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Effect of Nitrogenous Fertilizer Fertilization on Mineral Metabolism in Grazing Guizhou Black Goats

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Abstract: To assess the impact of N fertilization on contents of mineral elements in herbage and the effect of increased forage S on the copper metabolism of grazing Guizhou black goats, study was conducted during the summer grazing season (2010, 2011 and 2012). Pasture replicates (30 ha; n = 3/treatment) received the same fertilizer treatment in each growing season, consisting of: 100 kg N/ha from quickly available nitrogen, 100 kg N/ha from ammonium nitrate, 100 kg N/ha from ammonium sulfate and control (no fertilizer). Forage sampling was collected at 60 days intervals following fertilization (10 samples per pasture) for Cu, Mo, Mn, Se, Fe, Zn, Ca and P. To determine the effect of fertilizer treatment on mineral metabolism in grazing guizhou black goats, liver and blood samples were collected at the start and end of the study period in 2010, 2011 and 2012. Ammonium sulfate fertilization increased ($p<0.01$) forage S concentration. Plant tissue N concentrations were increased by N fertilization, regardless of source in 2010, 2011 and 2012. Guizhou black goats grazing S fertilization pastures had lower ($p<0.05$) liver and blood Cu concentrations at the end of the study period in 2010, 2011 and 2012, compared with urea, ammonium nitrate and control. Nominal increases in forage *in vitro* organic matter digestibility were realized by fertilization, regardless of N source in each year.

Key words: Guizhou black goats, nitrogen fertilizer, meadow, forage, mineral element metabolism

INTRODUCTION

The study area is located in a region adjoined by the provinces of Guizhou, Yunnan and Sichuan (26°56'-27°47'N, 103°56'-104°51'E). In the region, the average elevation is 2100 m above the sea level, the annual precipitation is 956 mm and the average atmospheric temperature is 9-11°C. The main grassland species include 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 (*Elymus nutans*), Common leymus (*Leymus secalinus*) and June grass (*Koeleria cristata*). Most of the plants are herbaceous and good resources for grazing animals. Thirty percents of the pasture are saltmarsh and swamp meadows. Soils in the area are saline and alkaline. Since, 2000s, pasture is fertilized with large amounts of nitrogenous fertilizer in an attempt to improve productivity of grassland. The use of N fertilization would be of benefit if they increased forage yields without causing mineral metabolism disorder. Tiffany *et al.* (2002) reported on a 3 years study investigating the effect of municipal sewage sludge containing high sulfate in bahiagrass at a site in South-central Florida. In their study, the application of municipal sewage sludge contains high sulfate resulted in higher forage S concentration (0.23 and 0.30% for applications of 86 and 174 kg of S/ha, respectively) compared with bahiagrass fertilized with ammonium nitrate (0.10% S).

Studies of Guizhou black goats reared in confinement suggest that they may be more susceptible to copper (Cu) deficiency than other species of ruminants (Shen and Zhang, 2012). There are two types of Cu deficiency-induced or direct. The former occurs when contents of Mo, S and (or) Fe are very rich in forage. The latter occurs when the Cu content in forage is lower than normal. Impaired immune competence is the probably result of Cu metabolism disorder in Guizhou black goats (Shen and Du, 2006). Cu deficiency has a direct impact on the ability of cattle to mount a normal response to viral infection (Tiffany *et al.*, 2002). This alteration in immune competence may result in failure to respond to vaccination along with increased energy losses during disease exposure.

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MATERIALS AND METHODS

Pastures and fertilizer treatment: This study was conducted over 3 years (2010, 2011 and 2012). Pastures replicates (20 ha, n = 3/treatments) received the same fertilizer treatment in each growing season. The treatments consisted of, 100 kg N/ha from Urea (U), 100 kg N/harom Ammonium Nitrate (AN), 100 kg N/ha from Ammonium Sulfate (AS), Control (C). Date of fertilizer application varied each year as dictated by precipitation (May 10, May 3 and May 21 for 2010, 2011 and 2012, respectively). Sampling of forage was conducted at 60 days interval and analyzed for *In Vitro* Organic Matter Digestibility (IVOMD), S, Fe, Mn, Zn and Cu. To reduce soil contamination, the herbage sample were cut 1-2 cm above ground level in randomly distributed 1m^2 grazing exclusion cages. Additionally, a 1.08 m^2 area outside of but adjacent to each cage was sampled for an estimation of forage availability. The forage samples were dried at 60-80°C for 48 h. Forage N and IVOMD analysis were conducted at the animal nutrition laboratory for N analysis; samples were digested using a modification of the aluminum block digestion procedure of Tiffany *et al.* (2002). For IVOMD analysis, a modification of the two stages technique was used as described previously (Tiffany *et al*., 2002). Soil samples were taken from the surface layer (0-30 cm) of each pasture at the start and end of the study period using a 30 mm diameter cylindrical corer. Four cores per paddock were bulked and placed in polythene bags. The soil samples were dried out at 60-80°C for 48 h and passed through a 10 mm sieve. Rumen fluid was obtained from guizhou black goats consuming hay and provided 450 g of soybean meal 1 h prior to collection.

Animal: Eighty growing Guizhou black goats weighing 21±1.2 kg were randomly allotted to fertilized pasture (twenty per part) where they grazed for 180 days. The grazing experiment began on May 10, May 3 and May 21 for 2010, 2011 and 2012, respectively. The effect of fertilizer treatment on yak liver Cu concentrations was evaluated. The animals utilized in these studies 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. Jugular blood was collected in heparinized, trace mineral-free Vacutainer tubes at the start and end of the study period in 2010, 2011 and 2012. Blood was kept cool at the collection site and subsequently transported to the animal nutrition laboratory for further preparation and analysis. Liver biopsy was performed by a trained technician using techniques described previously at the start and end of the study period in 2010, 2011 and 2012 (Wang *et al*., 1996). Random liver tissue samples were collected, handled and analyzed for elements concentrations. Hair was sampled from each yak's neck and washed as described by Wang *et al.* (1996).

Analysis of mineral contents: Zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) were determined by atomic absorption spectrophotometry. The accuracy of the analytical values was checked by reference to certified values of elements in the National Bureau of Standards (NBS), Standard Reference Material bovine liver SRM 1577a.

Hematological and biochemical examination: Hemoglobin (Hb), Red Blood Cell (RBC) and White Blood Cell (WBC) values were determined by routine methods. Biochemical analyses included Glutathione Peroxidase (GSH-Px), Superoxide Dismutase (SOD), Ceruloplasmin (Cp), Lactate Dehydrogenase (LDH) and Creatinine (Crt) were determined using test kits provided by the Nanjing Jiancheng Bio-Engineering Institute. r-Glutamyl-Transferase (r-GGT) and Cholesterol (Chol) in serum were determined using test kits provided by Matsuda Planning and Industrial Co., Ltd.

Statistical analysis: 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

Forage and soil elements: The contents of elements in forage are similar and within the normal range in AS, AN, U and C at the start of the study period in 2010, 2011 and 2012 (Table 1). Compared with unfertilized pasture, plant tissue N was higher ($p<0.05$) in the fertilized pastures at the end of the study period in 2010, 2011 and 2012 (Table 2). Nominal increases in forage IVOMD were realized by fertilization regardless of N source in each year (Table 2). Forage Mn was increased ($p<0.05$) in ammonium sulfate fertilized pastures compared with other fertilized pastures and control at the end of the study period (Table 3). Similarly, forage Zn concentration was significantly greater ($p<0.01$) in fertilized pastures compared with control (Table 3). Plant tissue S was significantly greater ($p<0.01$) in pastures fertilized with ammonium sulfate compared with other pastures at the end of the study period in the 2010, 2011 and 2012 (Table 2). Fertilizer treatment did not affect other forage mineral concentrations analyzed for this study. The contents of elements in soil are given in Table 1. Soil Mn

Table 1: Contents of mineral element in soil at the start and end of the study period

Elements	Time	AS	AN	U	C
S (%)	Start	1.37±0.26	1.29±0.31	1.27±0.36	1.30±0.29
	End	1.49±0.31	1.26±0.45	1.23±0.32	1.28±0.37
Cu (μg g^{-1})	Start	5.3±2.2	5.5±2.6	5.0±2.7	5.3±2.8
	End	5.4±1.9	5.2±2.1	5.3±2.3	5.4±2.6
Mn (μg g^{-1})	Start	1.57±0.36^a	1.53±0.53^a	1.49±0.28^a	1.53±0.39
	End	1.27±0.35^a	1.32±0.61^a	1.23±0.52^a	1.54±0.56
Fe (μg g^{-1})	Start	7765±356	7697±371	7793±397	7812±378
	End	7675±297	7753±365	7732±382	7697±452
Zn (μg g^{-1})	Start	2.71±0.89	2.72±0.76	2.57±0.76	2.68±0.69
	End	2.71±0.82	2.76±0.67	2.55±0.56	2.69±0.76

^{a}p<0.05

Table 2: Effect of N fertilizer source on mean nutritive value of herbage

Items	Time	AS	AN	U	C
IVOMD	2010	58.20±5.20	58.30±4.80	58.70±5.30	47.50±4.60
	2011	57.80±5.30	58.60±6.20	59.30±5.90	47.30±6.20
	2012	59.80±6.30	57.90±5.60	57.20±4.90	47.80±5.80
Sulfur	2010	0.49±0.12^b	0.23±0.03	0.21±0.01	0.23±0.06
	2011	0.52±0.21^b	0.21±0.05	0.23±0.06	0.21±0.03
	2012	0.57±0.16^b	0.22±0.02	0.23±0.03	0.24±0.04
Nitrogen	2010	1.87±0.32^a	1.89±0.29^a	1.91±0.19^a	1.21±0.27
	2011	1.89±0.25^a	1.99±0.52^a	1.97±0.45^a	1.34±0.36
	2012	1.89±0.43^a	2.01±0.27^a	1.99±0.63^a	1.31±0.42

^{a}p<0.05; ^{b}p<0.01

Table 3: Effect of N fertilizer source mean mineral concentration of forage

Elements	Time	AS	AN	U	C
Cu (μg g^{-1})	2010	5.92±1.25^b	6.93±1.38	6.95±1.58	6.92±2.17
	2011	5.73±1.52^b	6.96±1.75	6.98±1.37	6.95±1.92
	2012	5.56±1.83^b	6.93±1.26	6.99±1.62	6.97±2.21
Mn (μg g^{-1})	2010	68.7±5.7^a	51.7±6.3	51.5±5.8	52.6±6.7
	2011	67.2±6.2^a	51.9±6.9	51.6±7.5	51.7±7.2
	2012	69.2±5.9^a	52.9±5.7	52.9±6.4	51.5±5.6
Fe (μg g^{-1})	2010	681±35	669±56	667±46	648±43
	2011	679±36	682±46	686±53	656±52
	2012	682±47	676±38	668±51	666±48
Zn (μg g^{-1})	2010	93.8±8.7^a	93.9±7.6^a	92.9±7.8^a	73.9±9.8
	2011	95.6±9.3^a	95.2±8.5^a	96.9±7.2^a	72.8±8.2
	2012	99.8±7.9^a	97.9±7.9^a	99.2±6.9^a	74.8±7.9

^{a}p<0.05; ^{b}p<0.01

concentrations were greater ($p<0.05$) in control compared with fertilized pastures in the end of experiment period. Soil pH was not affected by fertilizer treatment (average soil pH = 7.2). Other soil mineral contents evaluated in this study were not affected by fertilizer treatment.

Mineral metabolism status: A random collection of 20 liver samples at the start of study period revealed an initial liver Cu content of 71.6±8.1 μg g^{-1}. These results suggest that the Guizhou black goats had marginal liver Cu stores when they initially entered the study (Liu *et al*., 1994). Contents of other elements analyzed in blood, hair and liver are similar and within the normal range in AS, AN, U and C at the start of the study period in 2010, 2011 and 2012. The Cu contents in blood, hair and liver of guizhou black goats grazing pasture fertilized with ammonium sulfate were significantly lower than ($p<0.01$) those of animals grazing ammonium nitrate and urea fertilized pastures and control at the end of the grazing season in 2010, 2011 and 2012 (Table 4- 6). The

Table 4: Blood trace mineral concentrations at the end of the study period

Elements	Time	AS	AN	U	C
Cu (μg g^{-1})	2010	0.23±0.07^b	0.69±0.06	0.68±0.03	0.65±0.09
	2011	0.21±0.05^b	0.66±0.07	0.67±0.08	0.68±0.07
	2012	0.22±0.08^b	0.65±0.04	0.66±0.05	0.67±0.05
Mn (μg g^{-1})	2010	0.45±0.12^a	0.36±0.13^a	0.36±0.08^a	0.26±0.12
	2011	0.41±0.09^a	0.38±0.08^a	0.37±0.07^a	0.25±0.09
	2012	0.43±0.13^a	0.39±0.12^a	0.35±0.12^a	0.25±0.11
Fe (μg g^{-1})	2010	423±45	429±51	436±35	426±51
	2011	435±37	441±46	429±42	431±45
	2012	431±36	431±53	419±37	433±39
Zn (μg g^{-1})	2010	0.36±0.12	0.35±0.11	0.32±0.07	0.21±0.13
	2011	0.32±0.09	0.33±0.08	0.35±0.13	0.23±0.15
	2012	0.35±0.11	0.36±0.09	0.34±0.08	0.25±0.17

^{a}p<0.05; ^{b}p<0.01

Table 5: Liver trace mineral concentrations of Grazing guizhou black goats at the end of the study period

Elements	Time	AS	AN	U	C
Cu (μg g^{-1})	2010	38±11^b	83±12	82±15	87±21
	2011	36±16^b	82±17	85±13	88±19
	2012	33±13^b	89±19	87±12	91±22
Mn (μg g^{-1})	2010	6.7±1.2	5.9±1.3	6.3±1.3	6.1±1.5
	2011	6.8±1.6	5.6±1.1	6.1±1.2	6.3±1.4
	2012	6.6±1.3	5.3±1.0	5.8±0.9	6.2±1.6
Fe (μg g^{-1})	2010	332±35	388±45	371±41	387±39
	2011	352±42	378±47	367±39	368±44
	2012	377±37	365±36	389±37	357±45
Zn (μg g^{-1})	2010	139±23	131±27	133±18	106±31
	2011	133±31	129±23	139±21	107±28
	2012	135±29	136±31	133±27	105±24

^{b}p<0.01

Table 6: Hair trace mineral concentrations of Grazing guizhou black goats at the end of the study period

Elements	Time	AS	AN	U	C
Cu (μg g^{-1})	2010	3.78±0.71^b	5.76±0.69	5.77±0.81	6.13±0.97
	2011	3.36±0.75^b	5.78±0.73	5.71±0.69	6.15±0.89
	2012	3.48±0.68^b	5.95±0.82	5.97±0.72	6.49±0.79
Mn (μg g^{-1})	2010	38.5±7.6	38.2±8.2	38.8±6.9	32.6±8.8
	2011	39.2±8.9	39.9±7.9	39.8±8.1	32.2±9.1
	2012	39.7±6.8	39.1±8.1	39.1±7.5	31.9±7.6
Fe (μg g^{-1})	2010	538±23	557±26	539±36	546±34
	2011	557±31	553±28	551±29	556±27
	2012	539±19	547±31	538±32	565±41
Zn (μg g^{-1})	2010	153±17.6^a	158±11.9^a	1256±15.8^a	153±18.5
	2011	155±15.7^a	157±12.7^a	157±17.2^a	152±16.8
	2012	151±16.6	153±13.6	154±15.8	155±15.9

^{a}p<0.05; ^{b}p<0.01

contents of Zn and Mn in blood and hair of Guizhou black goats grazing in fertilized pastures were significantly higher ($p<0.01$) than those of control at the end of the grazing season in 2010, 2011 and 2012 (Table 4 and 6). The Zn contents in liver of control were significantly lower than ($p<0.01$) those of animals grazing fertilized pasture at the end of the grazing season in 2010, 2011 and 2012. Guizhou black goats assigned to both treatments (Cu sulfate and Availa-Cu) had severe Cu deficiency. Liver and blood Cu increased over time ($p<0.01$) in all Guizhou black goats regardless of treatment. However, Guizhou black goats supplemented with organic Cu tended to have higher mean liver Cu values than those receiving inorganic Cu.

Table 7: Hematological values in Guizhou black goats at the end of the study period

Items	AS	AN	U	C
Hb (g L^{-1})	97.1±21.1^b	118.10±6.3	118.20±6.30	117.90±7.10
RBC (10^{12} L^{-1})	9.8±0.30^b	8.35±2.1	8.46±0.21	8.59±0.26
WBC (10^9 L^{-1})	8.7±2.40^a	9.70±0.5	9.60±0.60	9.70±0.70

^{a}p<0.05; ^{b}p<0.01

Table 8: Serum biochemical values in kuizhou black goats at the end of the study period

Items	AS	AN	U	C
SOD (NU mL^{-1})	77.1±12.1^b	116.1±16.7	118.2±17.1	102.1±16.7
GSH-PX (U mL^{-1})	41.2±11.2^b	66.7±11.2	67.8±12.1	68.2±7.1
LDH (U dL^{-1})	176±72	179±81	177±76	177±72
r-GGT (mg dL^{-1})	13.6±4.2	13.7±3.2	13.9±3.9	13.8±4.3
Chol (mg dL^{-1})	120±13^b	118±12^b	121±16^b	89.2±7.2
Crt (μmol L^{-1})	173±7.7^b	178±8.9^b	176±11^b	119.6±11.2
CP (BU dL^{-1})	2.1±0.71^b	3.96±0.71	4.27±0.7^6	4.67±0.86

^{b}p<0.01

Hematological and biochemical value: The hematological values are similar and within the normal range in AS, AN, U and C at the start of the study period in 2010, 2011 and 2012. The hematological values for affected Guizhou black goats by fertilization treatment are given in Table 7 and compared with the normal values reported previously. The average Hb concentration from Guizhou black goats grazing pasture fertilized with ammonium sulfate were significantly reduced. The abnormal blood indices indicated a hypochromic microcytic anemia in Guizhou black goats. The biochemical values were similar and within the normal range in AS, AN, U and C at the start of the study period in 2010, 2011 and 2012. The corresponding biochemical values are given in Table 8. All the values were within the normal range except for the elevated serum cholesterol and the decreased Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-Px) and Ceruloplasmin (Cp) in serum of animals grazing pasture fertilized with ammonium sulfate.

DISCUSSION

Forage quality: Forage N concentration was higher in fertilized than in unfertilized pastures in 2010, 2011 and 2012, regardless of N source. Ammonium sulfate fertilization resulted in substantially greater ($p<0.01$) forage S concentrations compared with unfertilized pastures or pastures fertilized with ammonium nitrate and urea. Tiffany *et al.* (2002) made similar conclusions for results obtained from municipal sewage sludge containing high sulfate-fertilized small grains whereas ammonium sulfate application significantly increased forage S concentration without improving the yield. Nominal increases in forage IVOMD were realized by fertilization regardless of N source in each year. Slight improvements in the digestibility of municipal sewage sludge containing high sulfate-fertilized herbage have been reported previously (Tiffany *et al.*, 2002). This improvement in digestibility may be related to increased forage S which may have a complementary impact on the ruminant microbial environment.

Animal mineral status: Cu concentrations of blood from guizhou black goats grazing ammonium sulfate fertilized pasture were significantly lower than ($p<0.01$) those of animals grazing other fertilized pastures and control at the end of study period. Decreases in circulating blood Cu are associated with liver Cu contents of approximately 40 μg g^{-1} and lower (Liu *et al.*, 2010; Shen, 2009). Once these concentrations are achieved, animals are considered severely deficient because availability of Cu to peripheral tissue sites is compromised. Liver Cu concentrations are the most reliable indicator of Cu status in cattle. Bovine livers generally contain 100-300 μg g^{-1} of Cu (Wang *et al.*, 1996; Shen, 2009) and concentrations below 75 μg g^{-1} may indicate onset of deficiency. Concentrations below 25 μg g^{-1} indicate severe deficiency (Auza *et al.*, 1999).

Suttle and Shen reported that initially deficient blood and live Cu concentrations declined at calving in pregnant heifers receiving sulfated water (0.35% S in diet). They concluded that S intake had a negative effect on the Cu status of beef cows and that 10 mg Cu/kg of diet was not enough to improve the deficient status of these cattle. The low liver Cu stores of guizhou black goats may reflect low forage Cu or the high forage S concentrations (Shen *et al.*, 2006). In the treated pasture with ammonium sulfate; S interferes with Cu metabolism. Forage Mo of treated pastures (Tiffany *et al.*, 2002; Shen and Zhang, 2012) was very low (<0.6 μg g^{-1}) and there is little evidence to suggest that Mo at these concentrations would have more effect on Cu status. Suttle reported a 56% reduction in the increase in blood Cu following Cu sulfate supplementation of Cu deficient sheep fed with a diet containing 0.4 vs. 0.1% S. Liu (2010) and Shen *et al.* (2010) suggests that the maximum limit for potential S toxicity in ruminants is 0.4% and S toxicity in ruminants has been reviewed previously. Even though this threshold was exceeded in guizhou black goats grazing ammonium sulfate pastures, no signs of S toxicity were noted in this study. Excessive dietary S is often associated with a neurological disease in feedlot cattle called polioencephalomalacia (Shen and Du, 2006). Even though cattle grazing AS pastures consumed large amounts of S, the only indicator of S excess was their failure to respond to Cu supplementation.

Hematological and biochemical values: Normal Hb concentrations for most mammals are 13-15 g/day/L (Hawkey *et al*., 1983; Youde, 2002). Lower concentrations may be linked to Cu deficiency (Shen, 2011). However, low Hb concentrations do not always occur when Cu status is low. Hemoglobin was significantly low for most guizhou black goats. Under normal conditions, most of the Cu in serum is present as ceruloplasmin (Cp) which plays an essential role in promoting the rate of iron saturation of transferrin and so in the utilization of iron by the bone marrow. For this reason, Cu deficiency not only markedly reduces the content of Cp but also causes anemia which varies between and with in species. In rats, lambs, rabbits and pigs, the anemia is hypochromic and normocytic. In cattle and adult sheep, the anemia is hypochromic and macrocytic. In affected Guizhou black goats, researchers concluded that the anemia was hypochromic and micocytic.

CONCLUSION

The objectives of this study were to determine the effect of agronomic level of N fertilization on mineral element content of herbage and mineral metabolism in guizhou black goats.

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