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Original research

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Keywords
Przewalski's gazelle, Mo poisoning, Copper sulfate, Qinghai Lake basin

Abstract
This paper aimed to study the effect of copper sulfate fertilization (90 kg/hm2) on molybdenum (Mo) poisoning of the Przewalski's gazelle□P. przewalskii□ in the Qinghai Lake basin. Soil, pasture, and the Przewalski's gazelle's blood and hair samples were collected from the animal rescue center in this study. The biochemical parameters of the Przewalski's gazelle's blood and the mineral contents of the fodder and soil were ascertained. The findings demonstrated that the impacted pastures' soil and forage had considerably greater Mo levels ($p < 0.01$) than those of the healthy pastures. Compared to the control group, the experimental gazelles had significantly lower Mo content in the blood and hair, while the impacted gazelles had significantly higher copper and sulfur contents in the blood and hair ($p < 0.01$). The levels of blood Hb, PCV, MCV, and MCH of the Przewalski's gazelle in the fertilized group had been extensively greater than those in the control group ($p < 0.01$). The tiers of blood AST, LDH, CPK, and ALP of the Przewalski's gazelle in the fertilized group had been extensively greater than those in the control group. The contents of TP, ALB, and GLB in the fertilized group had been substantially decreased than those in the control group. In comparison to the control group, the fertilized group exhibited considerably higher activity of serum GSH-Px, SOD, T-AOC, and CAT, and a significantly lower MDA concentration. The results showed that the appropriate application of copper sulfate could significantly increase the copper content in forage, regulate the molybdenum-copper ratio in soil, and thus effectively improve the molybdenum toxicity symptoms of Przewalski's gazelle, improve the antioxidant ability of the gazelle, and alleviate the molybdenum toxicity condition.

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Effects of fertilized copper sulfate on the Przewalski's gazelles of molybdenosis in the Qinghai Lake Watershed

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Abstract:

This paper aimed to study the effect of copper sulfate fertilization (90 kg/hm^2) on molybdenum (Mo) poisoning of the Przewalski's gazelle（*P. przewalskii*） in the Qinghai Lake basin. Soil, pasture, and the Przewalski's gazelle’s blood and hair samples were collected from the animal rescue center in this study. The biochemical parameters of the Przewalski's gazelle's blood and the mineral contents of the fodder and soil were ascertained. The findings demonstrated that the impacted pastures' soil and forage had considerably greater Mo levels ($p < 0.01$) than those of the healthy pastures. Compared to the control group, the experimental gazelles had significantly lower Mo content in the blood and hair, while the impacted gazelles had significantly higher copper and sulfur contents in the blood and hair ($p < 0.01$). The levels of blood Hb, PCV, MCV, and MCH of the Przewalski's gazelle in the fertilized group had been extensively greater than those in the control group ($p < 0.01$). The tiers of blood AST, LDH, CPK, and ALP of the Przewalski's gazelle in the fertilized group had been extensively greater than those in the control group. The contents of TP, ALB, and GLB in the fertilized group had been substantially decreased than those in the control group. In comparison to the control group, the fertilized group exhibited considerably higher activity of serum GSH-Px, SOD, T-AOC, and CAT, and a significantly lower MDA concentration. The results showed that the appropriate application of copper sulfate could significantly increase the copper content in forage, regulate the molybdenum-copper ratio in soil, and thus effectively improve the molybdenum toxicity symptoms of Przewalski's gazelle, improve the antioxidant ability of the gazelle, and alleviate the molybdenum toxicity condition.

Keywords: Przewalski's gazelle; Mo poisoning; Copper sulfate; Qinghai Lake basin

Introduction

The Przewalski's gazelle is a member of the genus Procapra, kindred Bovidae, subfamily Antelopidae, the order Artiodactyla. Its range of activity was very small and had appeared in Qinghai, Ningxia, Inner Mongolia, and Gansu[1]. Due to the fragmentation, degradation, and reduction of its natural habitat as a result of increased human activities, the variety of the Przewalski's gazelle has declined drastically, and it is now only found in parts of the area around the Qinghai Lake[2, 3]. The Przewalski's gazelle is one of the most endangered species on the IUCN Red List, as well as a national first - class wildlife - protected animal in China and a flagship species unique to Qinghai Lake[4]. The 2023 Qilian Mountain News reported that Przewalski's gazelle population has now reached more than 3,500, from less than 300 at the beginning of the monitoring period to more than 3,000 at present[5]. Although the Przewalski's gazelle population is growing at the moment, yet is still additional work to be done to preserve and boost this species' numbers[6].

Mo is one of the rare elements that are refractory to melt in nature, and in areas with high Mo content, due to the high Mo content in the soil, forage, and water sources, the Mo content in animals also increases, and Mo poisoning is prone to occur[7]. Due to the large amount of Mo in the discharge of municipal sewage and industrial pollutants and its refractory nature, there is still a huge risk of discharging into soil and rivers after treatment.[8] The level of Mo in the buffalo's hair and hooves was discovered to be significantly higher than normal when the grass was planted with the soil followed sewage treatment and fed to the animals. Also, the amount of Mo in the surrounding plants and soil at the sewage treatment discharge point exceeded compared to what was permitted worldwide. The best test for Mo poisoning is a blood test[9]. A comparable state of affairs befell at the Qinghai Lake Animal Rescue Centre, the place of excessive Mo levels in the soil and forage resulted in greater Mo levels in the hair and blood of the Przewalski's gazelle than in different healthful areas, leading to Mo toxicity in the Przewalski's gazelle[10]. Distribution of Mo content in the soil of China, the Mo content is high in parts of northeast, northwest, and south China. The Qinghai Lake, located in northwestern China, has areas of excessively high Mo content in the soil and pasture, resulting in Mo poisoning after animal foraging[11].

Some studies have found that ruminants have a more pronounced response to Mo poisoning, with symptoms such as diarrhoea, hair discolouration, lethargy, osteoporosis, neurological disorders, and death, which then results in reduced immune function and impairment of physiological and reproductive functions[12, 13]. It has also been shown that excessive Mo level in animals can block the absorption of copper (Cu) and sulfur (S), resulting in Cu and S deficiencies, as well as cause apoptosis and increase the apoptotic index of splenocytes and thymocytes[14, 15]. Excessive Mo affects the micro-ecological balance in the rumen and interferes with the absorption and utilization of other elements[16]. The purpose of this study was to investigate the Mo content in the Qinghai Lake Animal Rescue Center and to explore ways to reduce the Mo content in the Przewalski's gazelle and prevent Mo poisoning. This

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furnished a basis for in addition perception of the metabolic mechanism of the Przewalski's gazelle in a high Mo environment.

Experimental

2 Materials and methods

2.1. Forage study

This study was conducted at the Qinghai Lake Animal Rescue Center. At a point of more than 3,200 meters above the ocean's surface, the Qinghai Lake basin endures an average yearly temperature of -1.5 to 1.5 °C. The average annual rainfall ranges from 350 to 450 mm, and most of the rainfall is concentrated from July to September[17, 18]. The unique geographic location of Qinghai Lake has formed grassland-based vegetation types, mainly Achnatherum splendens (temperate steppe type), Thermopsis lanceolata, Stipa purpurea Griseb (alpine steppe type), Artemisia frigida, Polygonum viviparum (alpine meadow type), and many other types of forage resources[19, 20]. The variety and nutrient-rich herbs provide a good source of food for the animals.

2.2. Experimental design

This study was conducted at the Qinghai Lake Animal Rescue Centre. CuSO4 was purchased from Lanno Chemical Reagent Distribution Department, Chengbei District, Xining City, Qinghai Province, with a purity of more than 99 %. Previous to the trial, the mineral content of the soil and forage was determined by testing and analysis (Table 1). The experiment was conducted by fencing 32 hm^2 of grassland with high Mo content, divided into two groups (control group and fertilizing group), four replicates/groups, and 2 hm^2/replication. The application rate of CuSO4 was 90 kg/hm^2 (the fertilizer rate of CuSO4 application was 45 kg/hm^2, and 1 kg was irrigated with a ratio of 600 kg of water). Twenty Przewalski's gazelles (similar weight and size) were selected as test carriers, randomly divided into two groups with ten animals in each group, and arbitrarily assigned to the grazing land for 120 days. The take a look at samples have been all gathered at the end of the experiment.

2.3. Sample collection

The soil and forage samples were accumulated randomly, 18 soil samples (9 samples/group) were gathered from the soil surface (0 - 30 cm) of the forage the usage of a 31-mm-diameter cylindrical corer, dried at 60 - 80 °C for 48 h, comminuted, then the soil samples have been got by way of the use of a 0.06 mm mesh sieve to do away with other impurities. To prevent soil contamination, 18 forage samples (9 samples per group) were cut 1 to 2 cm above the ground. They were then collected and dried for eighty hours at 40 °C. The dried soil and forage samples were analyzed for chemical and mineral contents. Samples from both blood and hair were taken from the Przewalski's gazelle's neck. Upon collection, samples of hair were rinsed six times with distilled water and cleaned with acetone before being placed on silica gel in a desiccator to be detected and inspected. Using sterile vacuum blood collection tubes filled with sodium heparin and EDTA-K2, blood samples were drawn from the jugular vein and kept at 4 °C for assay analysis. After centrifuging the serum samples for ten minutes at 3500 rpm, they were kept at -80 °C for further examination.

2.4. Determination of samples

Manganese (Mn), zinc (Zn), copper (Cu), and iron (Fe) contents in the soil, forage, and serum were determined by AA-1800H (Meixi, Analytical Instruments, Shanghai, China) atomic absorption spectrometer. Mo content was determined using an AAS6000 (Tianrui Instrument Co, Jiangsu, China) flame atomic absorption spectrometer. For the determination of elemental contents, multiple small amounts were used to minimize errors. S content was determined and analyzed using the barium sulfate gravimetric method. Haemoglobin (Hb), erythrocyte pressure-volume (PCV), mean corpuscular volume of erythrocytes (MCV), red blood cells (RBC), and white blood cells (WBC) were determined by using an animal hematology analyzer (Pucon PE-6800VET). Then, using the formulas MCH = HB/RBC and MCHC = HB/(RBC × MCV), the mean hemoglobin content of erythrocytes (MCH) and mean hemoglobin concentration of erythrocytes (MCHC) were obtained. Albumin (ALB), alanine aminotransferase (ALT), total protein (TP), alkaline phosphatase (ALP), aspartate aminotransferase (AST), cholesterol (CHOL), phosphocreatine kinase (CPK), creatinine (CR), globulin (GLB), and lactate dehydrogenase (LDH) were measured using a fully automated biochemical analyzer (URIT-8401) (Jumo Medical Devices, Shanghai, China). The oxidative indexes were measured by Nanjing Jiancheng reagent kits (Institute of Biological Engineering, Nanjing， China). Oxidative indexes were determined using Nanjing built kit for glutathione peroxidase (GSH-Px), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (T-AOC).

2.5. Statistical analyses

Data were analyzed using the Statistical Package (SPSS, version 23.0, Inc., Chicago, Illinois, USA) and presented in the form of mean ± standard deviation (SD). Significant differences between groups were assessed using Student's t-test and the $p < 0.01$ indicated a very significant difference.

Results and Discussion

3 Results

3.1.The mineral contents in the soil and forage

The Mo concentration in the soil and forage from the ranches of rescue center was 4.32 ± 0.34 μg/g and 4.12 ± 0.31 μg/g, respectively, which was noticeably higher than that in the healthy ranches ($p < 0.01$ Table 1).

The Mo content in the fertilized pasture was significantly lower than that in the control pasture. The Cu and S contents in the fertilized pasture have been substantially greater than those in the control group (Table 2).

The concentrations that Cu in *P. przewalskii* blood and hair were 1.67 ± 0.59 μg/g and 3.66 ± 0.72 μg/g, respectively. Table 3 shows that these values were much greater than those in the control group ($p < 0.01$). The Mo material was found to be considerably greater in the *P. przewalskii* blood and hair, measuring 0.13 ± 0.01 μg/g and 1.53 ± 0.05 μg/g, respectively, compared to the control group ($p < 0.01$, Table 3). The S content material in the blood and hair of the *P. przewalskii* was once 3.11 ± 0.43 μg/g and 4.33 ± 0.41 μg/g, respectively, which was once appreciably improved than that in the control group ($p < 0.01$, Table 3). There had been no considerable variations in other elements.

3.2. The physiological levels in the blood of the Przewalski's gazelle

Compared with the control group, the degrees of Hb, PCV, MCV, and MCH in the fertilizing group were substantially improved than those in the control team (p < 0.01). There have been no significant variations in the levels of RBC, MCHC, and WBC (Table 4).

3.3. The blood biochemical parameters of the Przewalski's gazelle after $CuSO_4$ application

Compared with the control group, the levels of AST, LDH, CPK, and ALP in the fertilizing group were significantly increased. The levels of TP, ALB, and GLB in the fertilizing group had been extensively reduced than those in the control group ($p < 0.01$). There had been no enormous variations in the levels of ALT, CR, and Chol (Table 5).

3.4. Effect of $CuSO_4$ application on the antioxidant index of the Przewalski's gazelle

In comparison to the control group, the fertilized group displayed appreciably decreased ($p < 0.01$) MDA levels, but appreciably improved ($p < 0.01$) GSH-Px, SOD, T-AOC, and CAT levels (Table 6).

4 Discussion

Mo, one of the dietary trace element components, is essential for the healthy survival of animals and humans. It is obtained primarily that high levels of Mo in the diet, soil and pasture can lead to illness and death of animals[21]. Mo toxicity in ruminants usually occurs on pastures where Mo is naturally concentrated or industrially contaminated, and studies of the mineral content in the soil, pasture, and animal (blood, hair) can help to understand an animal's health status and growth needs[22, 23]. It is well established that Cu and Mo are reciprocally antagonistic in animal metabolism[24]. High Mo level in the soil and forage, leads to increased Cu deficiency in the animal. The Cu content material in the hair and blood is additionally a touchy indicator for diagnosing Cu deficiency. After fertilizing the soil with $CuSO_4$ compared to the unfertilized group, the Cu content in the blood and hair of the Przewalski's gazelle increased significantly and the fertilization of $CuSO_4$ prevented and cured diseases caused by Mo poisonin[25]. Studies have shown that moderate copper supplementation on Mo-contaminated pastures does not adversely affect the health of dairy cows. Reduced Mo level in animals, thus reducing symptoms of Mo toxicity[26, 27]. Other studies have shown that dietary molybdenum concentrations of 5- 50 mg Mo/kg DM reduce copper levels in ruminants[28]. In this study, the Mo level was significantly higher in the test sites than that in healthy pasture. In addition, increased Mo levels in the soil and pasture affected the absorption of Cu in the animals. The Cu awareness in the hair and blood of Przewalski's gazelle used to be extensively decreased than that in the control group, and the Mo attention in the affected gazelles used to be appreciably greater than that in the unaffected gazelles. This leads to impaired absorption and secondary Cu deficiency[29]. Another test used to assess an animal's mineral nutritional condition is the Cu content in its blood and hair[22]. Generally, Cu is present in the blood in the form of ceruloplasmin (Cp), which influences Fe absorption and utilization. Meanwhile, when Cu level is lowered, Cu deficiency coupled with impaired Fe absorption and utilization significantly reduces Cp and is associated with anemia[30]. When the mean value of Cu in the blood was less than 0.05 μg/g, it indicated that the animal was severely Cu deficient[31]. In this study, the application of $CuSO_4$ reduced the Mo level in Przewalski's gazelle, which is consistent with the results of previous studies[32]. As Mo intake increases, the concentration of Mo in the animal's blood rises rapidly. Mo that enters the bloodstream becomes a highly dialysable cation that participates in blood circulation and enters tissues such as bone, muscle, and fur[10, 33].

A defense system against oxidative stress is built into all living organisms, which consists of a variety of antioxidant[34]. The antioxidant system is a free radical scavenging defense system consisting of T-AOC, GSH-Px, MDA, and other antioxidant enzymes[18], The activities of these enzymes are closely related to mineral nutrition[35]. The increase of T-AOC can expand the antioxidant potential of the liver and spleen, and decorate the antioxidant popularity of the spleen and liver below oxidative stress, as properly as alleviate the manufacturing of giant quantities of lipid peroxides and free radicals[36, 37]. GSH-Px defends against cellular damage caused by reactive oxygen specie[12]. Less lipid oxidation took place in meat samples with greater GSH-Px activity, whereas accelerated lipid oxidation in salted meat may also be related to decreased GSH-Px recreation[38]. Consistent with the outcomes of the current study, the GSH-Px content material of the Przewalski's gazelle used to be improved through fertilization with $CuSO_4$, which enhanced the body's ability to resist oxidative losses. MDA is one of the representative end products of lipid peroxidation and can mirror the diploma of lipid peroxidation in the body[39]. In this study, the activities of GSH-Px, T-AOC, SOD, and CAT of the Przewalski's gazelle in the fertilized group had been substantially greater than those in the control group. The content material of MDA used to be drastically decreased than that in the control group. The application of $CuSO_4$ had a vast impact on the content of mineral factors in the pasture. The Cu content material in the hair and blood of the Przewalski's gazelle in the fertilized group was once greater than in the control group. This indicated that the application of $CuSO_4$ increased the Cu content and decreased the Mo content in the animal's body, thus alleviating the Mo poisoning of the Przewalski's gazelle. Therefore, the application of $CuSO_4$ in the soil can indirectly improve the antioxidant capacity of the Przewalski's gazelle.

The results of the study confirmed that the Cu content material of Mo-poisoned Przewalski's gazelle was once extensively decreased than the normal level, while the Mo content material used to be drastically higher than that in the control group. However, after the application of $CuSO_4$, the Cu content of the Przewalski's gazelle in the fertilization group increased significantly, while the Mo content decreased significantly. This suggested that the application of $CuSO_4$ could effectively regulate the balance of Cu and Mo in the Przewalski's gazelle and alleviate the symptoms of Mo toxicity. In conclusion, after the application of the appropriate amount of $CuSO_4$ in the Qinghai Lake Animal Rescue Centre, the Cu content of plants in the area increased significantly. This indicates that the application of $CuSO_4$ can effectively improve the Mo poisoning symptoms of the Przewalski's gazelle.

5 Conclusion

The results showed that high Mo concentration in the soil and pasture significantly increased the Mo level in the blood and hair of the Przewalski's gazelle, leading to Mo toxicity in the Przewalski's gazelle. Physiology, immune function, and antioxidant capacity were reduced in the Mo-poisoned Przewalski's gazelles. Soil fertilized with CuSO4 significantly reduced Mo content in forage and Przewalski's gazelles' blood and hair, thus alleviating the Mo poisoning phenomenon.

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Conflict of Interest

The authors declare no conflict of interest.

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Table 1. The mineral contents in soil and forage

Elements	Soils		Forages	
	Effected ranches	Healthy ranches	Effected ranches	Healthy ranches
Cu (µg/g)	14.73±1.22	14.23±1.31	5.37±0.33	5.47±0.31
Mo (µg/g)	4.32±0.34*	1.31±0.12	4.12±0.31*	1.41±0.12
Fe (µg/g)	4251.35±56.14	4261.67±54.69	341.25±25.44	329.71±25.92
Mn (µg/g)	61.37±6.43	62.47±5.83	14.55±1.23	14.37±1.34
Co (µg/g)	4.66±0.47	4.41±0.39	1.23±0.07	1.24±0.06
S (%)	1.23±0.12	1.22±0.14	0.21±0.09	0.20±0.02

Cu, copper; *Mo*, molybdenum; *Fe*, iron; *Mn*, manganese; *Co*, cobalt; *S*, sulfur
*indicates significant differences at the level of $p < 0.01$.

Table 2. Effects of fertilized $CuSO_4$ on mineral contents in the forage

Elements	Forages	
	Control ranch	Fertilized ranch
Cu (µg/g)	5.37±0.33	7.47±0.31*
Mo (µg/g)	4.11±0.32	1.43±0.14*
Fe (µg/g)	332.27±27.33	331.23±27.55
Co (µg/g)	1.35±0.08	1.31±0.08
S (%)	0.21±0.02	0.31±0.03*

*indicates significant differences at the level of $p < 0.01$.

Table 3. Effects of fertilized $CuSO_4$ on mineral contents in the blood and hair of the *P. przewalskii*

Elements	Blood		Hairs	
	Control group	Fertilized group	Control group	Fertilized group
Cu (µg/g)	0.13±0.02	1.67±0.59*	2.37±0.23	3.66±0.72*
Mo (µg/g)	2.37±0.13	0.13±0.01*	5.57±0.38	1.53±0.05*
Fe (µg/g)	439.33±27.56	438.72±25.78	317.22±25.31	317.41±28.33
Mn (µg/g)	0.57±0.03	0.53±0.04	13.62±1.33	13.44±1.91
Co (µg/g)	0.47±0.03	0.43±0.05	1.23±0.03	1.31±0.01
S (%)	1.22±0.11	3.11±0.43*	1.83±0.73	4.33±0.41*

*indicates significant differences at the level of $p < 0.01$.

Table 4. Effects of fertilized $CuSO_4$ on hematological levels in the *P. przewalskii*

Parameters	Control group	Fertilized group
Hb (g/L)	97.56±5.37	117.22±11.32*
RBC (10^{12}/L)	5.97±0.53	5.89±0.53
PCV (%)	37.87±3.33	56.77±5.31*
MCV (fL)	47.32±3.27	55.51±4.55*
MCH (pg)	11.55±1.23	15.77±1.57*
MCHC (%)	24.47±2.32	24.32±2.33
WBC (10^9/L)	7.65±0.72	7.54±0.68

Hb, hemoglobin; *RBC*, red blood cell; *PCV*, packed cell volume; *MCV*, mean corpuscular volume; *MCH*, mean corpuscular hemoglobin; *MCHC*, mean corpuscular hemoglobin; *WBC*, white blood cell

*indicates significant differences at the level of $p < 0.01$.

Table 5. Effects of fertilized $CuSO_4$ on biochemical parameters in the *P. przewalskii*

Parameters	Control group	Fertilized group
AST (U/L)	97.34±7.33	123.71±11.43*
ALT (U/L)	45.77±4.37	44.76±4.32
LDH (U/L)	429.33±37.63	543.77±52.33*
CPK (U/L)	273.56±27.33	373.55±31.33*
ALP (U/L)	568.23±54.97	856.77±63.77*
CR (µmoL/L)	77.73±7.01	75.67±7.57
TP (g/L)	57.47±3.33	35.77±3.25*
ALB (g/L)	43.33±3.23	23.01±2.12*
GLB (g/L)	27.55±2.33	15.43±1.34*
Chol (mmol/L)	2.37±0.21	2.43±0.25

AST, aspartate aminotransferase; *ALT*, alanine aminotransferase; *LDH*, lactate dehydrogenase; *CPK*, phosphocreatine kinase; *ALP*, alkaline phosphatase; *CR*, creatinine; *TP*, total protein; *ALB*, albumin; *GLB*, globulin; *Chol*, cholesterol

*indicates significant differences at the level of $p < 0.01$.

Table 6. Effects of fertilized CuSO4 on antioxidant parameters in the *P. przewalskii*

Items	Control group	Fertilized group

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GSH-Px (U/mL)	141.33±16.89	339.71±23.31*
SOD (U/mL)	57.43±5.33	78.35±7.33*
T-AOC (U/mL)	3.23±0.13	6.77±0.63*
CAT (U/mL)	5.77±0.53	15.77±1.63
MDA (nmol/L)	37.47±3.65	16.55±1.77*

GSH-Px， glutathione peroxidase; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; CAT, catalase; MDA, malondialdehyde

*indicates significant differences at the level of $p < 0.01$.

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