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Original Research

Effect of Different Copper Levels in Feed on Antioxidant Capacity in Stocking the Native Sheep in the Wumeng Prairie

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Abstract

The purpose of this research is to better understand the effect of different copper (Cu) levels of feed on the antioxidant capacity in the native sheep, solve the adverse effects of local seasonal Cu deficiency on sheep, and provide a reasonable reference for Cu supplementation. 10 healthy native sheep were selected as the control group (C group). 40 Cu-deficient native sheep were selected as tested group, and divided randomly into 4 groups (n = 10), supplied by $CuSO_4$ (0, 20, 40, 60 mg/kg for group I, group II, group III and group IV, respectively). The results showed that the level of Cu, hemoglobin (Hb), packed-cell volume (PCV), albumin (ALB), the activities of ceruloplasmin (CP), Cu/Zn-superoxide dismutase (Cu/Zn-SOD) and catalase (CAT) in group I were significantly lower than those in C group, group II, III, IV (P<0.05), but malondialdehyde (MDA) concentration was higher. The activities of these antioxidant enzymes and MDA concentration had no significant difference between C group, group II, III, IV (P>0.05), but group III had the highest antioxidant enzymes activity and the lowest MDA concentration. There was no significant difference in manganese (Mn), zinc (Zn), iron (Fe), molybdenum (Mo), selenium (Se), red blood cell count (RBC), white blood cell count (WBC), blood urea nitrogen (BUN), alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (GOT), total cholesterol (TC), manganese-superoxide dismutase (Mn-SOD) between all groups (P>0.05). Conclusion: The function of antioxidant system of the native sheep in Weining County have seriously reduced. Added $CuSO_4$ to the diet increase the activity of antioxidant enzymes and improve the health parameters, but them did not increase linearly. Added 40 mg/kg $CuSO_4$, the activity of antioxidant enzymes was the highest and the content of MDA was the lowest. Therefore, it is recommended to add 40 mg/kg $CuSO_4$ to diet of the native sheep in Weining County.

Keywords: the native sheep, different copper level, physiological parameter, biochemical parameter, antioxidant system

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Introduction

The native sheep, whose original name was Guizhou semi-fine wool sheep, is a sheep breed hybrid developed by crossing local Tibetan sheep with other breeds [1]. Hence, it has some excellent characteristics, such as good adaptability, greater resistance to disease, great ability of enduring roughage, obedient disposition, easy management, and the quality requirements of forage is not high [2, 3].

Copper (Cu) as one of the essential trace elements of animal, plays an irreplaceable role in hematopoiesis, metabolism, growth and reproduction, maintenance of growth performance and enhancement resistance in body [4]. Lack or excess of Cu will affect the health and performance in animals [5]. Cu deficiency in animals can lead to tissue damage, and reduce meat, wool, milk and lambing rate, etc. Severe Cu deficiency in animals can cause obvious clinical symptoms, such as lackluster clothing hair, joint deformity, decreased reproductive capacity, neurological disorders, ataxia, anemia, even death [6,7]. Cu excesses can also cause loss of appetite, bradykinesia, hemolytic anemia and other symptoms [8-10]. Cu nutrient not only participates in the production of superoxide dismutase (SOD) and ceruloplasmin (CP) but also plays a key role in antioxidant system [11, 12]. Antioxidant system, a defense system of the organisms to free radicals, include the enzymatic and non-enzymatic system [4, 13, 14]. The non-enzymatic system comprise mainly vitamin, cysteine, glutathione (GSH) and mineral element. The enzymatic system consists of antioxidant enzymes, including mainly CAT, SOD and GSH-Px. Antioxidant system can catalyze rapidly the superoxide anion (O^{2-}) to produce disproportionation reaction, eliminate superoxide anion, and protect cells of organism from damage of free radicals [15-18]. Cu supplementation, in the range of growth promotion, can increase activities of GSH-Px, SOD and CP in serum in cattle, pigs and chickens [17]. Li et al. [19] reported that when the Mo level was 0.27 mg/kg in beef cattle diet, adding 10 mg/kg Cu could meet the needs of the body, when the Mo level was 5.27 mg/kg in beef cattle diet, adding 25 mg/kg Cu was more appropriate. Zhang et al. [20] pointed out that the optimum additive amount of Cu based on basic diet (actual measured Cu content in the basic diet was 4.85 mg/kg) was 30.0 mg/kg, which was the most beneficial to immune function and antioxidant enzyme activity of the growing laying hens. Zhang et al. [21] added different levels of Cu to the diet of White Cashmere Goats during the cashmere growing period, the results showed that the growth rate of villi in the 20 mg/kg Cu group was significantly higher than that in the 10 mg/kg group, but there was no significant difference between the 30 mg/kg Cu and the 20 mg/kg Cu. However, the effects of different Cu content in diet on antioxidant enzymes of native sheep were not clear, and the appropriate supplemental level of Cu in diet was not reported in Cu-deficient native sheep [22].

The objective of this experiment is to solve the problem of seasonal Cu deficiency causing adverse effects on antioxidant capacity in the native sheep and provide a scientific basis for the rational addition of Cu in feeds for the native sheep.

Materials and Methods

Experimental Design

10 healthy native sheep, 6-month-old, were selected as the control group (C group) in Dushan County, Guizhou Province, Southwest China, where the environment mineral elements are within the normal range value. 40 Cu-deficient native sheep, 6-month-old, were selected as tested group in Weining County, Guizhou Province, where Cu is lacking. Cu-deficient native sheep were divided randomly into 4 tested groups (n = 10), and supplying $CuSO_4$ (0, 20, 40, 60 mg/kg for group I, group II, group III and group IV, respectively) [23]. The feeding test was carried out for 60 days, and the jugular vein blood was collected in the morning at the end of the feeding experiment, to measure the blood indexes. During the 60-day feeding experiment, C group and group I native sheep maintained the original growth environment and feeding level, as reference.

Sample Collection

On the morning of the end of the feeding trial, the jugular blood of the animals used was collected by aseptic vacuum tubes, collected 20 mL per sheep, keep it at low temperature (4-8ºC), and transported back to the laboratory within 4 hours.

Determination and Method

Mineral Element Content

The contents of manganese (Mn), zinc (Zn), copper (Cu), iron (Fe), molybdenum (Mo) and selenium (Se) in blood were determined by XDY-2A atomic absorption spectrometer (PerkinElmer, Inc., Waltham, MA, USA).

Blood Physiological and Biochemical Indicators

Hemoglobin (Hb), red blood cell count (RBC), packed-cell volume (PCV) and white blood cell count (WBC) in blood were measured by using automatic blood cell analyzer (SF-3000; Sysmex Corporation, Kobe, Japan). The activity or content of albumin (ALB), blood urea nitrogen (BUN), alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (GOT), total cholesterol (TC), ceruloplasmin (CP), copper/zinc-Superoxide dismutase (Cu/Zn-SOD), manganese-superoxide

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Table 1. Effect of Cu level on mineral content in blood in native sheep.

Element	C group	Group I	Group II	Group III	Group IV
Mn ($\mu g \cdot g^{-1}$)	0.242±0.031	0.249±0.025	0.243±0.025	0.243±0.032	0.244±0.031
Zn ($\mu g \cdot g^{-1}$)	0.523±0.023	0.516±0.024	0.529±0.048	0.518±0.038	0.517±0.030
Cu ($\mu g \cdot g^{-1}$)	1.28±0.19a	0.63±0.18b	1.21±0.15a	1.29±0.18a	1.34±0.19a
Fe ($\mu g \cdot g^{-1}$)	437.3±9.0	431.4±9.1	436.3±12.2	432.5±10.6	433.6±9.8
Mo ($\mu g \cdot g^{-1}$)	0.231±0.018	0.215±0.025	0.228±0.023	0.228±0.025	0.218±0.023
Se ($\mu g \cdot g^{-1}$)	0.087±0.016	0.0912±0.012	0.0927±0.013	0.087±0.005	0.084±0.003

Mn = manganese, Zn = zinc, Cu = copper, Fe = iron, Mo = molybdenum, Se = selenium
Note: different little letters show significant difference (P<0.05).

dismutase (Mn-SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were detected by using automatic biochemical analyzer (MindrayBS-420, China).

Data Analysis

SPSS 20.0 (version 20.0 for windows, Chicago, Illinois, USA) was used to analyze the effects of the dietary Cu levels on measures. Multiple mean comparisons were performed using one-way ANOVA. Data are presented as (means±S.D.).

Results

Effect of Cu Level on Mineral Element Content in Blood

The effect of feed with different Cu level on the mineral content in blood is shown in Table 1. The content of Cu in blood in group I (deficient sheep) was significantly lower than that in C group (healthy sheep) (P<0.05), while the contents of Mn, Zn, Mo, Fe and Se were no significant difference between group I and C group (P>0.05). With the increase of Cu content in diet, the content of Cu in blood gradually increases, group IV>group II>C group>group II (P>0.05), group II> group I (P<0.05), while the contents of Mn, Zn, Mo, Fe and Se was no significant change (P>0.05).

Effects of Cu Levels on Blood Index

The effect of feed with different Cu level on blood index in native sheep is shown in Table 2. The Hb, and PCV in group I were significantly lower than those in C group (P<0.05). There was no significant difference in the number of RBC and WBC between two groups (P>0.05). With the increases of Cu content in diet, Hb and PCV were increased (P<0.05), and there was a tendency to increase with the increase of Cu dosage, but no significant increase between Cu addition groups (P>0.05). In addition, the change of Cu level did not cause significant changes in the number of RBC and WBC (P>0.05).

Effect of Different Cu Levels on Blood Biochemical Value

The effect of feed with different Cu level on blood biochemical value in native sheep is shown in Table 3. The content of ALB in group I were significantly lower than those in C group (P<0.05). With the dietary Cu content increase, the content of ALB increased gradually, but there was no significant difference between the $CuSO_4$ addition groups (P>0.05). There were no significant differences in BUN, ALT, ALP, GOT and TC activities between C group, group I, group II, group III and group IV (P>0.05).

Table 2. Effect of Cu level on blood index in native sheep.

Blood indices	Control group	Group I	Group II	Group III	Group IV
Hb ($g \cdot L^{-1}$)	138.2±3.2a	101.4±3.2b	129.1±3.1a	135.1±3.3a	135.9±3.1a
RBC ($\times 10^{12} \cdot L^{-1}$)	17.91±3.15	16.92±1.79	17.48±1.92	17.98±2.80	17.44±1.81
PCV (%)	43.11±3.57a	31.89±2.04b	41.01±2.36a	42.32±3.48a	41.92±2.11a
WBC ($\times 10^{9} \cdot L^{-1}$)	8.43±2.37	9.26±1.93	9.05±1.79	8.93±2.12	9.23±1.75

Hb = hemoglobin, RBC = red blood cell count, PCV = packed-cell volume, WBC = white blood cell count
Note: different little letters show significant difference (P<0.05).

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Table 3. Effect of Cu level on biochemical value in blood in the native sheep.

Biochemical values	C group	Group I	Group II	Group III	Group IV
ALB ($g \cdot L^{-1}$)	28.73±2.87a	27.45±3.25b	29.73±2.84a	31.45±3.15a	30.95±3.20a
BUN ($mmol \cdot L^{-1}$)	6.12±2.01	7.12±1.85	6.57±1.92	6.24±1.80	5.77±1.72
ALT ($IU \cdot L^{-1}$)	11.41±1.87	12.39±2.08	11.91±2.51	11.11±2.42	10.31±1.96
ALP ($IU \cdot L^{-1}$)	291.61±13.21	281.84±19.52	287.33±18.83	291.01±19.25	295.87±17.26
GOT ($IU \cdot L^{-1}$)	36.24±2.90	34.94±3.19	35.84±2.40	35.34±2.98	36.14±3.09
TC ($mmol \cdot L^{-1}$)	2.67±0.28	2.55±0.26	2.61±0.24	2.64±0.18	2.59±0.20

ALB = albumin, VUN = blood urea nitrogen, ALT = alanine transaminase, ALP = alkaline phosphatase,
GOT = aspartate aminotransferase, TC = total cholesterol
Note: different little letters show significant difference ($P<0.05$).

Effects of Different Cu Levels on Antioxidant Capacity

The effect of feed with different Cu level on antioxidant index in native sheep is showed in Table 4. The activity of CP, Cu/Zn-SOD, CAT and GSH-Px in blood in group I were significantly lower than those in C group ($P>0.05$), and the content of MDA was significantly higher than that in C group ($P>0.05$), while the activity of Mn-SOD had no significant difference between two groups ($P>0.05$). After adding different level $CuSO_4$ to diet, the activity of CP, Cu/Zn-SOD, CAT and GSH-Px in blood in the native sheep was group III>group IV>group II>group I ($P>0.05$), and the MDA concentration in blood was group III< group IV<group II<group I ($P>0.05$). In addition, Mn-SOD was little affected by the change of Cu level, had no significant difference between the C group and the experimental groups ($P>0.05$).

Discussion

Oxygen free radicals are the main free radicals in animal body, and even accounting for 95% of the total. There is a dynamic balance between oxygen free radicals' generating and scavenging in health body to ensure body's health [24]. However, when the generation of oxygen free radicals exceeds the body's scavenging capacity, there will be a disorder of redox balance, resulting in damage to a variety of macromolecules (DNA, protein, lipid), and then lead to the occurrence and development of diseases [25, 26].

The antioxidant system of organism is the scavenging system of oxygen free radicals, including enzymatic scavenging system and non-enzymatic scavenging system. It plays a key role in resist antioxidant damage and maintaining oxidation-reduction balance. The enzymes involved in enzymatic scavenging system include GSH-Px, SOD and CAT [27]. Cu is an essential element for animal growth and development. As a component of enzymes, Cu participates in animal metabolism and plays an important role in enhancing the function of antioxidant system [28]. The results shown that the activity of CP, Cu/Zn-SOD, GSH-Px and CAT in blood in group I were significantly lower than those in C group, while the MDA content was significantly higher than that in C group, which indicated that when the animal lack Cu in their diet, the body's antioxidant ability will be reduced. The activity of CP, Cu/Zn-SOD, GSH-Px and CAT in group III were kept at the highest level, while the contents of MDA were at the lowest

Table 4. Effect of Cu level on antioxidant index in blood in the native sheep.

Antioxidant indexes	C group	Group I	Group II	Group III	Group IV
CP ($mg \cdot L^{-1}$)	50.68±2.19a	44.21±1.97b	49.07±3.17a	53.57±2.69a	52.68±1.74a
Cu/Zn-SOD ($U \cdot L^{-1}$)	18.58±1.71a	15.32±1.32b	17.77±2.06a	18.72±2.15a	18.54±1.38a
Mn-SOD ($U \cdot L^{-1}$)	11.22±1.60	9.92±1.64	10.81±1.86	11.37±1.61	11.36±1.65
CAT ($U \cdot L^{-1}$)	1.97±0.18a	1.54±0.18b	1.88±0.19a	1.96±0.23a	1.92±0.15a
GSH-Px ($U \cdot L^{-1}$)	23.48±2.13a	17.74±2.04b	23.07±2.54a	23.88±1.92a	23.25±2.47a
MDA ($nmol \cdot L^{-1}$)	22.05±2.17a	31.87±3.64b	23.34±3.02a	21.95±2.46a	22.07±2.53a

CP = ceruloplasmin, Cu/Zn-SOD = copper/zinc-Superoxide dismutase
Mn-SOD = manganese-superoxide dismutase, CAT = catalase, GSH-Px = glutathione peroxidase MDA = malondialdehyde
Note: different little letters show significant difference ($P<0.05$).

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level. It showed that the antioxidant function of this group was the best. The antioxidant capacity of group I was lower than that of group II and group III, while the antioxidant capacity of group IV was lower than that of group III showed that the function of the antioxidant system is closely related to the content of Cu in the diet, but the antioxidant capacity of the body was not linear with the level of Cu, only by increasing the content of Cu in the diet within a certain range can effectively improve the antioxidant capacity of the body [29, 30].

SOD is an essential component of the antioxidant system, it can antagonize and block the damage of free radicals to cells by scavenging free radicals in animals, and repair the damage in time [31, 32]. SOD can be classified into 4 groups based on the metal residue that binds to the active site: Mn-SOD, Cu/Zn-SOD, iron-superoxide dismutase (Fe-SOD) and nickel-superoxide dismutase (Ni-SOD) [33,34]. Mn-SOD helps to protect the cells from the adverse effects of excess ROS in aerobic organisms. It has the capacity to convert the superoxide anion into H_2O_2 and O_2 [35, 36]. Cu/Zn-SOD is the most common of the four kinds of SOD, so increasing the level of Cu in vivo is helpful to synthesis and improve Cu/Zn-SOD [37]. When Cu nutrient is deficient or excessive, lipid peroxidation produces a lot of MDA, consumes a large amount of SOD and GSH-Px, and finally cause a significant decrease of SOD and GSH-Px content, and free radical scavenging capacity decreased [38, 39]. CP is the main carrier protein of Cu in blood, synthesized by Cu and protein in liver. It has the similar effect as superoxide dismutase in scavenging superoxide anion free radicals and inhibiting lipid peroxidation [40]. The results showed that CP activity increased with the increase of Cu content in diet. However, when 60 mg/kg was added to the diet, the activity of CP decreased significantly. The mechanism may be that the high Cu diet increased the deposition of Cu in the liver of native sheep, reduced the ability of liver to convert Cu to CP, resulting the activity of CP decreased [41]. The results showed that the dosage of 60 mg/kg $CuSO_4$ had exceeded the optimum range for native sheep to copper.

Conclusion

The function of antioxidant system in Cu deficiency native sheep was severely reduced. Adding appropriate amount of Cu can improve the function of antioxidant system. According to the experimental results, added 40 mg/kg $CuSO_4$ to the diet, the activity of antioxidant enzyme was the highest and concentration of MDA was the lowest in grazing the native sheep, and other blood indexes were the closest to those in healthy sheep. Therefore, the optimum dietary $CuSO_4$ level to native sheep was 40 mg/ kg. It is suggested that this dosage be added.

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Conflict of Interest

The authors declare no conflict of interest.

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