Journal of Animal and Veterinary Advances 13 (8): 519-523, 2014
ISSN: 1680-5593

Effect of High Content of Sulfur in Forage on Serum Biochemical Values in Grazing Oula Sheep

[1,4]Shen Xiao Yun, [2]Xiong Kang Ning, [3]Wang Xiao Li, [2]Chen Yong Bi and [2]Zhang Meng
[1]Chongqing University of Science and Technology, 401331 Chongqing, China
[2]Guizhou Normal University, 550001 Guiyang, China
[3]Guizhou Institute of Prataculture, 550006 Guiyang, China
[4]Office of Poverty Alleviation, 550004 Guiyang, Guizhou Province, China

Abstract: The Oula sheep was affected by high level of sulfur in forage in the Huangheshouqu Pasture in Gansu Province in the South of the Qinghai-Tibetan Plateau of China. The related blood indices were measured. The mineral composition of forages, blood and liver in Oula sheep from ranches of high sulfur were compared with those of samples from normal areas. The concentration of copper in soil and forage was 16.8±7.1 and 6.39±1.26 μg g^{-1} (dry matter), respectively compared with 16.7±5.6 and 7.12±2.86 μg g^{-1}. The mean concentration of Cu in blood and liver from the affected Oula sheep was 0.28±0.05 and 13.9±3.7 μg g^{-1}, respectively compared with 0.86±0.16 and 107.6±11.2 μg g^{-1} for non-affected Oula sheep. The concentration of sulfur in soil and forage was 1.97±0.26 and 1.36±0.17%, respectively compared with 10.8±0.31 and 0.57±0.16% (dry matter) for normal animals. The content of S in blood and liver was 6.37±1.7 and 1.58±3.6%, respectively compared with 4.12±0.86 and 1.32±0.35% (in fresh and soft tissues), respectively. The 12 Cu-deficient sheep were obtained from the same location allocated to one of two treatments, consisting of supplement providing 42 mg day^{-1} of Cu sulfate or tribasic copper chloride. Treatment were delivered for 90 days. Liver Cu increased over time in all sheep regardless of treatment, however, sheep supplemented with tribasic copper chloride tended ($p<0.01$) to have higher mean liver Cu concentrations than those receiving Cu sulfate.

Key words: Oula sheep, sulfur, forage, biochemical values, blood

INTRODUCTION

The Oula sheep is one of the most important populations of Tibetan sheep in the Qinghai-Tibet Plateau and is also vital to the production system of the Huangheshouqu Grass land. Animals provide meat, wool and hides for local people. Since the 2000s, the local government allocated the pastures to each family for use in all four seasons in an attempt to improve the local herdsmen's nomadic life and productivity. All the animals have to graze on the same pasture and water throughout the year. As a result, Oula sheep in the Huangheshouqu pasture have been affected by high sulfur forage characterized by pica, emaciation, dyskinesia and anemia. The incidence was estimated at 10-15% and the mortality reached 50%. Forage Mo is a commonly recognized contributor to Cu deficiencies in ruminants but adequate dietary S is required in this antagonism. Molybdenum combines with S to form a thiomolybdate complex. Thiomolybdates bind with Cu to form an insoluble complex, rendering Cu unavailable for absorption (Suttle, 1991). The purpose of this study reported here was to determine the cause and pathogenesis of ailment.

MATERIALS AND METHODS

Study area: The Huangheshouqu Grass land is located at 33°12'-34°8'N latitude and 99°11'-101°2'E longitude where the three Chinese Provinces of Qinghai, Gansu and Sichuan meet and is important pasture land for Oula sheep which is at an average elevation of 3300 m earlier sea level 1. The annual precipitation is 760 mm. The average atmospheric temperature is only 1.1°C and there are no completely frost-free seasons. The 30% of the pasture is swamp meadows, the grassland vegetation being mainly *Polygonum viviparum* L., *Anemone rivularis* var. flore-minore Maxim, *Potentilla bifurca* L., *Potentilla fragarioides* L., *Potentilla anserina* L., *Astragalus ploycladus* Bur et Franch, *Elymus nutans* Griseb, *Festuca sinensis* Keng, *Festuca rubra* L., *Koeleria cristata* L. Pers, *Festuca ovina* L., *Poa annua* L., *Poa pratensis* L., *Stipa aliena* Keng, *Kobresia bellardii* (All.) Degl, *Helictotrichon tibeticum* (Roshev.) Holub, grows well in the pasture.

Ethics statement: The 60 sheep selected for this study came from six ranches in the Huangheshouqu Pasture of

Corresponding Author: Xiong Kang Ning, Guizhou Normal University, 550001 Guiyang, China

China and were all showing signs of pica, emaciation, dyskinesia and anemia. Table 1 shows the results of clinical examination on these animals. The animals used in these experiments were cared as per outlined by FASS (2010).

Sample collection: All samples were taken in July 2010. Blood samples for analysis of mineral contents and for hematological and biochemical examination were obtained from the jugular vein using trace mineral-free Vacutainer tubes. Blood samples were kept cool at the collection site. Live biopsy was also sampled by a trained technician using techniques previously described (Arthington and Corah, 1995). Hair was sampled from each camel's neck and washed as described by Salmela *et al.* (1981). Multiple small portions of forage were sampled from each pasture and mixed. To reduce soil contamination, the herbage samples were cut 1-2 cm earlier ground level. The forage samples were dried at 60-80°C for 48 h and ground to facilitate chemical analysis (Wang *et al.*, 1996). Soil samples were taken from the surface layer (0-30 cm) of each pasture 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.

Analysis of mineral contents: Sulfur (S) was determined by nephelometry (Wen *et al.*, 1983). Copper (Cu), iron (Fe), Cobalt (Co) and Calcium (Ca) were measured using a Perkin-Elmer AAS5000 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, Connecticut, USA). Molybdenum (Mo) content was determined using the flameless atomic absorption spectrophotometry (Perkin-Elmer 3030 graphite furnace with a Zeeman background correction). Phosphorus (P) was determined by spectrophotometry and fluorine by the method described by Wang *et al.* (1996). Also to ensure the overall reliability of the analytical methods certified National Bureau of Standards (NBS) Bovine Liver SRM1577.

Hematological 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 using an automated hematology analyzer (SF-3000, Sysmex-Toa Medical Electronics, Kobe, Japan). The serum content of Ceruloplasmin (Cp), Lactate Dehydrogenase (LDH), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (AKP), γ-glutamyl transferase (γ-GT), Creatinine (Crt), Cholesterol (Chol), Blood Urea Nitrogen (BUN), Sodium (Na), Potassium (K), Magnesium (Mg), Calcium (Ca), Inorganic Phosphorus (IP) were determined on an automatic analyser (SF-1, Shanghai Medical Apparatus and Instruments Factory, Shanghai, China) using commercial test kits (Nanjing Medicine University Biochemical Co., Nanjing, China). Quality control serum (Shanghai Biochemical Co., Shanghai) was used to validate the blood biochemistry data.

Statistical analyses: The data are presented as means±standard deviation. The differences were assessed by Student's t-test. Experiment data were analyzed by SPSS Version.

Table 1: The results of clinical examination of 60 Oula sheep

Characteristics	Male	Non-pregnant	Pregnant
Pica	20	20	20
Emaciation	20	20	20
Dyskinesia	0	3	4
Fracture	0	1	0
Respiratory rate (breaths/min)	16.7±4.1	18.5±5.1	19.6±7.2

RESULTS

The concentrations of the mineral element in the soil and forage samples are given in Table 2. The sulfur contents of soils and forages from the affected areas were significantly different ($p<0.01$) from those of unaffected samples. The phosphorus contents of the forages from both the affected and the normal areas were below the reference values reported by Wang *et al.* (1996). Other values were within the normal ranges.

The concentrations of mineral elements in the blood and liver samples are given in Table 3. The copper contents in the blood and liver of affected camels were significantly lower than those of control animals ($p<0.01$). The sulfur contents of the liver and blood were also significantly raised in the affected as compared to the normal animals. The Mo and Fe contents of the liver and blood were within the normal ranges as compared with non-affected animals.

Table 2: The contents of mineral elements in soil and forage sample

	Soil		Forage	
Elements	Affected area	Unaffected area	Affected area	Unaffected area
S (%)	1.97±0.26	1.08±0.31	1.36±0.17	0.57±0.16
Cu (μg g^{-1})	16.8±7.1	16.7±5.6	6.39±1.26	7.12±2.86
Mo (μg g^{-1})	1.43±0.31	1.46±0.29	1.21±0.13	1.12±0.12
Fe (μg g^{-1})	8676±971	8762±861	461±78	469±82
Se (μg g^{-1})	0.08±0.051	0.086±0.026	0.088±0.026	0.098±0.016
Co (μg g^{-1})	6.63±1.22	5.28±1.63	1.30±0.45	1.33±0.21
Ca (μg g^{-1})	16178±889	14397±746	6813±746	5528±725
P (μg g^{-1})	58±11	63±12	432±81	452±51
F (μg g^{-1})	23.6±8.7	18.9±4.6	10.9±1.76	9.6±2.1

Table 3: The contents of mineral elements in blood and liver samples

	Animals			
	Blood		Liver	
Elements	Affected	Unaffected	Affected	Unaffected
S (%)	6.37±1.7	4.12±0.86	1.58±0.36	1.32±0.35
Cu (μg g^{-1})	0.27±0.03	0.86±0.16	13.6±3.7	107.6±11.2
Mo (μg g^{-1})	0.18±0.10	0.19±0.09	2.79±0.61	2.87±0.72
Fe (μg g^{-1})	456±61	427±76	131±17	146±18
Co (μg g^{-1})	0.56±0.39	0.67±0.12	0.71±0.36	0.65±0.21
F (μg g^{-1})	17.6±3.1	13.9±2.8	6.21±0.31	4.13±0.12
Ca (μg g^{-1})	129±31	131±26	187±19	196±27
P (μg g^{-1})	279±33	286±26	871±87	869±37
Se (μg g^{-1})	0.096±0.04	0.097±0.03	1.26±0.91	1.29±0.86

Table 4: The contents of mineral elements in hair samples

	Animals	
Elements	Affected	Unaffected
S (%)	5.87±2.3	4.67±7.21
Cu (μg g^{-1})	3.37±0.71	6.52±1.26
Fe (μg g^{-1})	559±97	532±72
Mo (μg g^{-1})	2.61±1.72	2.32±0.81
Se (μg g^{-1})	0.182±0.076	0.186±0.061
Co (μg g^{-1})	1.15±0.62	0.99±0.23
F (μg g^{-1})	116±35	98±25
Ca (μg g^{-1})	2127±661	1989±326
P (μg g^{-1})	112±31	129±23

Table 5: Hematological values in Oula sheep

	Animal	
Items	Affected	Unaffected
Hb (g L^{-1})	96.7±20.1[b]	127.0±9.8
RBC (10^{12} L^{-1})	12.8±3.60	11.7±1.5
PCV (%)	31.6±4.16[b]	39.6±3.1
MCV (fl)	24.7±5.10[b]	33.9±5.1
MCH (pg)	7.5±2.10[c]	11.0±1.2
MCHC (%)	30.6±4.60	32.4±3.2
WBC (10^9/L)	6.9±2.30[b]	12.9±2.9

[b]p<0.01; [c]p<0.05

Table 6: Serum biochemical values in Oula sheep

	Animal	
Items	Affected	Normal
Cp (mg L^{-1})	37.3±8.9	51.5±11.5
LDH (μmol L^{-1})	3.71±0.37	3.61±0.51
AKP (IU L^{-1})	236±37	258±42
AST (IU L^{-1})	31.7±6.9	31.8±6.8
ALT (IU L^{-1})	12.7±3.7	13.7±3.9
γ-GT (IU L^{-1})	15.3±3.7	15.2±3.5
SOD (μmol L^{-1})	16.3±1.9	23.5±2.1
CAT (μmol L^{-1})	25.3±2.6	25.2±2.9
GSH-Px (μmol L^{-1})	29.5±2.1	29.9±3.1
BUN (mmol L^{-1})	6.68±2.31	6.57±2.26
Crt (μmol L^{-1})	273±27	276±27
Chol (mmol L^{-1})	2.55±0.39	2.58±0.37
K (mmol L^{-1})	3.53±0.37	3.42±0.47
Na (mmol L^{-1})	119±32	127±38
Ca (mmol L^{-1})	2.27±0.27	2.25±0.21
IP (mmol L^{-1})	1.53±0.31	1.61±0.39
Mg (mmol L^{-1})	0.81±0.17	0.83±0.27

The concentrations of mineral element in the hair were given in Table 4. The copper contents in the hair of affected camels were significantly lower than those of control animals ($p<0.01$). The sulfur content of hair was also significantly raised in the affected as compared to the normal animals.

The hematological values given in Table 5. Hb, PCV, MCV and MCH were significantly reduced. The abnormal blood indices indicated a hypochromic microcytic anemia. Biochemical values given in Table 6. Activities of Cp and SOD were significantly reduced.

DISCUSSION

The copper contents in soil and forage >6 and 5 μg g^{-1} (dry matter) are safe for ruminants (Li and He, 1990). In the study, the copper levels in soil and forage were 16.8±7.1 and 6.39±1.26 μg g^{-1}, respectively higher than this safe level but sulfur contents were significantly higher than unaffected areas (Table 2). Suttle (1974) reported a 56% reduction in the increase in plasma Cu following Cu sulfate supplementation of Cu-deficient sheep fed a diet containing 0.40 vs. 0.1% S. A review of the literature (NRC, 1996) suggests that the maximum limit for potential S toxicity in cattle is 0.40% and S toxicity in ruminants has been reviewed previously (Kandylis, 1984). Even though this threshold was exceeded in Oula sheep grazing high S pastures in the study, no signs of S toxicity were noted.

In this study, the mean copper contents in the liver only was 13.6±3.1 μg g^{-1} were significantly lower than unaffected animal. The mean contents of copper in the blood only were 0.28±0.05 μg g^{-1}, markedly lower than normal animals. The normal concentration of copper in blood in Oula sheep is 0.86 μg g^{-1}. Decreases in circulating plasma Cu are associated with liver Cu concentrations of approximately 40 μg g^{-1} and lower (Claypool *et al*., 1975). The concentration of copper in blood depends on the amount of copper stored in the liver (Li and He, 1990) low concentrations of copper in the blood indicating exhaustion of the liver reserves. Liver Cu contents are the most reliable indicator of status in ruminant in general, dry liver Cu concentrations below 25-75 μg g^{-1} are considered deficient for ruminant (McDowell, 1992). Therefore, the results showed that the copper status of sheep in the affected regions was severely deficient.

The copper content of hair is also a sensitive indicator for diagnosing copper deficiency since as previously reported in cattle, the values for liver copper and hair copper or blood copper are positively correlated (Wang, 1988; Wahbi *et al*., 1984). In addition, once removed from the pastures containing high concentrations of S, Cu-deficient Oula sheep were able to rapidly respond to Cu supplementation from both

inorganic ($CuSO_4$) and tribasic copper chloride source. Oula sheep receiving tribasic copper chloride tended to have higher mean liver-Cu concentrations than animals supplement with Cu sulfate. Thus, it is probable that the disease of Oula sheep in the Huangheshouqu Pasture is a secondary copper deficiency caused by the high sulfur in the soils and forage. Anaemia can be a feature of copper inadequacy in all species but usually develops only where deficiency has been severe or prolonged (Baumgartner *et al*., 1978). Normal Hb concentrations for most mammals are 13-15 g dL^{-1} (Swenson and Reece, 1993). Lower concentration may be linked to Cu deficiency (Sanders and Sanders, 1983). However, low Hb concentrations donot always occur when Cu status is low (Tiffany *et al*., 2002). In current study, the mean Hb concentration, PCV, MCV and MCH were significantly reduced. 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 (Williams *et al*., 1974). For this reason, Cu deficiency not only markedly reduces the content of Cp but cause anemia which varies between and within species. In rats, lambs, rabbits and pigs, the anemia is hypochromic and microcytic as is found in iron deficiency but in chickens and dogs, it is normochromic and normocytic. In cattle, yak and adult sheep, the anemia is hypochromic and macrocytic (Brewer, 1987; Suttle, 2010).

CONCLUSION

In affected Oula sheep, researchers concluded that the anemia was hypochromic and microcytic. Activities of SOD is a sensitive indicator for diagnosing Cu deficiency, since as previously reported in cattle, the activities of SOD in serum and the Cu values of the liver or blood are positively correlated (Shen *et al*., 2006; Wang, 1988). In this study, the activities of SOD in serum were significantly lower in the affected camels than those in the healthy animals.

ACKNOWLEDGEMENTS

This research was financially supported by the West light foundation of Chinese Academy of Science (2010-2), the cooperation projects of Chinese Academy of Science (2010-4) and the China Agriculture Research System (CARS-40-30).

REFERENCES

Arthington, J.D. and L.R. Corah, 1995. Liver biopsy procedures for determining the trace mineral status in beef cows: Part II (Video, AI 8134). Extension TV, Deptarment of Communications, Cooperative Extension Service, Kansas State Univeristy, Manhattan, KS., USA.

Baumgartner, S., D.J. Brown, E. Salevsky and R.M. Leach, 1978. Copper deficiency in the laying hen. J. Nutr., 108: 804-811.

Brewer, N.R., 1987. Comparative metabolism of copper. J. Am. Vet. Med. Assoc., 190: 654-658.

Claypool, D.W., F.W. Adams, H.W. Pendell, N.A. Hartman Jr. and J.F. Bone, 1975. Relationship between the level of copper in the blood plasma and liver of cattle. J. Anim. Sci., 41: 911-914.

FASS, 2010. Guide for the Care and use of Agricultural Animals in Research and Teaching. 3rd Edn., FASS, Champaign, Illinois, pp: 169.

Kandylis, K., 1984. Toxicology of sulfur in ruminants: Review. J. Dairy Sci., 67: 2179-2187.

Li, G.H. and P.X. He, 1990. Diseases Caused by Trace Elements in Animals. Anhui Science and Technology Press, Hefei, pp: 100-206.

McDowell, L.R., 1992. Copper and Molybdenum. In: Minerals in Animal and Human Nutrition, Cunha, T.J. (Ed.). Academic Press, San Diego, CA., pp: 175-204.

NRC, 1996. Nutrient Requirements of Beef Cattle. 7th Edn., National Academy Press, Washington, DC., USA.

Salmela, S., E. Vuori and J.O. Kilpio, 1981. The effect of washing procedures on trace element content of human hair. Anal. Chim. Acta, 125: 131-137.

Sanders, D.E. and J.A. Sanders, 1983. Diagnosis and management of copper deficiency in dairy cattle. Mod. Vet. Pract., 64: 63-65.

Shen, X.Y., G.Z. Du and H. Li, 2006. Studies of a naturally occurring molybdenum- induced copper deficiency in the yak. Vet. J., 171: 352-357.

Suttle, N.F., 1974. Effects of organic and inorganic sulphur on the availability of dietary copper to sheep. Br. J. Nut., 32: 559-568.

Suttle, N.F., 1991. The interactions between copper, molybdenum and sulphur in ruminant nutrition. Ann. Rev. Nutr., 19: 121-140.

Suttle, N.F., 2010. Mineral Nutrition of Livestock. 4th Edn., CABI Publishing, London, ISBN: 9781845934729, Pages: 587.

Swenson, M.J. and W.O. Reece, 1993. Physiological Properties and Cellular and Chemical Constituents of Blood. In: Dukes' Physiology of Domestic Animals, Swenson, M.J. and W.O. Reece (Eds.). 11th Edn., Cornell University Press, Ithaca and London, pp: 22-46.

Tiffany, M.E., L.R. Mcdowell, G.A. O'Connor, F.G. Martin, N.S. Wilkinson, S.S. Percival and P.A. Rabiansky, 2002. Effects of residual and reapplied biosolids on performance and mineral status of grazing beef steers. J. Anim. Sci., 80: 260-269.

Wahbi, A.G.A., S.A. Abdelgadir, N.A. Awadelseid and O.F. Idris, 1984. The Plasma Electrolytes and Minerals of Normal Camels in the Sudan. In: The Camelid, an All-Purpose Animal. Scandinavian Institute of African Studies, Cockrill, R. (Ed.). Uppsala University, Sweden, pp: 431-437.

Wang, K., R.H. Tang, H.B. Xu and X.M. Luo, 1996. Trace Elements in Life Science. Metrology Press, Beijing, China, pp: 138-289.

Wang, Z.Y., 1988. Diagnosis and surveillance of copper deficiency induced with molybdenum. Chin. J. Vet. Med., 14: 5-8.

Wen, F.W., S.D. Zhang, H.W. Zhang, T.A. Lu and D.R. Jiang, 1983. The study on total sulphur estimating method in wool, feeds and blood. J. Gansu Agric. Univ., 4: 29-37.

Williams, D.M., G.R. Lee and G.E. Cartwright, 1974. Ferroxidase activity of rat ceruloplasmin. Am. J. Physiol., 227: 1094-1097.