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Full Length Research Paper

Copper deficiency in Guizhou semi-fine wool sheep on pasture in south west China karst mountain area

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The Guizhou semi-fine sheep in the Weining County, Guizhou province, south west China karst mountain area were affected by an ailment characterized by pica, emaciation, dyskinesia, depressed appetites, unsteady gait and anemia. We found that concentrations of copper (Cu) in soil and forage from affected and unaffected areas were within the normal ranges, but concentrations of sulfur (S) and molybdenum (Mo) in soil and forage were significantly higher ($P<0.01$) in affected areas than in unaffected areas. Concentrations of Cu in blood, wool and liver from the affected Guizhou semi-fine sheep were significantly lower ($P<0.01$) than those in healthy animals. Affected Guizhou semi-fine sheep showed a hypochromic microcytic anemia and a low level of ceruloplasmin. Oral administration of $CuSO_4$ prevented and cured the disease. We conclude that the disorder of Guizhou semi-fine sheep is caused by secondary Cu deficiency, mainly due to high S and Mo content in forage.

Key words: Guizhou semi-fine sheep, copper, sulfur; molybdenum, South west China karst mountain area.

INTRODUCTION

The Guizhou semi-fine sheep is vital to the production system of the south west China karst mountain area. Animals provide meat, wool and hides for local people. Since the 2000s, Guizhou semi-fine sheep in Weining County, south west China karst mountain area have been affected by an ailment characterized by pica, emaciation, dyskinesia, unsteady gait, depressed appetites and anemia. The prevalence is estimated at 60 to 70% and the mortality may reach 50% (Bennetts and Chapman, 1937; Whitelaw, 1985). Affected area was an excellent autumn – winter range of native pasture for communal use until 2005, when the government allocated both the pasture and sheep to individual families for use in all 4 seasons, in an attempt to improve the local herdsmen's productivity. As a result, 20 families had 1859 sheep that were affected by copper deficiency, representing 65% of the total 2860 living the shoupu grassland in County south west China karst mountain area.

Forage molybdenum (Mo) is a commonly recognized contributor to copper (Cu) deficiencies in ruminants, but adequate dietary sulphur (S) is required in this antagonism. Mo combines with S to form a thiomolybdate complex. Thiomolybdates bind with Cu to form an insoluble complex, rendering Cu unavailable for absorption (Tiffany et al, 2002). The purpose of this study was to investigate the possibility that ailment is S and Mo-induced Cu deficiency and the effect of copper supplementation on the prevalence of ailment.

MATERIALS AND METHODS

Study area

The study area is located at 26°36' - 27°26' N latitude and 103°36' - 104°45' E longitude, where the provinces of Guizhou, Yunnan and Sichuan, meet an average elevation of 2200 m above sea level.

The annual precipitation is 960 mm. The average atmospheric temperature is only 10-12°C (Shen xiao yun, 2011). The grassland vegetation being mainly Puccinellia (*Chinam poensis ohuji*); Siberian Nitraria (*Nitraria sibirica pall*); Floriated astragalus (*Astragalus floridus*); Poly-branched astragals (*A. polycladus*); Falcate whin (*Oxytropis falcate*); Ewenki automomous banner

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(*Elymus nutans*); Common leymus (*Leymus secalinus*); June grass (*Koeleria cristata*) and the vegetation grows well in the pasture (Shen xiao yun 2011). Most of the plants are herbaceous and are good food resources for animals.

Animals

Twenty affected Guizhou semi-fine sheep, 2 to 3 years old, were selected for the study from 5 populations of 696 living in the *Suopu* grassland of the south west China karst mountain area. All showed signs of pica, emaciation, dyskinesia, depressed appetites and unsteady gait. Twenty healthy Guizhou semi-fine sheep also selected from *Liangshuiguo* pasture of the Weining County where the disease is not seen served as controls. The clinical signs were recorded by direct observation. Selective examinations were carried out on affected Guizhou semi-fine sheep by routine clinical diagnostic methods, including determination of pulse rate, body temperature and respiratory rate (Shi, 1990).

Sample collection

All samples were taken in July 2010. Blood samples for analysis of mineral contents and for hematological and 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). Wool samples from each affected Guizhou semi-fine sheep's neck was also sampled and washed as described by Salmela (1981). Samples of forage were sampled from 6 affected pastures. To reduce soil contamination, herbage samples were cut 1-2 cm above ground level. The forage samples were dried at 60-80℃ for 48 h and ground to facilitate chemical analysis. Soil samples from affected areas were taken from the surface layer (0-30 cm) of 6 pastures, using a 30 mm diameter cylindrical core. Four cores per paddock were bulked and placed in polythene bags. Also, within the same period, 20 samples of soil, 20 samples of forage, and wool and blood from 20 Guizhou semi-fine sheep were also collected from the *liangshuiguo* pasture of the Weining County where the disease is not seen.

Analysis of mineral contents

S and phosphorus (P) levels were determined by nephelometry (Wen et al, 1983). Cu, iron (Fe), cobalt (Co) and calcium (Ca) levels were determined using a PerkinElmer AAS5000 atomic absorption spectrophotometer (PerkinElmer, Norwalk, Connecticut, USA). Selenium (Se) level was examined using atomic fluorescence spectrometer (AFS3000, Beijing Kechuang Haiguang Instrument CO., LTD, China). Mo contents were measured using flameless atomic absorption spectrophotometer with extra steps to produce reliable data. In brief, after adding the stannous chloride liquor, we extracted samples with isopentanol as soon as possible because the generated complex was unstable. Fluorine (F) level was determined by the method described by (Wang et al., 1996). The accuracy of analytical values was checked by reference to the certified values of elements in the National Institute of Standards and Technology Standard Reference Material bovine liver SRM1577(7).

Hematological and biochemical examination

Hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), packed cell volume (PCV), red blood cell (RBC), and white blood cell (WBC) values were determined using an automated hematology analyzer (SF-3000, Sysmex-Toa Medical Electronics, Kobe, Japan). Biochemical analyses, including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), ceruloplasmin (Cp), lactate dehydrogenase (LDH), alkaline phosphatase (AKP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamy1 transferase (γ-GT), creatinine (Crt), urea nitrogen (BUN), cholesterol (Chol), sodium (Na), potassium (K), magnesium (Mg), calcium (Ca) and inorganic phosphorus (IP) were determined with an automated biochemical analyzer (Olympus AU 640, Olympus Optical Co., Tokyo, Japan). Quality control serum (Shanghai Biochemical CO., LTD, China) was used to validate the blood biochemistry data. All serum biochemical values were measured at room temperature.

Prevention and treatment

Twenty affected Guizhou semi-fine sheep, average live weight 21 ± 3.2 kg were treated orally with 5 g of copper sulphate ($CuSO_4$), repeated once a week later.

Statistical analysis

The differences were assessed by Student's *t* test. Experimental data were analyzed by using a statistical package (SPSS version for Windows; SPSS, Chicago, Illinois, USA). The data were presented as means ± standard deviation.

RESULTS

Animals affected by disease demonstrated characteristic pica, emaciation, dyskinesia and anemia. The more seriously affected Guizhou semi-fine sheep had difficulty in keeping up with the populations and appeared to die of exhaustion. There were no significant differences in the clinical parameters of temperature, pulse and respiration between affected and healthy animals. Affected sheep do not change its coat color. All treated Guizhou semi-fine sheep, some signs of recovery were evident after one or two treatments, and appetite and vigor improved.

Concentrations of the mineral element in the soil and forage samples are given in Table 1. The contents of Cu in the soil and forage from affected and unaffected regions were similar and within normal ranges. The S and Mo contents of the soil and forage samples from the affected areas were significantly higher ($P<0.01$) than those from the unaffected areas. Other mineral concentrations in the soil and forage samples were within the normal ranges in all areas. The concentrations of mineral elements in the blood and liver are shown in Table 2. The Cu contents in the blood and liver of affected sheep were significantly lower than those in healthy animals ($P<0.01$). The S and Mo contents of the liver and blood were significantly higher in the affected Guizhou semi-fine sheep as compared with those in the unaffected sheep. Fe contents of the liver and blood were within the normal ranges as compared with those in the unaffected

Table 1. Contents of mineral elements in soil and forage samples (n=20).

Element	Soil		Forage	
	Affected area	unaffected area	Affected area	unaffected area
S (%)	1.87±0.35**	0.92±0.33	0.43±0.27**	0.18±0.15
Cu (µg/g)	15.6±3.1	15.7±2.6	6.37±1.55	6.41±1.86
Mo (µg/g)	4.12±1.23**	1.96±0.57	4.93±1.12**	1.32±0.13
Se (µg/g)	0.126±0.031	0.132±0.029	0.083±0.027	0.087±0.031
Co (µg/g)	5.63±1.53	5.58±1.67	1.35±0.43	1.37±0.42
Ca (µg/g)	14373±581	14537±612	5217±278	5138±269
P (µg/g)	52±13	55±12	442±52	452±47
F (µg/g)	13.6±2.7	14.1±2.6	9.3±1.7	9.6±2.1
Fe (µg/g)	14793±597	14837±631	686±31	678±30

**P<0.01.

Table 2. Contents of mineral elements in blood and liver samples (n=20).

Element	Blood		Liver	
	Affected	unaffected	Affected	unaffected
S (%)	5.63±1.3**	4.21±0.63	2.53±0.29**	1.37±0.33
Cu (µg/g)	0.23±0.03**	0.95±0.23	21.6±3.9**	102.1±13.2
Mo (µg/g)	0.37±0.19**	0.16±0.07	3.79±1.63**	2.85±0.65
Co (µg/g)	0.59±0.31	0.61±0.29	0.59±0.36	0.60±0.21
F (µg/g)	14.1±3.7	13.9±3.8	4.28±0.37	4.17±0.32
Ca (µg/g)	139±35	131±36	165±23	171±26
P (µg/g)	239±37	247±35	831±56	829±57
Se (µg/g)	0.086±0.03	0.089±0.02	1.21±0.31	1.19±0.36
Fe (µg/g)	676±75	671±83	736±69	748±65

**P<0.01.

Table 3. Contents of mineral elements in wool samples of Guizhou semi-fine sheep (n=20).

Element	Affected animals	unaffected animals
S (%)	4.57±2.3**	3.61±1.2
Cu (µg/g)	4.67±0.73**	6.52±1.26
Mo (µg/g)	3.71±1.32**	2.57±0.73
Se (µg/g)	0.163±0.061	0.159±0.071
Co (µg/g)	0.93±0.12	0.99±0.23
F (µg/g)	116±35	118±25
Ca (µg/g)	2113±361	1997±326
P (µg/g)	135±37	131±33
Fe (µg/g)	769±73	773±68

**P<0.01.

animals. The concentrations of mineral element in the wool were given in Table 3. The contents of Cu in the hair of affected Guizhou semi-fine sheep were significantly lower than those of healthy sheep (P<0.01). The S and Mo contents of the wool samples were significantly higher in the affected Guizhou semi-fine sheep as compared

Table 4. Hematological values in Guizhou semi-fine sheep (n=20).

Blood indices	Affected animal	Unaffected animal
Hb (g/L)	89.7±12.1**	123.8±13.8
RBC (10^{12}/L)	12.1±3.6	11.2±1.5
PCV (%)	32.3±4.1**	37.1±3.7
MCV (fl)	26.7±3.9**	33.1±4.1
MCH (pg)	7.4±2.1**	11.0±3.2
MCHC (%)	27.8±4.6**	33.4±3.9
WBC (10^9/L)	9.2±2.7	9.3±2.9

**P<0.01.

Table 5. Biochemical values in Guizhou semi-fine sheep (n=20).

Items	Affected animal	unaffected animal
Cp (mg/dL)	3.9±1.2	6.8±2.4
SOD (µmols^{-1}/l)	12.7±3.9	19.5±4.5
CAT (µmols^{-1}/l)	25.3±5.6	24.1±5.9
GSH-Px (µmols^{-1}/l)	27.5±3.3	28.9±3.7
LDH (U/dL)	7.31±1.27	7.41±1.35
AST (IU/l)	37.7±5.9	38.5±5.6
ALT (IU/l)	15.7±3.7	15.9±3.9
γ-GT (IU/dL)	25.6±5.7	26.3±5.3
AKP (IU/l)	116.8±23.7	118.9±23.5
Crt (µmol/L)	125.7±28.7	127.8±26.8
Ca (mmol/L)	2.76±0.35	2.67±0.39
IP (mmol/L)	2.85±0.53	2.83±0.46
K (mmol/L)	3.69±0.59	3.73±0.63
Na (mmol/L)	136.9±21.7	137.1±25.3
Mg (mmol/L)	0.93±0.27	0.91±0.31
BUN (mmol/L)	6.53±1.62	6.47±1.59

**P<0.01.

with those in the healthy animals. Other mineral concentrations in blood, liver and wool samples were within the normal ranges.

The hematological values are given in Table 4. The average Hb, PCV, MCV, MCH and MCHC in affected Guizhou semi-fine sheep were significantly lower (P<0.01) than those of unaffected animals. The abnormal blood indices indicated a hypochromic microcytic anemia in affected sheep.

The biochemical values are given in Table 5. The activities of SOD in serum from affected Guizhou semi-fine sheep were significantly lower than those in the healthy animals (P<0.01). The contents of Cp in the affected Guizhou semi-fine sheep were also significantly lower than those in the unaffected animals. There were no significant differences in other biochemical value between affected Guizhou semi-fine sheep and the healthy animals.

DISCUSSION

Previous studies showed that Cu levels greater than 5 µg/g of dry matters in forages are safe for ruminants (Shen et al., 2006; Wang et al., 1996). In the present study, the contents of Cu in the forage from affected and unaffected regions were within normal ranges by those standards, but the S and Mo levels of the forage in affected areas were significantly higher (P<0.01) than those of the unaffected areas. The S requirement of grazing ruminants in forage is only 0.13% of dry matter (Wang et al., 1995; Suttle, 2010). In this study, the S content in forage was 0.43 ± 0.27%, which would be excessive for Guizhou semi-fine sheep. In general terms, soils containing up to 2 µg/g of Mo and forages containing less than 3 µg/g of Mo in dry matter are considered to be safe (Laven et al., 2008; Shen 2009). In this study, the contents of Mo in the soil and forage from regions were 4.12±1.23 and 4.93±1.12 µg/g, respectively. Elevating the levels of S and Mo in the diet of yak, cattle, camel and goat has been shown to lower Cu absorption (Tiffany et al., 2002; Liu, 2005; Shen et al, 2006), so likely the elevated S and Mo levels in forage in areas where the affected Guizhou semi-fine sheep had the same effect. Various authors have reported that feeds and pastures with higher S and Mo interfered with the absorption of Cu, resulting in Cu deficiency for cattle, camel, goat and yak (Tiffany et al., 2002; Liu et al., 1995; Shen et al., 2006).

The concentration of Cu was very low in the whole blood, but the S and Mo levels were significantly higher than normal. The content of Cu in blood depends on the amount Cu stored in the liver, low content of Cu in the blood indicating exhaustion of the liver reserves (Bennetts and Chapman, 1937; Whitelaw, 1985). In ruminants, average blood Cu values of < 0.5 µg/ml are a sign of severe Cu deficiency (Arthington et al., 2002; Gengelbanch et al., 1997). The concentration of Cu in blood of unaffected sheep is 0.95 ± 0.23 µg/g. In this study, the contents of Cu in the blood from affected Guizhou semi-fine sheep are only 0.23±0.03 µg/g. Liver Cu contents are the most reliable indicator of status in ruminant. In general, dry liver Cu concentrations below 75 µg/g are considered deficient for ruminant (Mcdowell, 1992). In this study, the contents of Cu in the liver from affected animals are only 21.6 ± 3.9 µg/g. Therefore, our results show that the Cu status of Guizhou semi-fine sheep from the affected regions was severely deficient. The Cu content of wool is also a sensitive indicator for diagnosing Cu deficiency, since, as previously reported in cattle and yak, the Cu values for liver and hair or blood are positively correlated (Liu et al., 1995). The mean Cu concentration in the wool of the affected Guizhou semi-fine sheep of 3.67 ± 0.73 µg/g, well below the 5.5 µg/g characteristic of Cu deficiency in ruminant (Shen et al., 2006).

Under normal conditions, most of the Cu in serum is presented as Cp, which plays an essential role in promoting the rate of iron saturation of transferrin, and so in the absorption and transport of Fe and in the utilization of Fe by the bone marrow. For this reason, Cu deficiency not only markedly reduces the content of Cp but is accompanied by anemia.

The activities of SOD is also a sensitive indicator for diagnosing Cu deficiency, since, as previously reported in cattle, camel and yaks, the activities of SOD in serum and the Cu values of the serum are positively correlated (Shen 2009). In this study, the activities of SOD in serum were significantly lower in affected Guizhou semi-fine sheep than those in the healthy animals (P<0.01). Thus, it is reasonable to conclude that ailments of animals in the Weining County, south west China karst mountain area are caused by a secondary Cu deficiency, mainly due to high S and Mo content in soil and forage.

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Abbreviations:

Hb, Hemoglobin; **MCH,** mean corpuscular hemoglobin; **MCHC,** mean corpuscular hemoglobin concentration; **MCV,** mean corpuscular volume; **PCV,** packed cell volume; **RBC,** red blood cell; **WBC,** white blood cell; **SOD,** superoxide dismutase; **GSH-Px,** glutathione peroxidase; **CAT,** catalase; **Cp,** ceruloplasmin; **LDH,** lactate dehydrogenase; **AKP,** alkaline phosphatase; **AST,** aspartate aminotransferase; **ALT,** alanine aminotransferase; **γ-GT,** γ-glutamy1 transferase; **Crt,** creatinine; **BUN,** urea nitrogen; **Chol,** cholesterol; **IP,** inorganic phosphorus.

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