Journal of Animal and Veterinary Advances 13 (8): 514-518, 2014
ISSN: 1680-5593

Studies of Sulfur-Induced Copper Deficiency in the Bactrian Camel

Wang Xiao Li and Wu Jia Hai
Guizhou Province Institute of Prataculture, Guizhou University,
550006 Guiyang, Guizhou Province, China

Abstract: Bactrian camels in Haizi, Gansu China are affected by an ailment characterized by pica, emaciation, dyskinesia, deprived appetites and anemia. Researchers found that concentrations of Cu in soil and forage from affected areas and unaffected areas were similar and within the normal ranges but the concentrations of S in soil and forage were significantly higher ($p<0.01$) in affected than in unaffected areas. Concentrations of Cu in blood, hair and liver from the affected camels were significantly lower ($p<0.01$) than those in unaffected camels. Fifty affected Bactrian camels grazed on affected pastures consumed an average of 136 mg of Cu/day for 60 days by a free-choice, salt-based trace mineral supplement. Average liver Cu concentration was 37.9±5.5 μg g^{-1} at the end of the study. On the 62nd day, ten Bactrian camels were removed from the affected pastures and allocated to one of two treatments for 80 days, consisting of supplement providing 125 mg day^{-1} of either Cu sulfate or Availa-Cu. Liver Cu increased over time in all camels regardless of treatment, however, camels supplemented with Availa-Cu have higher mean liver Cu contents than those receiving Cu sulfate (163.6±13.5 and 228.9±26.7 μg g^{-1} for Cu sulfate and Availa-Cu, respectively) at the end of the study. Mean Cu content in the liver of Bactrian camels received Availa-Cu was significantly higher than that in supplemented camels with Cu sulfate. Thus, it is reasonable to conclude that ailments of Bactrian camels in the Haizi area are caused by a secondary Cu deficiency mainly due to high sulfur content in soil and forage.

Key words: Bactrian camels, deficiency, emaciation, dyskinesia, anemia

INTRODUCTION

Bactrian camel (*Camelus bactrianus*) is an important livestock to the production system of the Chinese desert and semi-desert areas. The camels not only provide meat, wool and hides for local farmers and herdsmen but also an indispensable means of transport in arid areas. Since the 1990s, Bactrian camels in the Haizi area, Gansu Province, China have been affected by an ailment characterized by pica, emaciation, dyskinesia, unsteady gait, deprived appetites and anemia. The incidence is estimated at 10-15% and the mortality may reach 50%. The affect area is located at 38.6°-39.3°N latitude and 93.6°-94.5°E longitude where the three provinces of Xinjiang, Qinhai and Gansu meet and is important pasture land for Bactrian camels. It is situated in the plateau valley between the mountains Arerjin and Saishiteng at an average elevation of 3200 m above sea level. The annual precipitation is 190 mm and the annual evaporative amount is 1900 mm. The average atmospheric temperature is only 3.8°C and the frost-free season is only 80 days long. The 30% of the pasture is swamp meadows, the soil is acid (pH 6.1-6.5) and abundant in humus. It is an excellent Autumn-Winter range of native pasture for communal use until 1999 when the government allocated both the pasture and the livestock to individual families for use in an attempt to improve the local herdsmen's nomadic life and productivity. As a result, ten villages have 899 Bactrian camels that were affected by ailment, making up 58% of the total of 1550 camels living the Haizi valley pasture area.

There are 2 types of Cu deficiency-induced or direct. The former occurs when sulphide is trapped as ferric sulfide by soluble iron (Fe) in the rumen and the Cu is adsorbed by insoluble ferric sulphide compounds (Shen *et al*., 2006) or S combines with molybdenum to form a thiomolybdate complex. Thiomolybdates bind with Cu to form an insoluble complex, rendering Cu unavailable for absorption (Tiffany *et al*., 2002; Arthington *et al*., 2002). The latter occurs when the Cu content in forage is lower than normal. Ruminants with Cu deficiency have lower percentages of lymphocytes than normal animals and tend toward decreased cytokine response to disease challenge (Gengelbach *et al*., 1997). The purpose of this study was to investigate the possibility that ailment is S-induced Cu deficiency and the effect of copper supplementation on the prevalence of ailment.

Corresponding Author: Wang Xiao Li, Guizhou Province Institute of Prataculture, Guizhou University, 550006 Guiyang, Guizhou Province, China

MATERIALS AND METHODS

Experiment 1

Sample collection: Experiment began on 21 July 2003. Fifteen affected Bactrian camels and five healthy Bactrian camels were selected for the study. Blood samples for analysis of mineral contents and for haematological 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 Maqu Biology Institute 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 camel's neck was also sampled and washed as described by Salmela *et al.* (1981). Multiple samples of forage (Puccinellia-*Chinampoensis ohuji*; Siberian Nitraria-*Nitraria sibirica pall* and Lovely Achnatherum-*Achnatherum splendens* (Trin) Neuski) were sampled from affected and unaffected areas and mixed. 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). Soil samples from affected and unaffected 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.

Analysis of mineral contents: Sulfur (S) and Phosphorus (P) levels were determined by nephelometry. Copper (Cu), iron (Fe), Cobalt (Co) and Calcium (Ca) levels were determined by atomic absorption spectrophotometry. The Selenium (Se) level was examined by hydride-generation atomic absorption spectrophotometry. The Molybdenum (Mo) level was determined for all treatments by using flameless atomic absorption spectrophotometry. The Fluorine (F) level was determined by the method described by Wang *et al.* (1996). 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).

Haematological and biochemical examination: Haemoglobin (Hb), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Haemoglobin 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 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), Inorganic Phosphorus (IP), Total Protein (TP), Albumin (Alb) and Globulin (Glob) were determined on an automatic analyser using commercial test kits (Nanjing Jiancheng Bio-Engineer Institute). Quality control serum (Shanghai Biochemical Co.) was used to validate the blood biochemistry data. Serum protein electrophoretic studies were performed on cellulose acetate (Shi, 1990). All serum biochemical values were measured at room temperature.

Experiment 2: The grazing experiment began on July 23, 2003. The affected pasture was provided a single mineral box providing free-choice access to a balanced salt-based trace mineral supplement containing 0.25% Cu from Cu sulfate. Fifty affected Bactrian camels (the mean content of Cu in liver was 13.6±3.1 $\mu g\ g^{-1}$) grazing affected pastures were consuming an average of 136 mg of Cu/day for 60 days by 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 $\mu g\ g^{-1}$ for Co, Cu, Fe, Mn, Mo and Zn, respectively). On the 62nd day, ten Bactrian camels with the lowest concentration of liver Cu were chosen to be transported to the Maqu Biology Institute. Following calving, Bactrian camels were assigned to one of two repletion treatments. Treatments were delivered for 80 days to pens (136 m^2), each pen housed two Bactrian camels. Two, 15 days enrollment periods were utilized to account for calving dates over a 30 days period. Within each period and after calving, Bactrian camels were allocated to receive 125 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 $\mu g\ g^{-1}$ for crude protein, total digestible nutrients, S, Mo and Cu, respectively) and were offered three times weekly (3.29 kg $camel^{-1}$ daily) in conjunction with free-choice access to Splendid achnatherum (*Achnatherum splendens*) hay (Nutrient composition was 11.3±1.5, 51.7±5.7, 0.31±0.05%, 0.65±0.14 and 8.67±0.67 $\mu g\ g^{-1}$ for Crude protein, total digestible nutrients, S, Mo and Cu, respectively). Liver tissue and jugular blood were collected on days 1, 10, 20, 30, 40, 50, 60, 70 and 80.

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

RESULTS

Experiment 1: Concentrations of the mineral element in the soil and forage samples are given in Table 1. Content of Cu in the soil and forage from affected and unaffected regions were similar and within normal ranges. S contents of the soil and forage samples from the affected areas were significantly higher ($p<0.01$) than those in the unaffected areas. P contents of the soil and forage from the affected areas were significantly lower than the normal values. Other mineral concentrations in the soil and forage samples were within the normal ranges in all areas. Concentrations of mineral elements in the blood and liver are shown in Table 2. Cu content in the blood and liver of affected camels was significantly lower than that in normal animals ($p<0.01$). S content of the liver and blood was significantly higher in the affected Bactrian camels as compared with that in the normal animals. P contents of blood and liver from the affected camels were significantly less than those from normal animals. Mo and Fe contents of the liver and blood were within the normal ranges as compared with those in the non-affected animals. Concentrations of mineral element in the hair were given in Table 3. Contents of both Cu and P in the hair of affected camels were significantly lower than those of healthy animals ($p<0.01$). S content of the hair samples was significantly higher in the affected Bactrian camels as compared with that in the healthy animals. Hematological values are given in Table 4. Average Hb concentration, PCV, MCV, MCH and MCHC in affected Bactrian camels were significantly lower ($p<0.01$) than those of normal animals. Abnormal blood indices indicated a hypochromic microcytic anemia in affected Bactrian camels.

Serum protein concentration and biochemical values are given in Table 5 and 6. Activities of SOD in serum from affected camels were significantly lower than those in the healthy animals ($p<0.01$). Contents of serum IP and Cp in the affected camels were also significantly lower than those in the normal animals. Values of serum AKP, LDH and Crt were significantly higher in the affected camels than those in the healthy animals. Concentrations of serum α-globulin and β-globulin were significantly

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

	Soil		Forage	
Elements	Affected area	Normal area	Affected area	Normal area
S (%)	1.99±0.76	0.96±0.31	0.46±0.17	0.17±0.16
Cu (μg g^{-1})	16.8±7.1	16.7±5.6	6.79±1.26	6.91±2.86
Mo (μg g^{-1})	1.43±0.31	1.46±0.29	1.21±0.13	1.12±0.12
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})	51±11	63±12	412±81	452±51
F (μg g^{-1})	23.6±8.7	18.9±4.6	10.9±1.76	9.6±2.1

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

	Blood		Liver	
Elements	Affected	Normal	Affected	Normal
S (%)	6.31±1.7	4.12±0.86	1.53±0.36	1.32±0.35
Cu (μg g^{-1})	0.27±0.03	0.93±0.16	13.6±3.1	105.6±11.2
Mo (μg g^{-1})	0.18±0.10	0.19±0.09	2.79±0.61	2.87±0.72
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})	239±33	286±26	821±87	869±37
Se (μg g^{-1})	0.096±0.04	0.099±0.03	1.26±0.91	1.29±0.86

Table 3: Contents of mineral elements in hair samples

Elements	Affected animals	Normal animals
S (%)	6.37±2.3	4.67±7.21
Cu (μg g^{-1})	3.37±0.71	6.52±1.26
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})	101±31	129±23

Table 4: Hematological values in Bactrian camels

Blood indices	Affected animals	Unaffected animals
Hb (g L^{-1})	96.7±20.1	1270±9.80
RBC (10^{12} L^{-1})	12.8±3.60	11.7±1.50
PCV (%)	31.6±4.16	39.6±3.10
MCV (fl)	24.7±5.10	33.9±5.10
MCH (pg)	7.5±2.10	11.0±1.20
MCHC (%)	30.6±4.60	32.4±3.20
WBC (10^{9} L^{-1})	8.9±2.30	9.80±2.90

Table 5: Serum protein concentrations in Bactrian camels

Items	Affected animals	Unaffected animals
Total protein (g L^{-1})	63.80±6.80	63.40±3.70
Albumin (g L^{-1})	45.30±5.80	46.50±8.30
α-globulin(g L^{-1})	3.90±0.90	2.90±0.81
β-globulin (g L^{-1})	4.80±1.30	3.50±1.30
γ-globulin (g L^{-1})	9.80±2.80	10.50±2.30
A/G	2.45±0.61	2.75±1.20

Table 6: Biochemical values in Bactrian camels

Items	Affected camels	Normal camels
Cp (mg/100 mL)	13.5±2.9	23.2±3.4
SOD (μmol L^{-1})	14.3±1.9	18.5±2.3
CAT (μmol L^{-1})	24.3±2.6	25.1±2.9
GSH-Px (μmol L^{-1})	28.5±2.1	27.9±3.1
LDH (μmol L^{-1})	3.57±1.21	5.41±1.36
AST (IU L^{-1})	39.7±5.6	39.5±4.9
ALT (IU L^{-1})	13.7±2.7	13.9±2.9
γ-GT (IU L^{-1})	21.6±3.1	19.3±3.7
AKP (IU L^{-1})	126.8±28.7	48.9±13.5
Crt (μmol L^{-1})	895.7±98.7	367.8±76.8
Ca (mmol L^{-1})	2.56±0.39	2.47±0.35
IP (mmol L^{-1})	1.85±0.21	2.57±0.31
K (mmol L^{-1})	3.97±0.76	3.89±0.83
Na (mmol L^{-1})	146.9±29.7	147.1±27.6
Mg (mmol L^{-1})	0.97±0.28	0.95±0.31
BUN (mmol L^{-1})	6.13±1.32	6.17±1.37

higher in the affected camels than those in the healthy animals ($p<0.01$). There were no significant differences in other biochemical values between the affected camels and the normal animals.

Experiment 2: Fifty affected Bactrian camels grazing affected pastures were consuming an average of 136 mg of Cu/day for 60 days by a free-choice, salt-based trace mineral supplement liver Cu increased over time in all camels. However, the mean Cu content of the liver was significantly lower in the Bactrian camels supplemented with salt-based trace mineral as compared with that in the healthy Bactrian camels at the end of the study (the Cu contents of liver were 37.9±5.5 and 105.6±11.2 μg g^{-1} for salt-based trace mineral and healthy, respectively). Over 60 days of supplementation the rate of liver Cu repletion was 0.41 μg/g/day. The syndrome described previously continued to development in many camels, 20% of the camels died of exhaustion. There were no significant differences in the clinical parameters of body temperature, pulse and respiration between affected and healthy animals. On the 62nd day, ten Bactrian camels assigned to both treatments were severe Cu deficiency (the mean Cu concentrations in the liver were 33.3±3.3 and 33.1±3.2 μg g^{-1} for Cu sulfate and Availa-Cu treatments, respectively). The Cu contents in the liver increased over time in all camels regardless of treatment (Fig. 1). However, the mean Cu concentrations from Bactrian camels supplemented with Availa-Cu in the liver were significantly higher than ($p<0.01$) those receiving Cu sulfate (the mean Cu contents in the liver were 144±69 and 104±47 μg g^{-1} for Availa-Cu and Cu sulfate treatments, respectively). Over 80 days of supplementation the rate of liver Cu repletion was 2.47 and 1.63 μg/g/day for Availa-Cu and Cu sulfate-supplemented Bactrian camels, respectively. No treatment differences were detected in plasma ceruloplasmin concentrations (25.7±7.3 and 26.9±6.7 mg/100 mL for Cu sulfate and Availa-Cu, respectively). In all treated Bactrian camels with Cu sulfate or Availa-Cu, some signs of recovery were evident in 25-30 days after and appetite and vigor improved. Cu content in the blood and hair reached normal values (Cu contents were 0.87±0.21 and 6.12±1.5 μg g^{-1} for blood and hair, respectively) within 30 days.

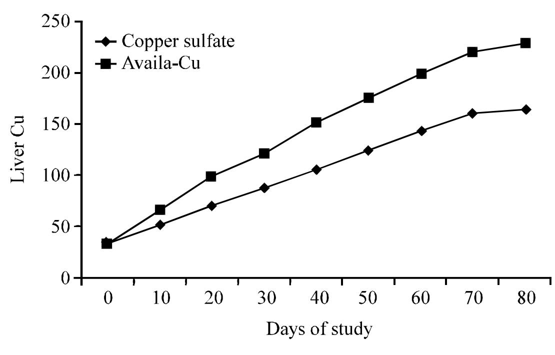

Fig. 1: Relationship between liver Cu concentration in Bactrian camels and supplementation of copper

DISCUSSION

Previous studies showed that Cu levels greater than 6 and 5 μg g^{-1} (dry matter) in soil and forage are safe for ruminants (Li and He, 1990). In the present study, the contents of Cu in the soil and forage from affected and unaffected regions were similar and within normal ranges by those standards but the S levels of the soil and forage in affected areas were significantly higher ($p<0.01$) than those of the unaffected areas. S requirement of grazing ruminants in forage is only 0.13% (dry matter) (Wang *et al*., 1995). In this study, the S content in forage was 0.46±0.17% which would be excessive for Bactrian camels. Elevating the levels of S in the diet of cattle and sheep has been shown to lower Cu absorption, the elevated S levels in soil and forage in areas where the affected Bactrian camels grazed had the same effect. Various researchers have reported that feeds and pastures with higher S interfered with the absorption of Cu, resulting in Cu deficiency for cattle (Jarvis and Austin, 1983; Li and He, 1990).

Concentration of Cu was very low in the whole blood but the S level was higher than normal. 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, average blood Cu values of <0.5 μg mL^{-1} are a sign of severe Cu deficiency. Normal concentration of Cu in blood of Bactrian camels is 0.86 μg g^{-1}. In this study, the contents of Cu in the blood from affected camels are only 0.27 μg g^{-1}. Liver Cu contents are the most reliable indicator of status in ruminant. In general, dry liver Cu concentrations below 75 μg g^{-1} are considered deficient for ruminant (McDowell, 1992; Shen, 2009). In this study, the contents of Cu in the liver from affected camel were only 13.6 μg g^{-1}. Therefore, Cu status of camels from the affected regions was severely deficient. 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). The mean Cu concentration in the hair of the affected Bactrian camels was 3.37±0.71 μg g^{-1} well below the 5.5 μg g^{-1} characteristic of secondary 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 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 (Shen, 2009; Shen *et al*., 2006). Anemia varies between and within species. In rats, lambs, rabbits and pigs, the anemia is hypochromic and microcytic as is found in Fe deficiency but in chickens and dogs, it is normochromic and normocytic. In cattle, yaks and adult sheep, the anemia is hypochromic and macrocytic (Suttle, 1999). In affected Bactrian camels, 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*., 2014; Wang, 1988). In this study, the activities of SOD in serum were significantly lower in the affected camels than those in the healthy animals.

Absence of Cu intake information in experiment 1 is a shortcoming of the present study when relating Bactrian camel Cu status to the pastures containing high concentrations of S. 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, fifty Bactrian camels grazing affected pastures were assessed. Despite the consumption of 136 mg of Cu/day these Bactrian camel found to have low liver Cu concentrations (37.9 ± 5.5 μg g^{-1}). Ten affected Bactrian camels removed from the pastures containing high concentrations of S were able to rapidly respond to Cu supplementation from both from Cu sulfate and Availa-Cu. Mean Cu content in the liver of Bactrian camels received Availa-Cu was significantly higher than ($p<0.01$) that in supplemented camels with Cu sulfate.

CONCLUSION

Thus, it is reasonable to conclude that ailments of Bactrian camels in the Haizi area are caused by a secondary Cu deficiency mainly due to high sulfur content in soil and forage.

ACKNOWLEDGEMENT

This research was supported financially by the National Natural Science Foundation of China (90202009).

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.

Arthington, J.D., J.E. Rechcig, G.P. Yost, L.R. McDowell and M.D. Fanning, 2002. Effect of ammonium sulfate fertilization on bahiagrass quality and copper metabolism in grazing beef cattle. J. Anim. Sci., 80: 2507-2512.

Gengelbach, G.P., J.D. Ward, J.W. Spears and T.T. Brown Jr., 1997. Effects of copper deficiency and copper deficiency coupled with high dietary iron or molybdenum on phagocytic cell function and response of calves to a respiratory disease challenge. J. Anim. Sci., 75: 1112-1118.

Jarvis, S.C. and A.R. Austin, 1983. Soil and plant factors limiting the availability of copper to a beef suckler herd. J. Agric. Sci., 101: 39-46.

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.

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.

Shen, X.Y., 2009. Effect of nitrogenous fertilizer treatment on mineral metabolism in grazing yaks. Agric. Sci. China, 8: 361-368.

Shen, X.Y., G.Z. Du, Y.M. Chen and B.L. Fan, 2006. Copper deficiency in yaks on pasture in Western China. Can. Vet. J., 47: 902-906.

Shen, X.Y., J.H. Zhang and R.D. Zhang, 2014. Phosphorus metabolic disorder of Guizhou semi-fine wool sheep. PLoS ONE, Vol. 9. 10.1371/journal.pone.0089472.

Shi, Y., 1990. Veterinary Clinical Diagnosis. Agricultural Press, Beijing, China, pp: 199-311.

Suttle, N.F., 1999. The Mineral Nutrition of Livestock. 3th Edn., CABI Publishing, London, UK., ISBN: 0-85199-128-9, pp: 243-303.

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.

Wang, F.Y., G.X. Cao, Z.Z. Hu and Y.W. Ding, 1995. Mineral Element Metabolism and Animal Diseases. Shanghai Science and Technology Press, Shanghai, China, pp: 106-188.

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.