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Studies of Anemia Ailment in Guizhou Black Goats in South West China Karst Mountain Area

[1,3]Shen Xiao Yun, [2,3]Jia Zhi-Hai and [4]Song De Rong
[1]Department of Biology, Chong Qing University of Science and Technology, 401331 Chongqing, China
[2]College of Animal Science and Technology, China Agricultural University, 100193 Beijing, China
[3]Pratacultural Ecology Institute, Bijie University, 551700 Bijie, P.R. China
[4]Bijie District Institute of Animal Husbandry and Veterinary, 551700 Bijie, China

Abstract: The mineral composition of soil and forages and samples of blood, hair and liver from Guizhou black goats in affected ranches were compared with those of samples from unaffected areas. Researchers have found that concentrations of Copper (Cu) in forage samples from affected areas and unaffected areas are similar and within the normal. The mean concentration of S in soil and forage were significantly higher ($p<0.01$) in affected than in unaffected areas. The Cu contents of blood, hair and liver from affected Guizhou black goats were significantly less than those in controls ($p<0.01$). Affected animals showed anemia and a low value of Ceruloplasmin (Cp). The content of S in blood, hair and liver from affected animals was significantly higher than that in unaffected animals. Eighteen Guizhou black goats grazing affected pastures and consuming an average of 32 mg of Cu day^{-1} for at least 50 days via a free-choice, salt-based trace mineral supplement. Average liver Cu concentration for 18 Guizhou black goats was 26.8±6.6 μg g^{-1}. Ten Guizhou black goats with the lowest content of liver Cu were transported to Bijie District Institute of Animal Husbandry and Veterinary and were assigned to one of two Cu repletion treatments consisting of supplements providing 35 mg day^{-1} of either inorganic (Cu sulfate; n = 5) or organic (Availa-Cu; n = 5) Cu. Treatments were delivered for 80 days. Liver Cu increased over time in all affected animals regardless of treatment however, black goats supplemented with Availa-Cu tended to have higher mean liver Cu concentrations than those receiving copper sulfate. In all treated animals, some signs of recovery were evident in 10-20 days after one or two treatments and appetite and vigor improved.

Key words: Guizhou black goat, sulfur, copper, nutrition, anemia ailment

INTRODUCTION

Since, the 2008, Guizhou black goats in the Bijie District have been affected by high S content in forage. Affected animals showed pica, emaciation, dyskinesia, deprived appetites, loss of coat color, diarrhea and anemia. The incidence was estimated at 20-30% and the mortality may reach 50%. The affected area is located at 26°32'-27°26'N latitude and 103°36'-104°45'E longitude at an average elevation of 2,200 m above sea level. The annual precipitation is 962 mm. The average atmospheric temperature is 10-12°C. The soil is acid (pH 6.5-6.7) and abundant in humus. The grassland vegetations are mainly Puccinellia (*Chinampoensis ohuji*), Siberian Nitraria (*Nitraria sibirica* Pall); floriated astragalus (*Astragalus floridus*); poly-branched astragals (*A. polycladus*), falcate whin (*Oxytropis falcate*), Ewenki automomous banner (*Elymus nutans*), common leymus (*Leymus secalinus*) and june grass (*Koeleria cristata*). It was an excellent Autumn to Winter range of native pasture for communal use until 2007 when the local government allocated both the pasture and the livestock to individual families for use in all 4 seasons in an attempt to improve the local herdsmen's productivity. As a result, ten villages have about 651 Guizhou black goats that were affected by forage of high S content making up 25% of the total of 2596 animals living the Yumaoshan pasture area in Heizhang County of Guizhou province, China.

Sulfur (S) forms ferric sulfide by soluble iron in the rumen and the Cu is adsorbed by insoluble ferric sulphide compounds (Shen *et al*., 2006) or S combines with Molybdenum (Mo) to form a thiomolybdate complex.

Corresponding Author: Shen Xiao Yun, Department of Biology, Chong Qing University of Science and Technology, 401331 Chongqing, China

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 caused by high S content in forage and the effect of copper supplementation on the prevalence of ailment.

MATERIALS AND METHODS

Experiment design (Experiment 1): Experiment began on 21 July, 2008. Eighteen affected Guizhou black goats were selected for the study. The animals utilized in these experiments were cared for by acceptable practices as outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching Consortium. About 18 blood samples from affected Guizhou black goats 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 Bijie District Institute of Animal Husbandry and veterinary for further preparation and analysis. About 18 liver biopsy from affected Guizhou black goats was also sampled by a trained technician using techniques described in this study (Arthington and Corah, 1995). Eighteen hair samples from affected Guizhou black goats was also sampled from each goat's neck and washed as described by Salmela *et al*. (1981). About 18 samples of forage from affected pasture were sampled. 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 (Wang *et al*., 1996). Eighteen soil samples from affected area were taken from the surface layer (0-30 cm) of each pasture using a 30 mm diameter cylindrical core. Four cores per paddock were bulked and placed in polythene bags. On 21 July, 2008 eighteen samples of soil, 18 samples of forage and hair, blood and liver from Guizhou black goats were also collected from a non-affected area to determine mineral content.

Experiment 2: Copper status was assessed by liver biopsy sampling of 18 Guizhou black goats grazing affected pastures. Guizhou black goats were consuming an average of 32 mg of Cu day^{-1} for at least 50 days via a free-choice, salt-based trace mineral supplement (Macro-mineral compositions of free-choice were 13.75, 13.01, 0.89, 1.11, 8.63 and 1.18% for Ca, P, K, Mg, Na and S, respectively. Micro-mineral compositions of free-choice were 57.9, 2455, 10105, 1335, 6.78 and 1150 μg g^{-1} for Co, Cu, Fe, Mn, Mo and Zn, respectively). Average liver Cu concentration for all 18 Guizhou black goats was 26.8±6.6 μg g^{-1}. Ten animals, 2-3 years old (21.8±5.2) with the lowest concentration of liver Cu were transported to Bijie District Institute of Animal Husbandry and Veterinary and were assigned to one of two repletion treatments. Treatments were delivered for 80 days to pens (6 m^2) housing two Guizhou black goats. All animals were allocated to receive 25 mg day^{-1} of supplemental Cu from either inorganic (Cu Sulfate; n = 5) or organic (Availa-Cu, Zinpro Corp., Eden Prairie, MN; n = 5) sources. Copper treatments were formulated into a corn meal (Nutrient composition was 19.1±1.3, 85.5±7.8, 0.26±0.03, 0.76±0.16 and 5.32±0.51 μg g^{-1} for crude protein, Total digestible nutrients, S, Mo and Cu, respectively). Liver tissue and jugular blood were collected on 0, 10, 20, 30, 40, 50, 60, 70 and 80 days.

Analysis of mineral contents: Copper (Cu), Cobalt (Co), Calcium (Ca) levels were determined, using a Perkin-Elmer AAS 5000 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, Connecticut, USA). Selenium (Se) was assayed using the modified fluorometric procedure of Whetter and Ullrey (1978). S levels in samples were determined by nephelometry (Wen *et al*., 1983). Molybdenum (Mo) was determined for all treatments using flameless atomic absorption spectrophotometry (Perkin-Elmer 3030 graphite furnace with a Zeeman background correction). Mo concentrations in tissue are difficult to determine accurately and extra steps are necessary to produce reliable data. When the graphite furnace is used for the determination of Mo concentration memory or carryover effects can occur after a sample is run. This happens when the intense heat of the graphite furnace allows the carbon in the graphite tube to combine with Mo in the sample to form Mo-carbides (Perkin-Elmer). Special steps were taken to eradicate this effect. After each sample was run, two blanks (deionized water) were run to help reduce the effects of memory. P was determined by spectrophotometry (Wang *et al*., 1996). Fluorine (F) was determined by an ashing, distilling and fluorine reagent colorimetric method (Wang *et al*., 1995). Assay variation was controlled using the bovine liver standards of National Institute of Standards and Technology (Gaithersburg, Maryland, USA).

Haematological and biochemical examination: Hemoglobin (Hb), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Packed Cell Volume (PCV) and Red Blood Cell (RBC) and White Blood Cell (WBC) values were determined by routine methods. Biochemical analyses which included Superoxide

Dismurase (SOD), Glutathione Peroxidase (GSH-Px), Catalase (CAT) Ceruloplasmin (Cp), Lactate Dehydrogenase (LDH), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), γ-Glutamyl 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 automatic biochemical analyzer (Olympus AU 640, Olympus Optical Co., Tokyo, Japan). All serum biochemical values were measured at room temperature.

Statistical analyses: The data are presented as means±standard deviation. 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).

RESULTS AND DISCUSSION

Experiment 1: The concentrations of the mineral element in the soil and forage samples are shown in Table 1. Content of Cu in the soil and forage from affected and unaffected regions were similar and within normal ranges. Sulfur contents of the soils and forages samples from the affected areas were significantly higher ($p<0.01$) than those in 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 content in the blood and liver of affected goats was significantly lower than that in normal animals ($p<0.01$). The S content of the liver and blood was significantly higher in the affected Guizhou black goats as compared with that in unaffected animals. The Mo and Fe content of the liver and blood were within the normal ranges as compared with that in the non-affected animals. The concentrations of mineral element in the hair were shown in Table 3. The copper contents in the hair of affected goats were significantly lower than those of control animals ($p<0.01$). The S content of the hair samples was significantly higher in the affected Guizhou black goats as compared with that in unaffected animals. The hematological values are shown in Table 4. The average Hb concentration, PCV, MCV and MCH in affected Guizhou black goats were significantly lower ($p<0.01$) than those of unaffected animals. The abnormal blood indices indicated a hypochromic microcytic anemia in affected Guizhou black goats.

The serum biochemical values are shown in Table 5. The activities of SOD and LDH in serum were significantly lower in the affected goats than those in unaffected animals ($p<0.01$). The contents of serum ceruloplasmin in the affected goats were also significantly lower than

Table 1: Contents of mineral elements in soil and forage sample

	Soil		Forage	
Elements	Affected area	Unaffected area	Affected area	Unaffected area
S (%)	1.89±0.56*	0.97±0.27	0.47±0.13*	0.18±0.07
Cu (ppm)	16.3±2.1	16.9±3.6	6.89±1.23	6.92±1.86
Mo (ppm)	1.53±0.23	1.56±0.22	1.21±0.15	1.18±0.13
Se (ppm)	0.083±0.013	0.086±0.015	0.087±0.021	0.084±0.019
Co (ppm)	7.63±1.22	7.27±1.63	2.39±0.45	2.37±0.21
Ca (ppm)	14178±889	14397±746	6813±746	6528±725
P (ppm)	63±11	61±12	478±81	472±51
F (ppm)	13.6±2.7	14.9±2.6	10.7±1.76	9.8±1.1

Table 2: Contents of mineral elements in blood and liver samples

	Blood		Liver	
Elements	Affected	Unaffected	Affected	Unaffected
S (%)	6.51±1.3*	4.23±0.66	1.83±0.39*	1.37±0.37
Cu (ppm)	0.23±0.03*	0.95±0.16	13.6±3.1*	105.6±11.2
Mo (ppm)	0.18±0.10	0.19±0.09	2.79±0.61	2.87±0.72
Co (ppm)	0.56±0.39	0.67±0.12	0.71±0.36	0.65±0.21
F (ppm)	15.6±3.1	15.9±2.8	4.21±0.31	4.13±0.12
Ca (ppm)	137±31	135±27	167±21	166±23
P (ppm)	278±37	276±29	831±37	829±39
Se (ppm)	0.093±0.04	0.095±0.03	1.27±0.78	1.23±0.77

Table 3: Contents of mineral elements in hair samples

Elements	Affected animal	Unaffected animal
S (%)	6.37±2.9*	4.67±0.77
Cu (ppm)	3.87±0.82*	6.59±1.56
Mo (ppm)	2.39±0.72	2.42±0.75
Se (ppm)	0.132±0.056	0.136±0.067
Co (ppm)	1.25±0.57	1.19±0.53
F (ppm)	72±17	75±15
Ca (ppm)	2057±171	2079±156
P (ppm)	112±23	117±25

Table 4: Hematological values in Guizhou black goats

Blood indices	Affected animals	Unaffected animals
Hb (g L^{-1})	89.30±9.30*	123.0±12.8
RBC (10^{12} L^{-1})	11.80±3.10	11.9±2.50
PCV (%)	29.60±4.26*	39.7±3.70
MCV (fl)	25.10±4.70*	33.4±5.70
MCH (pg)	7.57±2.30*	10.3±2.90
MCHC (%)	30.20±4.20	31.0±3.70
WBC (10^9 L^{-1})	8.50±2.30	8.7±2.90

Table 5: Biochemical values in Guizhou black goats

Items	Affected goats	Unaffected goats
Cp (mg/100 mL)	15.5±2.700*	23.20±3.40
SOD (μmol sec^{-1} L^{-1})	14.3±1.900*	18.50±2.30
CAT(μmol sec^{-1} L^{-1})	24.3±2.600	25.10±2.90
GSH-Px (μmol sec^{-1} L^{-1})	28.5±2.100	27.90±3.10
LDH (μmol sec^{-1} L^{-1})	3.57±1.21*	5.41±1.36
AST (IU l^{-1})	39.7±5.600	39.50±4.90
ALT (IU l^{-1})	13.7±2.700	13.90±2.90
γ-GT (IU l^{-1})	21.6±3.100	19.30±3.70
Crt (μmol L^{-1})	365.7±28.70	367.80±26.8
Ca (mmol L^{-1})	2.56±0.39	2.47±0.35
IP (mmol L^{-1})	2.65±0.21	2.57±0.31
K (mmol L^{-1})	3.97±0.76	3.89±0.83
Na (mmol L^{-1})	146.9±29.70	147.10±27.6
Mg (mmol L^{-1})	0.97±0.28	0.95±0.31
BUN (mmol L^{-1})	6.23±1.32	6.27±1.37

*Results between affected and unaffected area were significantly different ($p<0.01$)

those in unaffected animals. There were no significant differences in other biochemical values between the affected goats and unaffected animals.

Experiment 2: Copper status was assessed by liver biopsy sampling of 18 Guizhou black goats grazing affected pastures. All animals were consuming an average of 32 mg of Cu day^{-1} for at least 50 days via a free-choice, salt-based trace mineral supplement (Macro-mineral compositions of free-choice were 13.75, 13.01, 0.89, 1.11, 8.63 and1.18% for Ca, P, K, Mg, Na and S, respectively. Micro-mineral compositions of free-choice were 57.9, 2455, 10105, 1335, 6.78 and1150 ppm, for Co, Cu, Fe, Mn, Mo and Zn, respectively). Average liver Cu concentration for all 18 Guizhou black goats was 26.8±6.6 ppm. Ten Guizhou black goats with the lowest concentration of liver Cu were transported to Bijie District Institute of Animal Husbandry and Veterinary and assigned to both treatments had initial liver Cu concentrations (13.9±3.2 and 13.6±3.7 ppm, liver Cu for Cu sulfate and Availa-Cu treatments, respectively). Liver Cu increased over time ($p<0.01$) in all goats regardless of treatment (Fig. 1). However, Guizhou black goats supplemented with Availa-Cu tended to have higher mean liver Cu concentrations than ($p<0.01$) those receiving Cu sulfate (138.8±75.3 vs 100.1±53.1 ppm, respectively). About >80 days of supplementation the rate of liver Cu repletion was 1.57 vs. 1.08 ppm $days^{-1}$ for Availa Cu and Cu sulfate-supplemented Guizhou black goats, respectively. No treatment differences were detected in plasma ceruloplasmin concentrations (25.7±2.3 and 26.9±2.7 mg/100 mL for Cu sulfate and Availa-Cu, respectively). In all treated Guizhou black goats with Cu sulfate and Availa-Cu, some signs of recovery were evident in 15-25 days after and appetite and vigor improved. The Cu content in the blood reached normal values (0.87±0.21 ppm) within 30 days.

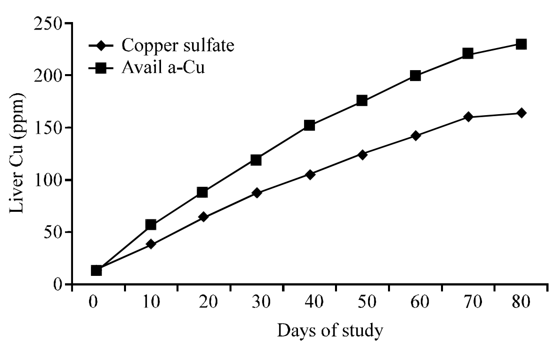

Fig. 1: The relationship between liver Cu concentration in Guizhou black goats and supplementation of copper

Chronic exposure to high S intakes in the diet or drinking-water can have adverse indirect effects on ruminants particularly by inducing copper deficiency with or without the assistance of molybdenum (Suttle, 2010). Previous studies showed that Cu contents in soil and forage >6 and 5 ppm (dry matter) are safe for ruminants (Li and He, 1990). In this study, the content of Cu in the soil and forage from affected and unaffected regions were similar and within normal ranges by those standards but the sulfur content of the soil and forage in affected areas was significantly higher ($p<0.01$) than those of the unaffected areas. The S requirement of grazing ruminants in forage is only 0.13% (dry matter) (Suttle, 1974). In this study, the S content in forage was 0.47±0.13% which would be excessive for Guizhou black goats. Elevating the levels of S in the diet of cattle and sheep has been shown to lower Cu absorption so, likely the elevated S levels in soil and forage in areas where the affected Guizhou black goats grazed had the same effect. Various researchers have reported that feeds and pastures with higher S interfered with the absorption of Cu, resulting in secondary Cu deficiency for ruminants.

The concentration of Cu was very low in the whole blood but the S level was 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. In cattle, blood Cu values of <0.5 $\mu g\ mL^{-1}$ are a sign of severe Cu deficiency. The normal concentration of Cu in blood in Guizhou black goats is 0.86 $\mu g\ mL^{-1}$ (Tian *et al*., 2011). Liver Cu contents are the most reliable indicator of status in ruminant. In general, dry liver Cu concentrations <75 ppm is considered deficient for ruminant (McDowell, 1992). Therefore, the results showed that the copper status of goats from the affected regions was severely deficient. The Cu content of hair is also, a sensitive indicator for diagnosing Cu deficiency since as previously reported in cattle, the Cu values for liver and hair or blood are positively correlated (Wang, 1988; Wahbi *et al*., 1984). The mean Cu concentration in the hair of the affected Guizhou black goats of 3.81±0.87 ppm, well <5.5 ppm characteristic of Cu deficiency in ruminant.

Under normal conditions, most of the Cu in serum is presented as Ceruloplasmin (Cp) which plays an essential role in promoting the rate of iron saturation of transferrin and so in the absorption and transport of iron and in the utilization of iron by the bone marrow. For this reason, Cu deficiency not only markedly reduces the content of Cp but is accompanied by anemia (Gengelbach *et al*., 1997). In affected Guizhou black goats, the average value of Cp in serum was15.5±2.7 mg/100 mL. The mean values of Hb concentration, PCV, MCV and MCH were significantly lower than the normal animals. Most research has suggested that the activities of SOD are more sensitive to Cu deficiency than to other parameters. In this study, the activities of SOD in serum were significantly lower in the affected goats than those in the healthy animals ($p<0.01$). Accordingly, it was concluded that the disease is mainly caused by high S content in forage.

The absence of Cu intake information in Experiment 1 is a shortcoming of the present study when relating Guizhou black goat Cu status to the pastures containing high concentrations of S. All Guizhou black goats were offered an equal opportunity to consume free-choice mineral. Researchers are not aware of any previous data suggesting that high S forages may limit free-choice mineral intake. However, researchers cannot preclude the potential influence of variable free-choice mineral intake on subsequent liver Cu concentrations reported in this study.

To provide further insight, a group of Guizhou black goats grazing affected pastures were assessed. Despite the consumption of 32 mg of Cu day^{-1}, these Guizhou black goats found to have low liver Cu concentrations (26.8±6.6 ppm). Once removed from the pastures containing high concentrations of S, Cu-deficient Guizhou black goats were able to rapidly respond to Cu supplementation from both from Cu sulfate and Availa-Cu. The mean Cu content in the liver of Guizhou black goats receiving Availa-Cu was significantly higher than ($p<0.01$) that in supplemented goats with Cu sulfate. Thus, it is reasonable to conclude that the anemia ailment of Guizhou black goats in the Bijie area is caused high S in forage.

CONCLUSION

Researchers conclude that the anemia disorder of Guizhou black goats was caused by high S content in forage.

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