in treated goat, indicating that there was a significant binding of free amino acid copper to thiomolybdate in the blood and sub clinical Cu deficiency was likely from copper thiomolybdate formation. It is suggested that small ruminants are prevented to intake more than 15 mg molybdenum and 2 g S daily for more than a few months.

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REFERENCES

Gengelbach, G.P., J. D. Ward and J.W. Spears, 1994. Effect of dietary copper, iron and molybdenum on growth and copper status of beef cows and calves. J. Anim. Sci., 72: 2722-2729.

Gooneratne, S.R., H.W. Symond, J.V. Bailey and D.A. Christensen, 1994. Effect of dietary copper metabolism and sulphur on biliary copper and zinc in Angus cattle. Can. J., 74: 315.

Hansen, S.L., P. Schlegel, L.R. Legleiter, E. Lioyed and J.W. Spears, 2008. Bioavailability of copper from copper glycinate in steers fed high dietary sulphur and molybdenum. J. Anim. Sci., 86 (1): 173-179.

Kellaway, R.C., P. Sitorus and J.M. Leiphlz, 1978. The use of copper levels in hair to diaghypocuprosis. Res. Vet. Sci., 24: 352.

Kendall, N.R., D.V. Illingworth and S.B. Telfer, 2001. Copper Responsive Infertility in British Cattle: The use of a Blood Caeruloplasmin to Copper Ratio in Determining a Requirement for Copper Supplementation. In: Fertility in the High-Producing Dairy Cow, Occasional Publication No. 26. Diskin, M.G. and U.K. Edinburgh (Eds.): British Society of Animal Science, 2: 429-432.

Kendall, N.R., P. Marsters, L. Guo, R.S. Scaramuzzi and B.K. Kampell, 2006. Effect of copper and thiomolybdates on bovine theca cell differentiation. J. Endocrinol., 189 (3): 455.

Lee, J. and N. Grace, 1988. Trace element and wool. Proceedings New Zealand trace element conference, 30 Nov. Lincoln College. Canterbury.

Mackenzie, A.M., D.V. Illingworth, D.W. Jackson and S.B. Telfer, 1997. The use of caeruloplasmin activities and plasma copper concentration as an indicator of copper status in ruminants. In: Trace element in man and animals-Tema, Ottawa, Canada, 9: 137- 139.

Mackenzie, A.M., M.M Moeini and S.B Telfer, 1999. Fertility in the High Producing Dairy Cow, BSAS Occasional Publication, No. 26, 2: 423-427.

Moeini, M.M., 1997. Copper, molybdenum, iron and sulphur interactions on clinical deficiency in dairy cattle and sheep. Ph.D Thesis, University of Leeds, UK.

Moeini, M.M. and S.B. Telfer and M.R. Sanjabi, 2003. The effect of Co secure supplementation on the copper status and fertility of grazing Holstein-friesian dairy. Acta Vet. Scand., 44 (Suppl. 1): 80 (abstract).

Moeini, M.M. and M. Souri, 2004. Copper, molybdenum, iron and sulphur interactions on clinical copper deficiency in sheep and goat, 23rd World Buiatrics Congress, Quebec, Canada (12-17 July).

Mulryan, G. and J. Mason, 1992. Assessment of liver copper status in cattle from plasma copper and plasma copper enzymes. Ann. Res. Vet., 23: 233-238.

Phillipoo, M., W.R. Humphrise and P.H. Garthwaite, 1987. The effect of dietary molybdenum and iron on copper status and growth in cattle. J. Agric. Sci., 109: 315.

Sanjabi, M.R., M.M. Moeini and S.B. Telfer, 2003. Thiomolybdate, the major factor on clinical copper deficiency. Acta. Scand., 44 (Suppl. 1): 81.

SAS, 1999. SAS/STAT Software, Release 8.1 SAS, Institute, Cary, NC.

Sorenson, J.R. and E.G. Melby, 1973. Interferences in the determination of metabolic elements in human hair-analytical methods. Arch. Environ. Health, Vol. 27.

Spears, J.W., E.B. Kegley, L.A. Mullis and T.A. Wise, 1997. Bioavailability of copper from tri-basic copper chloride in cattle. J. Anim. Sci., 75 (Suppl. 1): 265.

Spears, J.W., 2003. Trace mineral bioavailability in ruminants. J. Nutr. Am. Soc. Nutr. Sci., 133: 1506-1509.

Suttle, N.F., 1981. Comparison between parenterally administration of copper complexes of their ability to alleviate hypocupraemia in sheep and cattle. Vet. Res., 109: 34.

Suttle, N.F., 1991. The interaction between copper, molybdenum and sulphur in ruminants nutrition. Annu. Rev. Nutr., 11: 121.

Telfer, S.B., G. Zervas and G. Carlos, 1984. Curing or preventing deficiencies in copper, cobalt and Se in cattle and sheep. Can. J. Anim. Sci., 64 (Suppl. 1): 234-236.

Underwood, E.J. and N.E. Suttle, 1999. Mineral Nutrition of Livestock. CAB International, pp: 598.

Williams, C.L., 2004. The effects of molybdenum, iron and sulphur on copper metabolism and physiology of sheep. Ph.D Thesis, Harper Adams University College.

Woolliams, C., N.F. Suttle, J.A. Woolliams and G. Wiener, 1986. Studies on lambs from lines genetically selected for low and high copper status. Anim. Prod., 43: 293.

Zervas, G.P., 1983. The prevention of copper deficiency in ruminants by means of soluble glass rumen bullets. Ph.D Thesis, University of Leeds, UK.