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Seasonal Dynamics of Copper Deficiency in Wumeng Semi-Fine Wool Sheep

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Abstract

A study of the seasonal dynamics of copper (Cu) deficiency and its effects on the antioxidant system in Wumeng semi-fine wool sheep in Weining county was conducted. In addition, the concentrations of mineral elements in soil, grass, and blood of affected and healthy Wumeng semi-fine wool sheep were determined, and blood indices were measured. The results showed that Cu contents in soil, grass, and sheep blood from the experimental pasture were significantly lower than those from the control pasture ($P < 0.05$), while the Mo content in the samples from the experimental pasture were higher than those from the control pasture ($P < 0.05$). Hb, RBC, PCV, and MDA values in the blood of affected sheep were significantly higher than those of healthy sheep, the content of TP was significantly lower ($P < 0.05$), and the activities of CAT, GSH-Px, CP, SOD, POD, and TP in the blood of affected sheep were lower than those in healthy sheep ($P < 0.05$). A comparison of different months showed that the Cu content in soil was higher in November than in June and September. Hb, RBC, PCV, WBC, and the content of MDA in the blood of affected sheep was higher in November than in June and September; however, the change in CP, CAT, GSH-Px, POD, and TP showed the opposite trend. The content of Cu and Mo in soil, grass, and blood showed seasonal changes. Therefore, it is concluded that the seasonal dynamics of Cu deficiency in Wumeng semi-fine wool sheep in Weining was due to the content of Mo and Cu in soil and grass in different seasons, and high Mo concentration in grass affected Cu deficiency in sheep. The function of the antioxidant system of Wumeng semi-fine wool sheep was reduced as a result.

Keywords Wumeng semi-fine wool sheep · Soil · Grass · Copper deficiency · Antioxidant system

Introduction

Wumeng semi-fine wool sheep, formerly Guizhou semi-fine wool sheep, are bred both for mutton and wool and this hybrid was produced following the introduction of Corriedale blood from New Zealand in Weining local sheep (Tibetan Valley type coarse wool sheep), and has the characteristics of high production performance, strong adaptability, gentle temperament, and easy management. It is the material basis of human life in the Wumeng mountain area. The animal husbandry industry of Wumeng semi-fine wool sheep is economically important in the Wumeng mountain area. However, the occurrence of copper (Cu) deficiency disease has affected the development of animal husbandry [1]. Yuan et al. (2011) reported that the proportion of Cu deficiency in Guizhou semi-fine wool sheep in the southwestern China Karst mountain region reached 65% [2]. Zhang and Liu (2014) reported that the survival rate of Guizhou semi-fine wool lambs in Weining county was generally 40–60%, the lowest was only 13.5%, and the mortality rate was approximately 70% [3]. Symptoms of Cu deficiency include pica, depressed appetite, emaciation, anemia, and central nervous system dysfunction, resulting in diminished productive capacity.

Mineral elements play an important physiological role in animals. For example, ions are dissolved in body fluid to maintain the normal distribution of body water, body fluid acid-base balance, and normal excitability of nerve muscles, and are components of some enzymes or activators [4]. Moreover, mineral elements play a vital role in the function of the antioxidant system. An element concentration which is too high or too low not only affects its absorption and utilization but also affects the utilization of other elements. This

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eventually leads to destruction of the dynamic balance between antioxidant substances and oxygen-free radicals, and the function of the antioxidant system is reduced, causing damage to the body [5, 6]. Cu is an essential mineral element in animals [7]. As a component of metalloenzymes (such as cytochrome oxidase, amino acid oxidase, superoxide dismutase (SOD)), it can directly participate in body metabolism [8, 9], maintain iron (Fe) metabolism which is beneficial to hemoglobin (Hb) synthesis and red blood cell (RBC) maturation [10], and even takes part in bone formation [11]. Previous studies showed that Cu deficiency can lead to depressed appetite, emaciation, dyskinesia, unsteady gait, anemia, poor hair growth, and can influence the production of animals and thus affect the economic benefits of the industry [12, 13].

These mineral elements are mainly absorbed and utilized by animals through the soil-grass-animal ecological chain. Mineral element concentrations in soil are affected by factors such as temperature, precipitation, and season, and their concentrations in plants are influenced by soil background values and element species. The changes in mineral element concentrations in soil and plants can lead to seasonal differences in nutrient intake by animals and affect the health of animals [14, 15].

Based on the above findings, this study aimed to determine the seasonal contents of mineral elements in soil and grass of pasture where affected and healthy Wumeng semi-fine wool sheep were located. The mineral element concentrations and physiological and biochemical indices in blood in the affected and healthy Wumeng semi-fine wool sheep in different seasons were analyzed, and the seasonal dynamics of Cu deficiency in sheep and its effects on the antioxidant system were assessed.

Material and Methods

Study Area

Weining county, located between 103° 36′–104° 45′ E and 26° 36′–27° 26′ N, is the highest county in Guizhou province with an average elevation of 2200 m [16]. The county has a subtropical monsoon humid climate with 1812 h sunshine per year, 180 frost-free days per year, 926 mm of annual rainfall, small annual temperature differences, and large daily temperature differences.

Sample Collection

Soil Samples

In June, September, and November, 2018, using the serpentine method, 10 quadrats of 1 m^2 at intervals of 200 m were chosen in Yanchang town grassland (experimental pasture) and Liangshuigou grassland (control pasture), respectively. The topsoil of 0–30 cm was then collected from each sample using the diagonal method. The topsoil was then mixed and reduced to 200 g by the quartile method. After 48 h of drying in a 60–80 °C drying oven, the fine sand in the soil was removed using a 0.154 mm sieve and the remaining soil was used to determine the content of mineral elements.

Grass Samples

In June, September, and November, 2018, grass samples were collected at the same locations as the soil samples. The grass was gathered by the diagonal method and the collected grass was mixed and the surface soil shaken off. The sample was reduced to 200 g by the quadruple method and the grass was dried at 65 °C for 48 h in an oven in the laboratory. The fine soil from the grass samples was sieved through a 0.071 mm sieve. The contents of elements in the grass were then determined.

Blood Samples

In June, September, and November, 2018, 10 affected Wumeng semi-fine wool sheep and 10 healthy Wumeng semi-fine wool sheep were selected and blood samples were collected from these sheep in the experimental pasture and control pasture, respectively. Ten milliliters blood from the jugular vein of each sheep was collected into a vacutainer for mineral elements analysis, and 10 mL blood was collected into a vacutainer containing heparin sodium for measurement of blood indices.

Sample Analysis

Mineral Element Contents

The soil, grass, and blood samples were digested using the microwave digestion method, and treatment of the samples and the microwave digestion procedure are shown in Table 1. The content of elements such as Cu, manganese (Mn), cobalt (Co), iron (Fe), molybdenum (Mo), zinc (Zn), and sulfur (S) was measured by an inductively coupled plasma emission spectrometer (HK9600 Type Atomic Emission Spectroscopy, Huaketiancheng Co., Ltd., China), and the working conditions of the inductively coupled plasma emission spectrometer (ICP-AES) were the same as those described by Song and Shen (2019) [17].

Blood Indices

A fully automatic blood analyzer for animals (SF-3000, Sysmex-Toa Medical Electronics, Kobe, Japan) was used to

Table 1 Treatment of samples and procedure of microwave digestion

Sample	Sampling amount	Digestion reagent	Microwave digestion procedure				
			Procedure	Temperature/°C	Time/min	Power/kw	Pressure/atm
Soil	0.20 g	5 mL HNO_3	1	120	5	0.9	12
		2 mL HF	2	140	4	0.9	14
		1 mL H_2O_2	3	150	8	0.9	15
Grass	0.50 g	5 mL HNO_3	1	120	3	0.7	12
		1 mL H_2O_2	2	150	5	0.8	15
Blood	200 µL	4 mL HNO_3	1	120	4	0.9	12
		1 mL H_2O_2	2	140	4	1.2	15

determine the content of hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), and hematocrit (PCV).

An automatic biochemical analyzer (Olympus AU 640, Olympus Optical Co., Tokyo, Japan) was employed to measure the activity of catalase (CAT), glutathione peroxidase (GSH-Px), ceruloplasmin (CP), superoxide dismutase (SOD) and peroxidase (POD), the content of malondialdehyde (MDA), and total protein (TP). The kits used in these experiments were purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing City, P. R. China.

Data Analysis

SPSS 20.0 (version 20.0 for Windows, Chicago, IL, USA) was adopted to analyze the collected data and the differences were evaluated by the "*t* test." All data were expressed in the form of "average number ± standard deviation."

Results

Mineral Element Contents in Soil

The soil links together the lithosphere, the biosphere, the atmosphere, and the hydrosphere [18]. It is a bridge to the inorganic and organic communities and plays an important role in the ecosystem [19]. The content of nutrients in the soil directly affects the growth and quality of plants, and most of the nutrients needed for animal growth and development are obtained through the soil-grass-animal biological chain [20]. Therefore, the content of mineral elements in the soil can also affect the health of animals.

During the same season, compared with the control pasture (Table 2), the content of Cu in the experimental pasture soil was significantly lower ($P < 0.05$), while the content of Mo was significantly higher than that in the control pasture ($P < 0.05$). Other elements in the soil of the two pastures were not significantly different in different months ($P > 0.05$).

When the differences in mineral elements in soil in different months were compared (Table 2), it was found that the content of Cu, Fe, and Mo in soil in the experimental pasture increased in different months, but not significantly ($P > 0.05$). There was no obvious change in Mn, Co, Zn, and S concentrations between the 3 months studied ($P > 0.05$). The changes in elements in the soil of the control pasture were similar to those in the experimental pasture.

Mineral Element Contents in Grass

Grass is an important material basis for the development of herbivores. The mineral nutrient intake of herbivores is determined by the content of elements in grass.

Compared with the concentrations of elements in grass in the control pasture in the same season (Table 3), the contents of Cu and S in the experimental pasture were significantly lower than those in the control pasture ($P < 0.05$), while the contents of Fe and Mo in the experimental pasture were significantly higher ($P < 0.05$). The concentrations of Mn, Co, and Zn in the two pastures were not significantly different ($P > 0.05$).

The element contents in grass were compared in different months (Table 3), the content of each element in grass in the experimental pasture was lowest in November, and the contents in June and September were different due to the presence of different elements; however, the difference between these months was not significant ($P > 0.05$). The changes in elements in the grass of the control pasture were the same as those in the experimental pasture.

Mineral Element Contents in the Blood of Wumeng Semi-Fine Wool Sheep

The mineral elements in blood in affected Wumeng semi-fine wool sheep were compared with healthy sheep in the same season (Table 4), and it was found that the content of Cu in blood from affected sheep was significantly lower than that from healthy sheep ($P < 0.05$), while the content of Mo was

Table 2 Mineral element content in soil (DM)

Element	June		September		November	
	Experimental pasture	Control pasture	Experimental pasture	Control pasture	Experimental pasture	Control pasture
Cu ($mg{\cdot}kg^{-1}$)	19.05 ± 3.78a	23.20 ± 8.19b	20.84 ± 5.04a	24.96 ± 6.31b	21.41 ± 5.78a	25.83 ± 4.84b
Mn ($mg{\cdot}kg^{-1}$)	307.9 ± 18.8	305.9 ± 17.5	312.9 ± 15.8	309.4 ± 13.8	310.9 ± 14.5	306.6 ± 11.9
Co ($mg{\cdot}kg^{-1}$)	6.83 ± 1.17	7.04 ± 1.05	7.13 ± 1.12	6.85 ± 0.75	7.08 ± 1.01	7.18 ± 0.91
Fe ($mg{\cdot}kg^{-1}$)	290.3 ± 10.1	287.1 ± 9.5	293.9 ± 10.8	289.3 ± 10.9	295.7 ± 12.7	292.2 ± 9.8
Mo ($mg{\cdot}kg^{-1}$)	1.67 ± 0.13a	1.65 ± 0.14b	1.71 ± 0.14a	1.69 ± 0.15b	1.78 ± 0.14a	1.75 ± 0.14b
Zn ($mg{\cdot}kg^{-1}$)	52.76 ± 8.80	48.60 ± 8.77	53.60 ± 7.85	47.28 ± 8.03	54.90 ± 9.40	47.48 ± 7.71
S ($mg{\cdot}kg^{-1}$)	61.50 ± 2.99	45.86 ± 3.30	57.50 ± 3.03	43.89 ± 1.99	54.49 ± 2.24	50.94 ± 7.75

Different capital letters of peers indicate significant differences between different months, $P < 0.05$; different small letters of peers indicate significant differences between pastures, $P < 0.05$

Cu copper, *Mn* manganese, *Co* cobalt, *Fe* iron, *Mo* molybdenum, *Zn* zinc, *S* sulfur

significantly higher than that from healthy sheep ($P < 0.05$). The contents of Mn, Co, Fe, Zn, and S in the two groups were not significantly different ($P > 0.05$).

The differences in mineral element concentrations in blood in Wumeng semi-fine wool sheep in different months were assessed (Table 4). The results showed that the content of Cu in the blood of affected sheep was significantly lower in November than in June and September ($P < 0.05$), but there was no significant difference between June and September ($P > 0.05$), and there was no significant difference in the content of other elements between these 3 months ($P > 0.05$). The changes in elements in the blood of healthy sheep were similar to those in affected sheep, and no significant differences were observed in different months ($P > 0.05$).

Effect of Copper Deficiency on Blood Parameters in Wumeng Semi-Fine Wool Sheep

Compared with healthy sheep in the same season (Table 5), Hb, RBC, and PCV in the blood of affected sheep were significantly higher ($P < 0.05$), and WBC was not significantly different between the two groups ($P > 0.05$).

The blood parameters of sheep in different months were compared (Table 5) and the results showed that Hb, RBC, PCV, and WBC in the blood of affected sheep were higher in November than in June and September, but there was no significant difference in these parameters in different months ($P > 0.05$). The changes in blood parameters in healthy sheep were similar to those in affected sheep, and no significant differences in these parameters between months were observed ($P > 0.05$).

Effect of Copper Deficiency on Biochemical Parameters in Wumeng Semi-Fine Wool Sheep

CAT, GSH-Px, SOD, and POD are important enzymes in the antioxidant system of organisms. Changes in these parameters can reflect the functional status of a body's antioxidant system. In addition, the changes in SOD and MDA are a reflection of lipid peroxidation in liver tissue.

Table 3 Mineral element content in grass (DM)

Element	June		September		November	
	Experimental pasture	Control pasture	Experimental pasture	Control pasture	Experimental pasture	Control pasture
Cu $mg{\cdot}kg^{-1}$)	7.23 ± 0.72a	7.92 ± 0.49b	7.50 ± 0.66a	8.31 ± 0.47b	7.03 ± 0.73a	7.65 ± 0.58b
Mn ($mg{\cdot}kg^{-1}$)	36.64 ± 3.72	36.31 ± 2.91	34.43 ± 3.72	35.35 ± 3.95	33.14 ± 3.83	33.91 ± 2.84
Co ($mg{\cdot}kg^{-1}$)	0.173 ± 0.031	0.168 ± 0.031	0.164 ± 0.024	0.159 ± 0.014	0.145 ± 0.023	0.151 ± 0.015
Fe ($mg{\cdot}kg^{-1}$)	50.97 ± 6.95a	47.35 ± 3.9b	52.97 ± 6.24a	46.14 ± 3.8b	48.48 ± 7.11a	43.76 ± 3.4b
Mo $mg{\cdot}kg^{-1}$)	4.04 ± 0.43a	0.867 ± 0.141b	4.19 ± 0.37a	0.910 ± 0.163b	3.94 ± 0.44a	0.969 ± 0.226b
Zn ($mg{\cdot}kg^{-1}$)	30.53 ± 3.15	29.17 ± 2.96	28.55 ± 3.71	28.84 ± 2.62	26.29 ± 3.83	27.62 ± 2.42
S (%)	0.196 ± 0.032a	0.206 ± 0.032b	0.187 ± 0.032	0.201 ± 0.018	0.184 ± 0.021a	0.196 ± 0.022b

Different capital letters of peers indicate significant differences between different months, $P < 0.05$; different small letters of peers indicate significant differences between pastures, $P < 0.05$

Cu copper, *Mn* manganese, *Co* cobalt, *Fe* iron, *Mo* molybdenum, *Zn* zinc, *S* sulfur

Table 4 Mineral element content in blood

Element	June		September		November	
	Affected sheep	Healthy sheep	Affected sheep	Healthy sheep	Affected sheep	Healthy sheep
Cu ($mg \cdot kg^{-1}$)	0.185 ± 0.018Aa	0.494 ± 0.012b	0.170 ± 0.014Aa	0.553 ± 0.012b	0.145 ± 0.009Ba	0.595 ± 0.011b
Mn ($mg \cdot kg^{-1}$)	0.528 ± 0.054	0.539 ± 0.062	0.547 ± 0.055	0.549 ± 0.052	0.559 ± 0.053	0.564 ± 0.056
Co ($mg \cdot kg^{-1}$)	0.612 ± 0.091	0.633 ± 0.076	0.666 ± 0.056	0.653 ± 0.059	0.681 ± 0.061	0.675 ± 0.064
Fe ($mg \cdot kg^{-1}$)	386.4 ± 16.6	382.6 ± 17.0	381.8 ± 18.4	388.1 ± 15.3	373.4 ± 24.7	377.8 ± 16.7
Mo ($mg \cdot kg^{-1}$)	0.102 ± 0.008a	0.096 ± 0.007b	0.113 ± 0.007a	0.107 ± 0.009b	0.118 ± 0.009a	0.115 ± 0.009b
Zn ($mg \cdot kg^{-1}$)	6.84 ± 1.25	7.16 ± 1.102	6.77 ± 0.864	7.06 ± 0.879	6.67 ± 1.09	6.77 ± 1.194
S (%)	0.062 ± 0.005	0.059 ± 0.005	0.064 ± 0.006	0.062 ± 0.004	0.063 ± 0.005	0.064 ± 0.005

Different capital letters of peers indicate significant differences between different months, $P < 0.05$; different small letters of peers indicate significant differences between affected and healthy sheep, $P < 0.05$

Cu copper, *Mn* manganese, *Co* cobalt, *Fe* iron, *Mo* molybdenum, *Zn* zinc, *S* sulfur

Compared with healthy sheep in the same season (Table 6), the activities of CAT, GSH-Px, CP, SOD, POD, and TP in blood in affected sheep were lower ($P < 0.05$), and the content of MDA in affected sheep was much higher than that in healthy sheep ($P < 0.05$).

The activities of antioxidant enzymes in the blood of Wumeng semi-fine wool sheep were compared in different months (Table 6). The results showed that the activity of CP in the blood of affected sheep in November was significantly lower than that in June and September ($P < 0.05$), and there was no significant difference between June and September ($P > 0.05$). The changes in CAT, GSH-Px, and POD were similar to those in CP, but there was no significant difference between different months ($P > 0.05$). MDA content was highest in November ($P < 0.05$), but there was little difference between June and September ($P > 0.05$). The changes in biochemical parameters in the blood of healthy sheep were similar to those in affected sheep; however, there was no significant difference among these parameters in different months ($P > 0.05$).

Discussion

The content and proportion of mineral elements in grass are important factors, which affect their absorption and utilization by herbivores. However, the content of mineral elements in forage is affected by the concentrations of elements in soil. The content and distribution of mineral elements in soils will change due to the combination of climate, temperature, biology, and topography [21]. In addition to the influence of soil parent material and soil-forming conditions, the mineral element content in soil is also affected by season [22]. Therefore, Cu and Mo content in the soil were different in different pastures and in different months. The effects of soil element content, vegetation type, growth period, and fertilization lead to different contents of elements in grass from different pastures in the same season, and the content of elements in grass in the same pasture changes with the season and shows differences during growth [23, 24]. The differences in mineral element contents observed in this study between the two pastures and in different months were consistent with these findings. The contents of Cu, Fe, Zn, and Co in grass in November were

Table 5 Blood parameters in Wumeng semi-fine wool sheep

Items	June		September		November	
	Affected sheep	Healthy sheep	Affected sheep	Healthy sheep	Affected sheep	Healthy sheep
Hb ($g \cdot L^{-1}$)	136.01 ± 3.68a	117.41 ± 3.14b	134.70 ± 2.97a	112.42 ± 10.18b	138.01 ± 3.11a	118.32 ± 6.66b
RBC ($\times 10^{12} \cdot L^{-1}$)	17.42 ± 2.60a	11.33 ± 1.96b	17.69 ± 2.72a	11.24 ± 1.69b	17.92 ± 3.03a	11.84 ± 1.81b
PCV (%)	40.22 ± 3.64a	34.68 ± 2.86b	41.62 ± 3.77a	35.32 ± 2.96b	43.02 ± 3.64	36.92 ± 2.01b
WBC ($\times 10^{9} \cdot L^{-1}$)	8.61 ± 2.16	10.11 ± 1.36	8.43 ± 2.37	9.67 ± 1.77	8.25 ± 1.86	9.27 ± 1.95

Different capital letters of peers indicate significant differences between different months, $P < 0.05$; different small letters of peers indicate significant differences between affected and healthy sheep, $P < 0.05$

Hb hemoglobin, *RBC* red blood cell, *PCV* hematocrit, *WBC* white blood cell

Table 6 Biochemical parameters in Wumeng semi-fine wool sheep

Items	June		September		November	
	Affected sheep	Healthy sheep	Affected sheep	Healthy sheep	Affected sheep	Healthy sheep
CAT ($U\cdot L^{-1}$)	1.62 ± 0.09a	2.03 ± 0.19b	1.67 ± 0.12a	1.97 ± 0.13b	1.58 ± 0.11a	1.91 ± 0.17b
GSH-Px ($U\cdot L^{-1}$)	17.17 ± 1.49a	24.07 ± 2.38b	17.69 ± 1.56a	25.27 ± 2.02b	18.04 ± 1.33a	25.57 ± 2.17b
CP ($mg\cdot L^{-1}$)	46.37 ± 1.52Aa	51.98 ± 2.09b	45.21 ± 1.97Aa	53.38 ± 2.64b	42.37 ± 2.82Ba	52.86 ± 2.79b
SOD ($U\cdot L^{-1}$)	25.79 ± 1.97a	32.05 ± 2.46b	24.90 ± 2.17a	31.18 ± 2.47b	23.88 ± 2.72a	30.48 ± 2.53b
POD ($U\cdot L^{-1}$)	35.71 ± 1.79a	49.47 ± 2.75b	36.51 ± 2.18a	50.56 ± 2.34b	36.35 ± 1.74a	49.76 ± 2.13b
MDA ($nmol\cdot L^{-1}$)	34.62 ± 2.39Aa	24.43 ± 2.38b	34.13 ± 2.74Aa	25.63 ± 1.82b	40.76 ± 2.91Ba	26.23 ± 1.46b
TP ($nmol\cdot L^{-1}$)	49.23 ± 2.28a	53.15 ± 3.53Ab	48.63 ± 2.89a	51.25 ± 2.72Bb	47.23 ± 2.35a	50.53 ± 2.09Bb

Different capital letters of peers indicate significant differences between different months, $P < 0.05$; different small letters of peers indicate significant differences between affected and healthy sheep, $P < 0.05$

CAT catalase, *GSH-Px* glutathione peroxidase, *CP* ceruloplasmin, *SOD* superoxide dismutase, *POD* peroxidase, *MDA* malondialdehyde, *TP* total protein

lower than those in June, which was the same as those reported by Fleming and Murphy, Mayland and Reddy, et al. who believed that the contents of elements in grass decreased with the growth and maturity of grass [25, 26]. This should be the reason why Cu deficiency in Wumeng semi-fine wool sheep was more serious in winter.

Copper deficiency depends not only on the total Cu concentration in the diet, but also on other factors which affect Cu absorption and utilization [27]. Li et al. reported that Cu deficiency in Tibetan sheep was induced by S and Fe overdose [28]. Ward reported that a diet supplemented with 5 $mg\cdot kg^{-1}$ of Mo and 10 $mg\cdot kg^{-1}$ of Cu (126 days), and a diet without Cu caused severe Cu deficiency [29]. The influence of Fe on the absorption and utilization of Cu is mainly due to antagonism between Fe and Cu. As Fe competes with Cu for intestinal protein binding sites, it consequently reduces the rate of Cu absorption [30]. Therefore, many researchers believe that the best ratio of Fe to Cu is 10:1 and excessive Fe will lead to Cu deficiency. In the gastrointestinal tract of animals, Mo forms thiomolybdate with the participation of S, and becomes the Cu-Mo-S-protein complex and cupric thiomolybdate with Cu. The Cu-Mo-S-protein complex is relatively stable in blood, and Cu cannot be easily utilized by the tissue. As a result, Cu absorption is hindered. Cupric thiomolybdate is difficult to dissolve and is excreted directly through the intestine. When thiomolybdate enters the liver, it can strip Cu directly from metallothionein. The stripped Cu in the form of small molecule substances enters the blood and bile and is discharged with urine after metabolism. In this process, metallothionein transfers Cu from other proteins to supplement Cu, and then peels it off so that metallothionein can circulate in the body and at the same time reduce Cu in the body. Consequently, this process causes Cu deficiency in animals [31, 32]. For these reasons, it is suggested that the ratio of Cu to Mo should be between 6:1 and 10:1. If the ratio is less than 2:1, Cu deficiency will occur [33, 34]. Zn antagonizes Cu metabolism by displacing Cu from sulfhydryl binding sites on metallothionein [35, 36], and excessive Zn intake inhibits Cu uptake [37]. However, the absorption of Zn and Fe is mutually restricted [38]. In this study, the contents of S and Fe in grass were within the feed standard (NRC, 2007), but the content of Mo exceeded the recommended range, and even the ratio of Cu to Mo was less than 2:1 [39]. Therefore, the Cu deficiency in Wumeng semi-fine wool sheep in this area was caused by long-term ingestion of excessive Mo in grass, and these results are in line with existing research results [40, 41].

Cu participates in the production of SOD, CP, and other antioxidants, and plays an important role in the enzymatic system of the antioxidant system. SOD is a specific superoxide anion enzyme. CP has a similar function to SOD. MDA is a metabolite of lipid peroxidation with strong biological toxicity. Wang et al. established an experimental animal model by adding different doses of Mo to drinking water, and the results showed that the activities of SOD and GSH-Px in chicken serum were significantly inhibited by adding different doses of Mo [42, 43]. In the present study, the results showed that the MDA content in affected Wumeng semi-fine wool sheep was significantly higher than that in healthy sheep, and the activities of CAT, GSH-Px, CP, SOD, and POD in affected sheep were significantly lower than those in healthy sheep. These findings showed that Cu deficiency in Wumeng semi-fine wool sheep was caused by excessive Mo in grass, which seriously affected the antioxidation ability in Wumeng semi-fine wool sheep.

Conclusion

Copper deficiency in Wumeng semi-fine wool sheep was the result of secondary copper deficiency caused by excessive long-term Mo feeding. In addition, copper deficiency in these sheep changed with the season. In winter, the lack of copper resulted in serious disease, while in summer, the sickness was relatively mild. Copper deficiency seriously affected the activity of antioxidant enzymes and reduced the function of the antioxidant system in Wumeng semi-fine wool sheep. As the copper content in grass was lower in winter than in other seasons, it is suggested that copper sulfate should be added to the feed of Wumeng semi-fine wool sheep or nutritional licking bricks be placed in the pasture in winter to increase copper intake in Wumeng semi-fine wool sheep.

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Compliance with Ethical Standards

All protocols used in the study were approved by the School of Life Science and Engineering, Southwest University of Science and Technology Animal Care and Use Committee.

Conflict of Interest The authors declare that they have no conflict of interest.

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