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Full Length Research Paper

Studies of "emaciation ailment" in the Bactrian camel

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The clinical signs of disorder known locally as "emaciation ailment" in Bactrian camels in Haizi, Qinhai, China were defined. They included pica, emaciation, dyskinesia, deprived appetites and anemia. We found that concentrations of copper (Cu) in soil and forage from affected and unaffected areas were similar, but the concentrations of sulfur (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 camels grazing on affected pastures were consuming an average of 136 mg of Cu/d for 80 d 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 camels supplemented with salt-based trace mineral as compared with those in the healthy camels at the end of the study. Twelve affected camels were removed from the affected pastures and allocated to one of two treatments for 80 d, consisting of supplement providing 136 mg/d of either inorganic (Cu sulfate; n = 6) or organic (Availa-Cu n = 6) Cu. Liver Cu increased over time in all camels regardless of treatment; however, camels treated 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, for Cu sulfate and Availa-Cu, respectively) at the end of the study. Mean Cu content in the liver of camels received Availa-Cu was significantly higher than that in supplemented camels with Cu sulfate. In all treated camels, some signs of recovery were evident in 20 - 30 days after, and appetite and vigor were improved. Thus, it is reasonable to conclude that ailments of 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 camel, sulfur, copper, deficiency, "emaciation ailment".

INTRODUCTION

Bactrian camel (C*amelus bactrianus*) is an important livestock to the production system of the Chinese desert and semi-desert areas. Animals 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, Qinhai Province, China have been affected by an ailment characterized by pica, emaciation, dyskinesia, deprived appetites, and anemia. The incidence is estimated at 10 - 15% and the mortality may reach 50%. The affected area is located at 38.6° - 39.3°N latitude and 93.6° - 94.5°E longitude, and is an 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 690 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. Thirty percent 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 in the Haizi area.

There are two types of Cu deficiency-induced or

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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 healthy animals and tend toward decreased cytokine response to disease challenge (Gengelbanch 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.

MATERIALS AND METHODS

Experiment 1

Sample collection

Experiment began on 21 July, 2008. Fifteen affected Bactrian camels and fifteen unaffected Bactrian camels were selected for the study. 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 Maqu Biology Institute for further preparation and analysis. Liver biopsies were also sampled by a trained technician using techniques previously described by Arthington and Corah (1995). Hair samples from each Bactrian 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. 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 (Wen et al., 1983). Copper (Cu), cobalt (Co) and calcium (Ca) levels were determined by atomic absorption spectrophotometry, using a Perkin-Elmer AAS 5000 (Perkin-Elmer, Norwalk, CT), while selenium (Se) was assayed using the modified fluorometric procedure of Whetter and Ullrey (1978). 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". 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), and red blood cell (RBC) and white blood cell (WBC) values were determined using an automatic hematology analyzer (SF-3000, Medical Electronic Instrument Co., Japan). Biochemical analyses, which included superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) ceruloplasmin (Cp), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamy1 transferase (γ-GT), creatinine (Crt), blood urea nitrogen (BUN), cholesterol (Chol), sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), total protein (TP), albumin (Alb) and globulin (Glob) were determined on an automatic analyzer (OLYMPUS AU 640, Olympus Optical Co., Japan), 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 22°C.

Experiment 2

The grazing experiment began on 25 July, 2008. 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 µg/g) grazing affected pastures were consuming an average of 136 mg of Cu/d for 80 d 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 µg/g, for Co, Cu, Fe, Mn, Mo, and Zn, respectively). On the 80th day, twelve Bactrian camels with the lowest concentration of liver Cu were chosen to be transported to the Maqu Biology Institute (unaffected area). Bactrian camels were allocated to receive 136 mg/d of supplemental Cu from either inorganic (Cu Sulfate; n = 6) or organic (Availa-Cu, Zinpro Corp., Eden Prairie, MN; n = 6) 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 µg/g, and 5.32 ± 0.51 µg/g for crude protein, total digestible nutrients, S, Mo, and Cu, respectively) and were offered three times weekly (3.29 kg/day each Bactrian camel) 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 µg/g, and 8.67 ± 0.67 µg/g 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.

Table 1. Contents of mineral elements in soil and forage sample (n = 15).

Element	Soil		Forage	
	Affected area	Unaffected area	Affected area	Unaffected area
S (%)	1.99 ± 0.76**	0.96 ± 0.31	0.46 ± 0.17**	0.17 ± 0.16
Cu (μg/g)	16.8 ± 7.1	16.7 ± 5.6	6.79 ± 1.26	6.91 ± 2.86
Mo (μg/g)	1.43 ± 0.31	1.46 ± 0.29	1.21 ± 0.13	1.17 ± 0.12
Se (μg/g)	0.083 ± 0.031	0.086 ± 0.026	0.088 ± 0.026	0.091 ± 0.016
Co (μg/g)	6.63 ± 1.22	6.38 ± 1.63	1.30 ± 0.45	1.33 ± 0.21
Ca (μg/g)	16178 ± 889	16397 ± 746	6813 ± 746	6528 ± 725
P (μg/g)	51 ± 11	53 ± 12	417 ± 81	422 ± 51

**P<0.01.

Table 2. Contents of mineral elements in blood and liver samples (n = 15).

Element	Blood		Liver	
	Affected	Unaffected	Affected	Unaffected
S (%)	6.31 ± 1.7**	4.12 ± 0.86	2.53 ± 0.36**	1.32 ± 0.35
Cu (μg/g)	0.27 ± 0.03**	0.93 ± 0.16	13.6 ± 3.1**	105.6 ± 11.2
Mo (μg/g)	0.18 ± 0.10	0.19 ± 0.09	2.79 ± 0.61	2.87 ± 0.72
Co (μg/g)	0.56 ± 0.39	0.61 ± 0.12	0.71 ± 0.36	0.68 ± 0.21
Ca (μg/g)	129 ± 31	131 ± 26	187 ± 19	191 ± 27
P (μg/g)	239 ± 33	236 ± 26	857 ± 87	863 ± 37
Se (μg/g)	0.096 ± 0.04	0.099 ± 0.03	1.26 ± 0.91	1.29 ± 0.86

**P<0.01.

Table 3. Contents of mineral elements in hair samples (n = 15).

Element	Affected	Unaffected
S (%)	6.37±2.3**	4.67 ± 7.21
Cu (μg/g)	3.37 ± 0.71**	6.52 ± 1.26
Mo (μg/g)	2.31 ± 1.72	2.32 ± 0.81
Se (μg/g)	0.182 ± 0.076	0.186 ± 0.061
Co (μg/g)	1.15 ± 0.62	1.19 ± 0.23
Ca (μg/g)	2127 ± 661	2189 ± 326
P (μg/g)	119 ± 31	121 ± 23

**P<0.01.

RESULTS

Experiment 1

The affected Bactrian camels shown pica, emaciation, dyskinesia, deprived appetites, and anemia. 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. 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 Bactrian camels was significantly lower than that in healthy animals (P<0.01). S content of the liver and blood was significantly higher in the affected Bactrian camels as compared with those in the normal animals. Mo contents of the liver and blood were within the healthy ranges as compared with those in the non-affected animals. Concentrations of mineral element in the hair were given in Table 3. Contents of Cu in the hair of affected Bactrian camels were significantly lower than those of unaffected animals (P<0.01). S content of the hair samples was significantly higher in the affected Bactrian camels as compared with those in the unaffected 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 unaffected animals. Abnormal blood indices indicated a hypochromic microcytic anemia in affected Bactrian camels.

Serum protein concentration and biochemical values are given in Tables 5 and 6. Activities of SOD and contents of Cp in serum from affected Bactrian camels were significantly lower than those in the healthy animals (P<0.01). Values of serum ALP, LDH and Crt were signifi-

Table 4. Hematological values in Bactrian camels (n = 15).

Blood index	Affected	Unaffected
Hb (g/L)	96.7 ± 20.1**	127 ± 9.8
RBC (10^{12}/L)	12.8 ± 3.6*	11.7 ± 1.5
PCV (%)	31.6 ± 4.16**	39.6 ± 3.1
MCV (fl)	24.7 ± 5.1**	33.9 ± 5.1
MCH (pg)	7.5 ± 2.1**	11.0 ± 1.2
MCHC (%)	30.6 ± 4.6	32.4 ± 3.2
WBC (10^9/L)	8.9 ± 2.3	9.8 ± 2.9

*P<0.05; **P<0.01.

Table 5. Serum protein concentrations in Bactrian camels (n = 15).

Parameter	Affected	Unaffected
Total protein (g/L)	63.8 ± 6.8	63.4 ± 3.7
Albumin (g/L)	45.3 ± 5.8	46.5 ± 8.3
α-Globulin (g/L)	3.9 ± 0.9**	2.9 ± 0.81
β-Globulin (g/L)	4.8 ± 1.3**	3.5 ± 1.3
γ-Globulin (g/L)	9.8 ± 2.8	10.5 ± 2.3
A/G	2.45 ± 0.61	2.75 ± 1.2

**P<0.01.

cantly higher in the affected Bactrian camels than those in the unaffected animals. Concentrations of serum α-Globulin and β-Globulin were significantly higher in the affected Bactrian camels than those in the healthy animals (P<0.01). There were no significant differences in other biochemical values between the affected Bactrian camels and the healthy animals. There were no significant differences in the clinical parameters of temperature, pulse and respiration between affected and unaffected animals. Affected animals by disease demonstrate characteristic pica, emaciation, dyskinesia, and anemia. In all treated Bactrian camels, some signs of recovery were evident in 20 - 30 days after, and appetite and vigor improved.

Experiment 2

Fifty (50) affected Bactrian camels grazing affected pastures were consuming an average of 136 mg of Cu/d for 80 d by a free-choice, salt-based trace mineral supplement. Liver Cu increased over time in all Bactrian 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 those in the healthy Bactrian camels at the end of the study (the Cu contents of liver were 37.9 ± 5.5, 105.6 ± 11.2 µg/g, for salt-based trace mineral and healthy, respectively). Over 80 d of supplementation, the rate of liver Cu repletion was 0.41 µg/g/d. The syndrome described

Table 6. Biochemical values in Bactrian camels (n = 15).

Parameter	Affected	Unaffected
Cp (mg/100 mL)	13.5 ± 2.9**	23.2 ± 3.4
SOD (µmols^{-1}/l)	14.3 ± 1.9**	18.5 ± 2.3
CAT (µmols^{-1}/l)	24.3 ± 2.6	25.1 ± 2.9
GSH-Px (µmols^{-1}/l)	28.5 ± 2.1	27.9 ± 3.1
LDH (µmols^{-1}/l)	5.57 ± 1.21**	3.21 ± 1.36
AST (IU/l)	39.7 ± 5.6	39.5 ± 4.9
ALT (IU/l)	13.7 ± 2.7	13.9 ± 2.9
γ-GT (IU/l)	21.6 ± 3.1	19.3 ± 3.7
ALP (IU/l)	129.8 ± 28.7**	48.9 ± 13.5
Crt (µmol/l)	395.7 ± 98.7**	267.8 ± 76.8
Ca (mmol/L)	2.56 ± 0.39	2.47 ± 0.35
K (mmol/L)	3.97 ± 0.76	3.89 ± 0.83
Na (mmol/L)	146.9 ± 29.7	147.1 ± 27.6
Mg (mmol/L)	0.97 ± 0.28	0.95 ± 0.31
BUN (mmol/L)	6.13 ± 1.32	6.17 ± 1.37

**P<0.01.

previously continued to develop in many Bactrian camels, 20% of the Bactrian 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 80th day, twelve Bactrian camels of severe Cu deficiency in unaffected area were assigned to both treatments (The mean Cu concentrations in the liver were 33.3 ± 3.3 and 33.1 ± 3.2 µg/g, for Cu sulfate and Availa-Cu treatments, respectively). The Cu contents in the liver increased over time in all Bactrian camels regardless of treatment (Figure 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 ± 6.9 and 104 ± 4.7 µg/g, for Availa-Cu and Cu sulfate treatments, respectively). Over 80 d of supplementation, the rate of liver Cu repletion was 2.47 and 1.63 µg/g /d 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 were improved. Cu content in the blood reached normal values (Cu contents were 0.87 ± 0.21 µg/g) within 40 d.

DISCUSSION

Li and He (1990) showed that Cu levels greater than 6 and 5 µg/g (dry matter) in soil and forage are safe for ruminants. In the present study, the contents of Cu in the soil and forage from affected and unaffected regions were

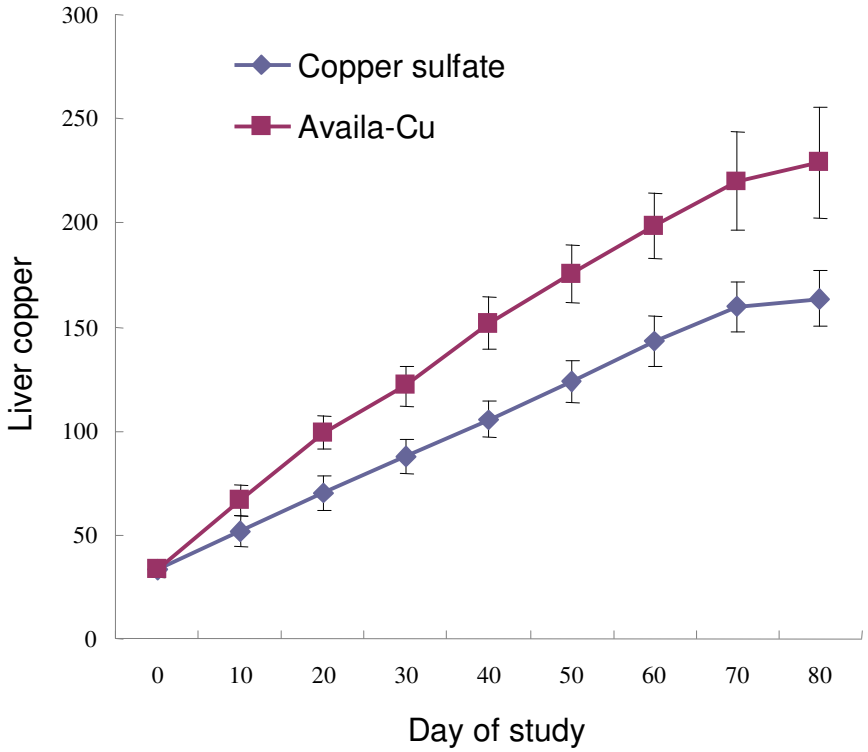

Figure 1. Relationship between liver Cu concentration (µg/g) in camels and supplementation of copper (n = 6).

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 (Jarvis and Austin, 1983). Various authors 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 of Cu stored in the liver, low content of Cu in the blood indicates exhaustion of the liver reserves. In cattle, average blood Cu values of < 0.5 µg/ml are a sign of severe Cu deficiency. Normal concentration of Cu in blood of Bactrian camels is 0.86 µg/g. In this study, the contents of Cu in the blood from affected Bactrian camels are only 0.27 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 (Mc Dowell, 1992). In this study, the contents of Cu in the liver from affected Bactrian camel were only 13.6 µg/g. Therefore, Cu status of Bactrian 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, well below the 5.5 µg/g 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 transferred 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. 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, we concluded that the anaemia 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 (Wang, 1988). In this study, the activities of SOD in serum were significantly

lower in the affected Bactrian 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. We are not aware of any previous data suggesting that high S forages may limit free-choice mineral intake. However, we 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 camels grazing on affected pastures were assessed. Despite the consumption of 136 mg of Cu/d, these Camels were found to have low liver Cu concentrations (37.9 ± 5.5 µg/g). Twelve affected camels removed from the pastures containing high concentrations of S were able to rapidly respond to Cu supplementation from both inorganic (Cu sulfate) and organic (Availa-Cu) sources. Mean Cu content in the liver of camels which received Availa-Cu was significantly higher than ($P<0.01$) that in supplemented camels with Cu sulfate. Thus, it is reasonable to conclude that ailments of camels in the Haizi area are caused by a secondary Cu deficiency, mainly due to high sulfur content in soil and forage.

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