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Effect of Fenced Pasture on Mineral Metabolic in Grazing Semi-Fine Wool Sheep in the Karst Mountain Areas of Southwest China

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Abstract: Mineral contents in the soil and forage are spatially distributed. Pasture fenced has created nutrition imbalance problems for animals. As a result, Semi-Fine Wool sheep are affected by a disease characterized by emaciation, lameness, stiffness in the gait, enlargement of the costochondral junctions and abnormal curvature in the long bones. The objective of this study was to determine possible relationships between the disease and pasture fenced. Results showed that Phosphorus (P) concentrations in forage samples from fenced areas were significantly lower than those from without fenced areas and the mean Calcium (Ca):P ratio in forage in fenced pasture was 11:1. Meanwhile, P concentrations of blood, bone, teeth and wool from fenced sheep were also significantly lower than those from without fenced group. Serum p levels of animals in fenced pasture were much lower than those of without fenced ones whereas serum alkaline phosphatase levels from fenced animals were significantly higher than those from the without fencing group. The disease could be alleviating with supplement of Disodium Hydrogen Phosphate (Na_2HPO_4) or demolish fence on pasture. The study clearly demonstrated that the disease of Semi-Fine Wool sheep was the P deficiency in forage mainly caused by pasture fenced.

Key words: Semi-Fine Wool sheep, pasture fenced, mineral metabolism, South West China Karst mountain area, animals

INTRODUCTION

Originally the affected area was an excellent autumn-winter range of native pasture. However, a few years ago, the pasture and livestock were allocated to individual families in an attempt to improve the local herdsmen's life and productivity. Because, mineral contents in the soil and forage are spatially distributed. Pasture fenced has created mineral nutrition imbalance problems for animals. As a result, Semi-Fine sheep have been affected in fenced pasture by a disease characterized by emaciation, lameness, enlargement of the costochondral junctions and abnormal curvature in the long bones. Severe cases included permanent recumbency, disability to standard up and eventual death. Based on preliminarily epidemiological and clinical data, this locally nutritional and metabolic disease may be associated with mineral P deficiency for the fenced animals. The objective of this study was to determine the pathogenesis of this disease and to establish possible relationships between the disease and fenced pasture.

MATERIALS AND METHODS

Epidemiological investigations: A detailed investigation on the epidemiology of the disease in Semi-Fine Wool sheep was carried out in the fenced area. Collected data included the history, incidence, character and regularity of the disease and the natural ecological conditions. Researchers interviewed many local herdsmen who had lived in the area for many years, asking for their advice and opinions and gathering background information on the disease. Data about the ecological and environmental conditions and their effects on the disease were obtained from local records and annual reports provided by the local government. Clinical signs were recorded by directly observing herd activities on the pasture.

Sample collections: Thirty (30) affected sheep were selected from 630 Semi-Fine Wool sheep in fenced pastures on 10 July of 2012. All the affected animals showed obvious clinical signs including lameness, weakness and enlargement of the costochondral

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junctions. Thirty unaffected Semi-Fine Wool sheep were selected from without fenced rang. All of the unaffected animals were judged to be in good health after clinical examination which were used as the control group.

Blood samples of the selected animals were obtained from the jugular vein using 1% sodium heparin as anticoagulant and stored at -10°C for analysis of trace elements (Youde, 2002; Yin *et al.*, 2010). Serum samples for biochemical analysis were taken in tubes without anticoagulant. The serum samples were separated by centrifugation and stored at -10°C in plastic vials. Wool samples were taken from the animal necks, washed and degreased (Heuer and Bode, 1998; Salmela *et al.*, 1981) and then kept in a desiccator over silica gel until analyzed. Thirty affected animals and thirty unaffected sheep were slaughtered by exsanguination. The animals used in these experiments were cared for by acceptable practices as outlined in the Guide for the Care and Use of Animals in Agricultural Research and Teaching Consortium (Yoyde, 2002). Thirty affected animals and thirty unaffected sheep were slaughtered by exsanguination which was approved by the Institute of Zoology, Chinese Academy of Sciences, Institutional Animal Care and Use Committee (Project A0668). Routinely post-mortem pathological examination was carried out by visually observing the tissues. Samples of ribs, hips and teeth were collected from the animals to determine minerals in the tissues.

Samples of forage and soil were collected in July 2012. Samples of forage were collected from 30 randomly distributed sites, i.e., from 6 fenced pastures and 5 samples/pasture. To reduce soil contamination, the herbage samples were cut 1-2 cm above the ground level. The forage samples were dried at 60-80°C for 48 h and ground to facilitate chemical analysis (McDowell, 1992; Wang *et al.*, 1996). At the same sites, 30 soil samples were taken from the surface layer (0-30 cm) using a 30 mm diameter cylindrical corer. Each soil sample was composited by four soil cores collected at the site. The soil samples were dried at 60-80°C for 48 h and passed through a 2 mm sieve. Thirty forage samples were also collected from without fenced rang.

Biochemical examination: Lactate Dehydrogenase (LDH), Alkaline Phosphatase (AKP), γ-Glutamyl Transferase (γ-GT), Calcium (Ca), Inorganic Phosphorus (IP) were determined using automatic biochemical analyzer (OLYMPUS AU 640, Olympus Optical Co., Japan). Quality control serum (Shanghai Biochemical Co.) was used to validate the blood biochemistry data. Serum protein electrophoretic studies were performed on cellulose acetate. All serum biochemical values were measured at 20°C.

Analysis of mineral contents: Concentration of copper (Cu), iron (Fe), manganese (Mn) and Ca in samples of animal tissues (blood, wool, ribs, hips and teeth) soil and forage were measured using a Perkin-Elmer AAS5000 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, Connecticut, USA). The accuracy of the analytical values was checked by reference to certified values of elements in the National Bureau of Standards (NBS) (bovine liver SRM 1577a).

Treatment and prevention: In one severely fenced area, 20 affected Semi-Fine Wool sheep were selected for the treatment experiment. Ten sheep were grazed on pasture demolished fence. Ten sheep were grazed on fenced pasture and given disodium hydrogen phosphate (Na_2HPO_4) orally at a dose of 60 g per animal. The treatment was repeated once a week between July and September of 2011. Clinical signs were recorded by directly observing the sheep activities on the pasture. Signs of lameness and gait stiffness and abnormal curvature in forelegs were specifically noted.

Statistical analyses: Data were analyzed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, USA) and presented in the form of mean±Standard Error (SE). Significant differences between groups were assessed using Student's t-test with least significant differences of 1% ($p<0.01$) and 5% ($p<0.05$).

RESULTS AND DISCUSSION

Epidemiology: The disorder mainly occurred in mature females of Semi-Fine Wool sheep throughout the year with a peak incidence in September. Pregnant and post-partum females were most commonly affected by the disease. The clinical signs were less obvious in mature males. The affected sheep were 30% and in fenced range, the mortality reached 15%. Besides the symptoms described above, long bones of the affected sheep were broken frequently, often without apparent stress. However, body temperature, respiratory rate and heart rate of the affected animals were normal.

Biochemical results: Serum AKP and LDH of the fenced sheep were significantly higher than those in without fenced animals ($p<0.01$) while IP levels were about half of those in the control group ($p<0.01$). The AKP values of the fenced animals were two times higher than those of without fenced sheep (Table 1). Likewise, concentrations of serum α-globulin and β-globulin of the fenced sheep were significantly higher than those of the without fenced group ($p<0.01$). There were no significant differences in other biochemical values between the fenced and without fenced sheep.

Minerals: Concentrations of P in the soil and forage samples in the fenced area were significantly lower than those in the without fenced area ($p<0.01$) (Table 2). The P concentrations in the forage samples of without fenced area were 5 times higher than those in the fenced area. The mean Ca:P ratio in forage of the fenced area was 11:1. Other values of the mineral elements were within the normal ranges. In addition, P concentrations in the blood and wool samples (Table 3) and in ribs, hips and teeth (Table 4) from the affected animals in fenced range were roughly half of those from without fenced animals ($p<0.01$).

Treatment and prevention: Animals treated with Na_2HPO_4 or animals grazed on pasture demolished fence recovered gradually within 10-20 days. Generally, vigor improved quickly and signs of lameness in most animals improved within 10 days after the treatment.

Semi-Fine sheep are vital to the production system of the Karst mountain areas of Southwest China. The local herd practices play an important role in mineral nutritional imbalance for grazing animals. Pasture fenced and animal habitat fragmentation has created nutrition imbalance problems for animals. Mineral nutrient in the soil and forage are spatially distributed. If animals graze in an extensive area they have chances to graze in poor as well as rich mineral element areas. Therefore, the mineral imbalance problem is minimal. In present study, P concentrations in the soil and forage from the fenced areas were significantly lower than those in the without fencing areas. Because of fenced pasture, the sheep grazed in the same pasture with P deficiency during the year. As a result, the Guizhou Semi-Fine Wool sheep were affected by P deficiency in forage.

A number of response criteria have been used to evaluate the P status of animals including serum levels of P, Ca and Alkaline Phosphatase (AKP). Earlier research suggests that bone criteria are more sensitive to P than to other elements. However, P levels of blood samples are not a good indicator for the P status because P levels can be normal for a long period after animals have been exposed to serious P deficiency (Zongping, 2005; Wang *et al*., 1995; Maduell *et al*., 2005). However, Heuer and Bode (1998) reported that P metabolism disease in water buffaloes was clearly associated with serum P levels below 0.97 mmol L^{-1} (FASS, 2010; Wang *et al*., 1995; Underwood and Suttle, 1999).

Table 1:Values of biochemical parameters in serum samples

Parameters	Affected in fenced pasture	Without fenced pasture
LDH (μmols L^{-1})	5.56±1.18[a]	3.21±0.52
γ-GT (IU L^{-1})	27.30±3.20	26.90±3.10
AKP (IU L^{-1})	129.30±11.3[a]	55.20±8.10
Ca (mmols L^{-1})	2.73±0.25	2.68±0.27
IP (mmols L^{-1})	1.25±0.12[a]	2.78±0.27

Table 2: Values of mineral element concentrations in soil and forage samples

	Soil		Forage	
Elements[a]	Fencing area	Without fencing area	Fencing area	Without fencing area
Ca (ppm)	13271±257	13719±219	2760±217	2513±112
P (ppm)	33.9±76.5[ab]	57.5±7.7	250±13[b]	1269±131
Cu (ppm)	16.7±2.9	16.9±2.7	6.9±2.3	6.3±2.7
Fe (ppm)	23527±271	23332±223	3752±131	3722±137
Mn (ppm)	57.7±12.5	57.8±12.2	14.2±4.9	14.3±4.6

[a]$p<0.01$

Table 3: Values of mineral element concentrations in the blood and wool samples

	Blood		Wool	
Elements	Affected animal in fenced pasture	Animal in without fenced pasture	Affected animal in fenced pasture	Animal in without fenced pasture
Cu (ppm)	0.71±0.27	0.73±0.28	5.28±1.27	5.25±1.37
Fe (ppm)	532±27	533±27	337±22	335±27
Mn (ppm)	0.57±0.11	0.56±0.13	5.87±1.26	5.79±1.27
Ca (ppm)	137±15	129±11	1028±141	1073±167
P (ppm)	263±22.4[a]	371±32.4	83±13[a]	153±13

Table 4: Values of mineral element concentrations in the bones and the teeth

	Rib		Hip		Teeth	
Elements[a]	Affected animal in fenced pasture	Animal in without fenced pasture	Affected animal in fenced pasture	Animal in without fenced pasture	Affected animal in fenced pasture	Animal in without fenced pasture
Cu (ppm)	7.7±2.72	7.5±2.3	5.88±1.26	5.79±1.17	4.62±0.73	4.67±0. 67
Fe (ppm)	199±11	193±12	197±14	195±15	177±11	173±12
Mn (ppm)	6.77±1.37	6.79±1.43	4.73±1.27	4.77±1.17	6.37±0.51	6.33±0.51
Ca (ppm)	138±12	138±15	127.6±18	125.6±23	177±22	173±23
P (ppm)	37±5[a]	75±12	35±3[b]	78±11	32±6[a]	77±7

[a]$p<0.01$

Shen and Zhang (2012) reported P metabolism disease in Wild yaks was clearly associated with serum P levels below 1.23 mmol L^{-1} (Shen and Zhang, 2012). Nevertheless, a marked hypophosphataemia is a good indicator of a severe P deficiency, even if serum levels of Ca are unaffected. To prevent P deficiency in grazing livestock, oral supplement of bone meal, phosphate and mineral mixtures is recommended (Youde, 2002; McDowell, 1992) or grazing extensive area.

Preliminary, epidemiological and clinical observations suggested that the disease of the Guizhou Semi-Fine Wool sheep in the Southwest China Karst mountain area is a nutritional metabolic disease associated with P deficiency mainly caused by fenced pasture. In general, P levels >0.005% in soils and >0.3% in forage should be sufficient for ruminants (Heuer and Bode, 1998). However, in present study, the P levels of the soils and forage in the affected areas were 0.0031 and 0.024%, respectively which were much lower than sufficient levels.

A Ca:P ratio of 1:1 to 2:1 is usually recommended for proper utilization of the elements by animals (Wang *et al*., 1995). Dietary Ca:P ratios <1:1 or >7:1 should adversely affect growth and feed efficiency of animals (Karn, 2001). In the study, the mean Ca:P ratio in forage from the fencing area was much lower than those of recommended values was only 11:1 which definitely had a negative impact on the Ca and P metabolisms of Semi-Fine Wool sheep in the area.

CONCLUSION

In this study, P levels in serum, wool, bones and teeth from the affected sheep were markedly lower and serum AKP level was significantly higher than those of the unaffected animals. The result was consistent with the response criteria in P deficiency disease of Bactrian camels (Zongping, 2005; Braithwaite, 1985; Yin *et al*., 2010). In the study, the oral supplement of Na_2HPO_4 appeared to cure the disease successfully. Based on earlier results, it was concluded that the disease of Guizhou Semi-Fine Wool sheep in fenced pasture was mainly caused by the P deficiency in forage and attributable to the current herding practices.

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