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Effect of Molybdenum on Sulfur Metabolism in Guizhou Semi-Fine Wool Sheep in South West China Karst Mountain Area

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Abstract: Guizhou semi-fine wool sheep has affected by forage of high molybdenum in the Hezhang County of Guizhou Province in South West China Karst Mountain area. The mineral composition of forage and samples of blood, liver and hair from Guizhou semi-fine wool sheep in affected ranches were compared with those from unaffected areas. Concentration of molybdenum in forage from affected pasture was significantly higher than that from unaffected pasture. The mean concentration of sulfur in forage from affected and unaffected pasture was similar. Mean concentrations of sulfur in blood, liver and hair from the affected Guizhou semi-fine wool sheep were significantly lower than those from the unaffected animals while the content of molybdenum in blood, liver and wool from the affected Guizhou semi-fine wool sheep was significantly higher than that in the unaffected animal. Oral administration of calcium sulfate prevented sulfur deficiency. Researchers concluded that S deficiency in Guizhou semi-fine wool sheep in this area was probably caused by high molybdenum in forage.

Key words: Guizhou semi-fine wool sheep, forage, sulfur, molybdenum, South West China Karst Mountain area

INTRODUCTION

The Guizhou semi-fine wool sheep is vital to the production system of South West China Karst Mountain area. The animals provide meat, hides, wool and farmyard manure. For several consecutive years prior to 2013, high Molybdenum (Mo) in forage caused much harm to Guizhou semi-fine wool sheep in Hezhang County of Guizhou Province in South West China Karst Mountain area. The local farmer had suffered seriously from this problem. The affected animals are characterized by lose wool, pica, emaciation, depressed, dyskinesia and appetites and gradually became thin and lost their appetite. The more seriously affected animals had difficulty in keeping up with the herd and appeared to die of exhaustion. The disease usually occurred during January and May, the peak time was between March and May. Incidence was estimated at 15-20% and the mortality may reach 20%. Affected area was an excellent Autumn to Winter range of native pasture for communal use until 2000 when the government allocated both the pasture and animal to individual families for use in all 4 seasons in an attempt to improve the local herdsmen’s productivity. As a result, Guizhou semi-fine wool sheep were affected by high Mo in forage.

Ruminants grazing forages of high Mo and (or) Copper (Cu) but adequate or deficient in S are at risk of molybdenosis or Sulfur (S) deficiency. Mo, Cu and S combines in the rumen to form a thiomolybdate complex, rendering S unavailable for absorption (Arthington *et al*., 2002). The purpose of this study was to investigate the possibility that S deficiency is caused by high Mo in forages.

MATERIALS AND METHODS

Study area: Affected area is located at 26°53'-27°15'N latitude and 103°37'-103°57'E longitude at an average elevation of 1550 m above sea level. The annual precipitation is 980 mm. The average atmospheric temperature is 8-11°C. The grassland vegetations are mainly Puccinellia (*Chinampoensis ohuji*) white clover (*Trifolium repens*); ryegrass (*Lolium multiflorum*); June grass (*Koeleria cristata*) and the vegetations grow well in the pasture.

Ethics statement: The Guizhou semi-fine wool sheep used in these experiments were cared as per outlined in the Guide for the Care and Use of Animals in Agricultural Research and Teaching Consortium (FASS, 2010). Liver

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biopsy collections were performed by a trained technician and approved by the Institute of Zoology, Chinese Academy of Sciences, Institutional Animal Care and Use Committee (Project: A0665).

Animals: Sixty affected Guizhou semi-fine wool sheep were selected for the study from 5 populations of 570 living in the Hezhang County. All showed signs of lose wool, pica, emaciation, dyskinesia and deprived appetites. Twenty healthy Guizhou semi-fine wool sheep also selected from the Dafang county where the disease is not seen. The clinical signs were record by direct observation. Selective examinations were carried out on affected Guizhou semi-fine wool sheep by Routine Clinical Diagnostic Methods including determination of pulse rate, body temperature and respiratory rate.

Sample collection: All samples were taken in May 2013. Blood samples for analysis of mineral contents and for biochemical examinations were obtained from the jugular vein using trace mineral-free vacutainer tubes. Blood was kept cool at the collection site and subsequently transported to the Animal Nutrition Laboratory at Guizhou University for further preparation and analysis. Liver biopsies were also sampled by a trained technician using techniques previously described (Arthington and Corah, 1995). Hair samples from each affected Guizhou semi-fine wool sheep were also sampled and washed as described by Salmela *et al.* (1981). Samples of forage were sampled from 10 affected pastures. To reduce soil contamination, herbage samples were cut 1-2 cm above ground level. The composite forage samples were dried at 60-80°C for 48 h and ground to facilitate chemical analysis. Soil samples from affected areas were taken from the surface layer (0-30 cm) of 10 pastures using a 30 mm diameter cylindrical core. Four cores per paddock were bulked and placed in polythene bags. Also in May 2013, 20 samples of soil, forage and wool and blood were also collected from the Dafang County of Guiozhou Province, China where the disease is not seen to determine mineral content.

Analysis of mineral contents: S and Phosphorus (P) levels were measured by nephelometry (Wen *et al.*, 1983). Copper (Cu), Zinc (Zn), Calcium (Ca) and iron (Fe) levels were determined using a PerkinElmer AAS5000 atomic absorption spectrophotometer (PerkinElmer, Norwalk, Connecticut, USA). Mo levels were measured using flameless atomic absorption spectrophotometry with extra steps to produce reliable data. In brief after adding the stannous chloride liquor, researchers extracted samples with isopentanol as soon as possible because the generated complex was unstable (Gallaher *et al.*, 1975; Hatzios and Bertozzi, 2011). Assay variation was controlled using the bovine liver standards of National Institute of Standards and Technology (Gaithersburg, Maryland, USA).

Biochemical examination: Biochemical analyses which included Catalase (CAT), Lactate Dehydrogenase (LDH), Alkaline Phosphatase (AKP), γ-glutamy1 transferase (γ-GT), Aspartate aminotransferase (AST), Creatinine (Crt), Calcium (Ca), sodium (Na), potassium (K), Inorganic Phosphorus (IP) were conducted with an automated biochemical analyzer (Olympus AU 640, Olympus Optical Co., Tokyo, Japan). Quality control serum (Shanghai Biochemical Co., Shanghai, China) was used to validate the blood biochemistry data.

Prevention and treatment: Forty affected Guizhou semi-fine wool sheep from affected area were allocated to one of two treatments, consisting of twenty affected Guizhou semi-fine wool sheep were fed 50 mg day^{-1} of $CaSO_4$ for 28 days. S was formulated into a corn meal carrier and was offered 0.5 kg per animal, 3 times weekly, twenty affected Guizhou semi-fine wool sheep were control for 28 days.

Statistical analysis: The differences were assessed by Student's t-test. Experiment data were analyzed by using a statistical package (SPSS Version for Windows; SPSS, Chicago, Illinois, USA). The data are presented as means±standard deviation.

RESULTS

The mineral contents in soil were given in Table 1. Contents of S in soil, no treatment differences were detected. Mo contents in soil from affected area were significantly higher than those from unaffected pasture ($p<0.01$). The contents of trace elements in forage are given in Table 2. The Mo contents in forages from the affected areas were significantly higher than those from the control samples ($p<0.01$). Other mineral contents in forage samples were within the healthy ranges in all areas. The concentrations of mineral elements in the blood samples were shown in Table 3. The S contents in the blood of affected Guizhou semi-fine wool sheep were significantly lower than those of control animals ($p<0.01$). The Mo contents in affected animals were significantly higher than those in healthy semi-fine wool sheep.

The concentrations of mineral elements in wool samples were shown in Table 4. The S content of wool

Table 1: Concentrations of mineral elements in soil

Elements	Affected area	Unaffected area
Cu (μg g^{-1})	27.7±3.7	29.1±3.3
Mo (μg g^{-1})	5.23±0.31[a]	2.15±0.27
Fe (μg g^{-1})	3529±135	3472±123
Zn (μg g^{-1})	23.0±6.3	22.8±7.1
Ca (μg g^{-1})	11233±307	11339±419
P (μg g^{-1})	53.8±6.9	52.5±7.8
S (%)	1.93±0.76	1.96±0.91

Table 2: Concentrations of mineral elements in forage

Elements	Affected area	Unaffected area
Cu (μg g^{-1})	17.9±1.3	17.3±2.7
Mo (μg g^{-1})	6.58±0.37[a]	3.23±0.48
Fe (μg g^{-1})	369±37	365±41
Zn (μg g^{-1})	6.1±2.2	6.2±2.1
Ca (μg g^{-1})	3166±247	3620±212
P (μg g^{-1})	1219±216	1189±221
S (%)	0.29±0.04	0.28±0.05

Table 3: Concentrations of mineral elements in the blood

Elements	Affected animals	Unaffected animals
Cu (μg mL^{-1})	0.68±0.27	0.71±0.21
Mo (μg mL^{-1})	0.89±0.17[a]	0.49±0.12
Fe (μg mL^{-1})	327±32	319±38
Zn (μg mL^{-1})	16.3±2.2	16.7±2.1
Ca (μg mL^{-1})	133.0±13.2	131.3±14.5
P (μg mL^{-1})	323.0±72.4	328.0±62.4
S (mmol L^{-1})	21.3±3.7[a]	42.6±7.3

Table 4: Concentrations of mineral elements in the hair

Elements	Affected animals	Unaffected animals
Cu (μg g^{-1})	4.68±1.52	4.75±1.83
Mo (μg g^{-1})	3.35±0.13[a]	1.37±0.11
Fe (μg g^{-1})	278±25	281±27
Zn (μg g^{-1})	127±14	129±13
Ca (μg g^{-1})	2119±47	1996±51
P (μg g^{-1})	138.9±15.7	135.7±15.9
S (%)	2.16±0.37[a]	3.98±0.57

Table 5: Concentrations of mineral elements in the liver

Elements	Affected animals	Unaffected animals
Cu (μg g^{-1})	98.6±13.1	113.6±11.2
Mo (μg g^{-1})	7.39±1.61[a]	5.29±0.62
Fe (μg g^{-1})	2376±79	2398±85
Zn (μg g^{-1})	417±48	129.9±13.8
Ca (μg g^{-1})	187±19	196±27
P (μg g^{-1})	871±87	869±37
S (%)	0.12±0.02[a]	0.21±0.03

[a]Results between the affected and unaffected animals were significantly different (p<0.01)

from affected Guizhou semi-fine wool sheep was significantly lower than that from healthy animal (p<0.01). The concentration of Mo from the affected Guizhou semi-fine wool sheep was significantly higher that of control animal. Other values were within normal ranges. The contents of mineral elements in the liver can be seen from Table 5. The concentrations of S were significantly lower in the affected animals than in the healthy Guizhou semi-fine wool sheep. Mo contents in affected animals were significantly higher than healthy animals. There were no significant differences in other elements between the affected and unaffected animals. The affected Guizhou semi-fine wool sheep were divided into two groups which were supplemented with $CaSO_4$ or control, respectively. Blood S was increased in all affected with $CaSO_4$. In all treated animals, some symptoms of recovery were evident in 20 days with appetite and vigor improved but untreated animals (20%) appeared to die of exhaustion (Table 6).

Table 6: Biochemical values in serum in Guizhou semi-fine wool sheep

Items	Affected animals	Unaffected animals
LDH (μmol L^{-1})	3.76±1.38	3.73±0.62
AST (IU L^{-1})	27.9±5.6[a]	36.7±5.2
γ-GT (IU L^{-1})	17.7±3.6	16.7±3.1
AKP (IU L^{-1})	255±15	261±13
Crt (μmol L^{-1})	309±34	311±38
Ca (μmol L^{-1})	2.13±0.29	2.21±0.27
IP (μmol L^{-1})	1.72±0.32	1.71±0.27
K (μmol L^{-1})	3.82±0.71	3.93±0.28
Na (μmol L^{-1})	128.8±23.8	127.7±21.9

[a]Results between the affected and unaffected animals were significantly different (p<0.01)

DISCUSSION

It is known that pica might be a natural response in animals to a mineral deficiency. The S level >0.14% (dry matter) in forage was safe for sheep (Guo, 2004; Shen and Jiang, 2013). The optimum dietary S was recommended to be 0.25% (Zhang *et al*., 2008; Shen *et al*., 2014). In present study, the contents of S in the forage were optimal by those standards in the affected areas. Mo levels from in forage from affected area were significantly higher than those of forage from the unaffected area. Forage Molybdenum (Mo) combines with S in the rumen to form a thiomolybdate complex, rendering S unavailable for absorption (Shen, 2011). As a result, the concentrations of S in blood from affected semi-fine wool sheep were significantly lower than those from unaffected animals but the Mo level in blood from affected animal was significantly higher than that from unaffected semi-fine wool sheep. In Guizhou semi-fine wool sheep, blood S values <43.67 mmol L^{-1} are a sign of severe S deficiency (Luo *et al*., 2001; Youde, 2002; Yang, 1998). Therefore, the results showed that the S status of Guizhou semi-fine wool sheep in the affected regions was severely deficient.

The S content of wool was also a sensitive indicator for diagnosing S deficiency since as previously reported in sheep, the values for wool S, blood S and liver S were positively correlated (Arthington *et al*., 2002; Youde, 2002; Kelly *et al*., 2012). The mean S concentration in wool of affected Guizhou semi-fine wool of 2.16±0.37% is well below 4.00±0.23% characteristic of S deficiency in wool (Bird, 1970; Youde, 2002). In addition, oral administration of calcium sulfate appeared to prevented S deficiency in Guizhou semi-fine wool sheep.

CONCLUSION

Therefore, it was reasonable to conclude that S deficiency in Guizhou semi-fine wool sheep was caused by high molybdenum in forages.

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