Effects of Different Copper Sources and Levels on Growth Performance, Nutrient Digestibility, and Serum Indices in Growing Blue Foxes (Postprint)

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Abstract

This experiment was conducted to investigate the effects of different copper sources and levels in diets on growth performance, nutrient digestibility, and serum indices of growing blue foxes. One hundred healthy 55-day-old blue foxes were selected and randomly divided into 5 groups with 20 replicates per group and one fox per replicate. The five groups were fed experimental diets supplemented with 0, 50, or 100 mg/kg (as copper) of copper sulfate pentahydrate (CuSO45H2O) or copper methionine (Met-Cu) based on the basal diet, with the 0 mg/kg group serving as the control for both copper sources. The preliminary period lasted 7 d, and the formal experimental period lasted 60 d. The results showed: 1) Dietary copper level had an extremely significant effect on average daily gain of growing blue foxes ($P<0.01$) and a significant effect on feed-to-gain ratio ($P<0.05$). Dietary copper source had no significant effects on average daily feed intake, average daily gain, or feed-to-gain ratio of growing blue foxes ($P>0.05$). 2) Dietary copper level had extremely significant effects on crude protein digestibility and fecal copper content ($P<0.01$) and significant effects on dry matter digestibility and crude fat digestibility ($P<0.05$). Dietary copper source had a significant effect on fecal copper content of growing blue foxes ($P<0.05$), with the copper methionine group having significantly lower fecal copper content than the copper sulfate group ($P<0.05$); dietary copper source had no significant effects on dry matter digestibility, crude fat digestibility, crude protein digestibility, or nitrogen retention of growing blue foxes ($P>0.05$). 3) Dietary copper source and level had extremely significant effects on serum urea nitrogen content ($P<0.01$), with the 50 and 100 mg/kg copper supplementation groups having extremely significantly higher serum urea nitrogen content than the control group ($P<0.01$), and the copper methionine group having extremely significantly higher serum urea nitrogen content than the copper sulfate group ($P<0.01$). Dietary copper source and level had no significant effects on serum total protein, albumin, globulin, copper, iron contents, or aspartate aminotransferase and alanine aminotransferase activities (P>0.05). In conclusion, supplementing copper methionine in diets of growing blue foxes can achieve similar growth-promoting effects as copper sulfate; furthermore, copper methionine has higher utilization efficiency and lower environmental emissions, making it a more efficient and environmentally friendly organic copper source for blue fox diets.

Full Text

Effects of Different Copper Sources and Levels on Growth Performance, Nutrient Digestibility and Serum Indices of Growing Blue Foxes

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Abstract: This experiment was conducted to investigate the effects of dietary copper sources and levels on growth performance, nutrient digestibility, and serum indices of growing blue foxes. One hundred healthy 55-day-old blue foxes were selected and randomly divided into 5 groups with 20 replicates per group and 1 fox per replicate. The five groups were fed experimental diets consisting of a basal diet supplemented with 0, 50, or 100 mg/kg copper (as copper) from either copper sulfate pentahydrate (CuSO ·5H O) or copper methionine (Met-Cu). The 0 mg/kg supplementation group served as the control for both copper sources. The pre-experimental period lasted 7 days, followed by a 60-day formal experimental period. The results showed: (1) Dietary copper level had a highly significant effect on average daily gain (P<0.01) and a significant effect on feed-to-gain ratio (P<0.05) of growing blue foxes. Dietary copper source had no significant effects on average daily feed intake, average daily gain, or feed-to-gain ratio (P>0.05). (2) Dietary copper level had highly significant effects on crude protein digestibility and fecal copper content (P<0.01) and significant effects on dry matter digestibility and ether extract digestibility (P<0.05). Dietary copper source had a significant effect on fecal copper content (P<0.05), with the copper methionine group showing significantly lower fecal copper content than the copper sulfate group (P<0.05). Dietary copper source had no significant effects on dry matter digestibility, ether extract digestibility, crude protein digestibility, or nitrogen retention (P>0.05). (3) Dietary copper source and level had a highly significant effect on serum urea nitrogen content

(P<0.01). The 50 and 100 mg/kg copper supplementation groups showed significantly higher serum urea nitrogen content than the control group (P<0.01), and the copper methionine group showed significantly higher serum urea nitrogen content than the copper sulfate group (P<0.01). Dietary copper source and level had no significant effects on serum total protein, albumin, globulin, copper, or iron contents, nor on aspartate aminotransferase or alanine aminotransferase activities (P>0.05). In conclusion, supplementing copper methionine in diets for growing blue foxes can achieve similar growth-promoting effects as copper sulfate. Moreover, copper methionine demonstrates higher utilization efficiency and lower environmental emissions, representing a more efficient and environmentally friendly organic copper source for blue fox diets.

Keywords: copper source; copper level; growing period; growth performance; blue fox

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Introduction

Copper is an essential trace element for animals that plays crucial physiological roles in maintaining normal iron metabolism, promoting bone development, and participating in intracellular energy metabolism. Long-term copper deficiency in animals leads to growth retardation, abnormal bone development, and ataxia, severely affecting growth performance and economic value [1]. Currently, copper sulfate (CuSO) is the most commonly used copper source in animal feed formulation due to its effective copper supplementation, low cost, and easy availability, making it widely adopted in production. Studies on weaned piglets have shown that dietary supplementation with copper sulfate at certain levels (125–250 mg/kg) significantly promotes growth [2]. Previous research on growing blue foxes indicated that a dietary copper level of 40 mg/kg (from copper sulfate) improved growth rate and feed conversion efficiency [3]. However, copper sulfate presents several practical problems. Research shows that animals have low utilization efficiency of inorganic copper, with most copper sulfate in feed being excreted in feces without being utilized by the body. This not only wastes copper but also causes heavy metal pollution in the environment [4]. Additionally, high copper levels reduce the absorption of other dietary mineral elements such as zinc and iron, and accelerate the oxidative deterioration of antioxidants and fats in feed [5].

Organic copper offers advantages including higher biological utilization, better palatability, reduced antagonism among mineral elements, and less damage to reducing substances in feed [6]. Using organic copper to replace copper sulfate can effectively improve copper utilization and reduce copper emissions. With increasing environmental pressures, the advantages of organic copper become more pronounced, leading to its gradual recognition and adoption by more feed producers. Copper methionine (Met-Cu) is a chelated product of methionine and copper that can be absorbed through amino acid pinocytosis, alleviating competitive antagonism among trace elements, greatly improving copper ion absorption and utilization, and reducing copper' s destructive effects on reducing substances such as vitamin C and selenium, as well as fat oxidation [7]. Deng et al. [8] compared copper methionine with copper sulfate and found that piglets had higher utilization efficiency of copper methionine. Wu [9] compared the biological utilization of three copper sources—copper sulfate, copper methionine, and basic copper chloride—in mink, reporting that the biological utilization of copper methionine was 124.35% relative to copper sulfate during the growing period. Currently, research on the biological utilization of different copper sources has mainly focused on pigs and poultry, while systematic studies on organic copper utilization in blue foxes have not been conducted. Therefore, this experiment used copper methionine as a representative organic copper source to investigate the effects of copper methionine and copper sulfate on growth performance, nutrient digestibility, and serum indices of growing blue foxes, providing theoretical guidance for the scientific application of organic copper in blue fox production.

Materials and Methods

1.1 Experimental Diets As there is currently no unified nutrient requirement standard for blue foxes, this study designed a basal diet for growing blue foxes based on the NRC [10] nutrient requirements for blue foxes and recent domestic and international literature on blue fox nutrition [11-13]. The composition and nutrient levels of the basal diet are shown in Table 1 . The measured copper content of the basal diet was 14.44 mg/kg dry matter (DM). Experimental diets were formulated by supplementing the basal diet with 0, 50, or 100 mg/kg copper (as copper) from either copper sulfate pentahydrate (CuSO ·5H O) or copper methionine. Since copper methionine introduces varying amounts of methionine along with copper, which would cause differences in dietary methionine content among groups, this experiment used the methionine amount introduced in the "highest copper methionine group" as the baseline. Additional methionine was supplemented in other groups to match this level, ensuring consistency in dietary methionine levels across all experimental diets.

1.2 Experimental Design and Management The experiment selected 100 healthy blue foxes at (55±5) days of age, which were randomly divided into 5 groups with 20 replicates per group (10 males and 10 females) and 1 fox per replicate. The five groups were fed experimental diets supplemented with 0, 50, or 100 mg/kg copper from either copper sulfate pentahydrate or copper methionine, with the 0 mg/kg supplementation group serving as the control for both copper sources. The pre-experimental period lasted 7 days, followed by a 60-day formal experimental period.

All experimental blue foxes were housed individually in cages (105 cm × 80 cm × 70 cm). Before the experiment began, foxes received routine immunization.

They were fed twice daily at 08:00 and 15:00 with ad libitum access to feed and water. Actual feed intake was recorded daily.

1.3 Digestion-Metabolism Trial Thirty days after the start of the formal experimental period, 8 healthy blue foxes were randomly selected from each group for a 5-day digestion-metabolism trial. The total feces collection method was used, with feces and urine collected daily using a fecal-urine separable collection tray. Management during the metabolism trial was identical to daily management. Feed samples, feces, and urine were collected daily, with detailed records of feed intake and leftover feed for each fox. After weighing, feces were preserved by adding 10% sulfuric acid solution at 5% of fresh weight plus a small amount of toluene as a preservative, and stored at -20°C. Urine was preserved by adding 10 mL of 10% sulfuric acid solution per 100 mL urine plus 4 drops of toluene, and stored at -20°C.

1.4.1 Growth Performance On the first morning of the formal experimental period, blue foxes were weighed after fasting to obtain initial body weight. Body weight was subsequently recorded every 15 days to calculate average daily gain and feed-to-gain ratio. Daily feed allowance and leftover feed were recorded for each fox to calculate average daily feed intake.

1.4.2 Nutrient Digestibility Dry matter content in diets and feces was determined using the 65°C drying method according to GB/T 6435-2006. Crude protein content was determined using the Kjeldahl nitrogen method according to GB/T 6432-1994. Ether extract content was determined using the Soxhlet extraction method according to GB/T 6433-2006. Copper content in diets, liver, and excreta was determined using flame atomic absorption spectrometry (GB/T 13885-2003).

Dry matter digestibility (%) = [(dry matter intake - dry matter excretion) / dry matter intake] $\times$ 100
Crude protein digestibility (%) = [(crude protein intake - fecal crude protein) / crude protein intake] $\times$ 100
Ether extract digestibility (%) = [(ether extract intake - fecal ether extract) / ether extract intake] $\times$ 100
Nitrogen retention (g/d) = nitrogen intake - fecal nitrogen - urinary nitrogen

1.4.5 Serum Indices Serum total protein (TP), albumin (ALB), globulin (GLO), urea nitrogen (UN), copper (Cu), and iron (Fe) contents, as well as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, were measured using a Hitachi 7020 automatic biochemical analyzer. Reagent kits were purchased from Zhongsheng Beikong Biotechnology Co., Ltd., and measurements were performed strictly according to kit instructions. Serum ceruloplasmin (Cp) and copper-zinc superoxide dismutase (Cu-Zn SOD) activities were measured using a UV/Vis spectrophotometer (Jena, Germany). Reagent kits were purchased from Nanjing Jiancheng Bioengineering Institute, and measurements were performed strictly according to kit instructions.

1.5 Data Processing and Statistical Analysis Experimental data are expressed as mean ± standard deviation. Data were analyzed using the GLM procedure of SAS 9.1.3 software. Two-way ANOVA was performed with copper source, copper level, and their interaction as factors. The model was:

Yijk = + Ai + Bj + (AB)ij + ijk

Where Yijk is the observed value, is the overall mean, Ai is the main effect of copper source, Bj is the main effect of copper level, (AB)ij is the interaction between copper source and copper level, and ijk is the random error. Duncan' s multiple comparison test was used to compare significant main effects, and the LSMEANS procedure with PDIFF method was used for significant interactions.

Results

2.1 Effects of Different Copper Sources and Levels on Growth Performance of Growing Blue Foxes The effects of different copper sources and levels on growth performance of growing blue foxes are shown in Table 2 . Dietary copper level had a highly significant effect on average daily gain ($P<0.01$), with the 50 and 100 mg/kg copper supplementation groups showing significantly higher average daily gain than the control group ($P<0.05$). Dietary copper level also had a significant effect on feed-to-gain ratio ($P<0.05$), with the 50 and 100 mg/kg copper supplementation groups showing significantly lower feed-to-gain ratio than the control group ($P<0.05$). Dietary copper source had no significant effects on average daily feed intake, average daily gain, or feed-to-gain ratio ($P>0.05$). No interaction between copper source and copper level was observed for average daily feed intake, average daily gain, or feed-to-gain ratio ($P>0.05$).

2.2 Effects of Different Copper Sources and Levels on Nutrient Digestibility of Growing Blue Foxes The effects of different copper sources and levels on nutrient digestibility of growing blue foxes are shown in Table 3 . Dietary copper level had highly significant effects on crude protein digestibility and fecal copper content ($P<0.01$) and significant effects on dry matter digestibility and ether extract digestibility ($P<0.05$). The 100 mg/kg copper supplementation group showed significantly higher dry matter digestibility and crude protein digestibility than the control group ($P<0.05$), while the 50 and 100 mg/kg copper supplementation groups showed significantly higher ether extract digestibility and fecal copper content than the control group ($P<0.05$). Dietary copper source had a significant effect on fecal copper content ($P<0.05$), with the copper methionine group showing significantly lower fecal copper content than the copper sulfate group ($P<0.05$). Dietary copper source had no significant effects on dry matter digestibility, ether extract digestibility, crude protein digestibility, or nitrogen retention ($P>0.05$). No interaction between copper source and copper level was observed for dry matter digestibility, ether extract digestibility, crude protein digestibility, nitrogen retention, or fecal copper content ($P>0.05$).

2.3 Effects of Different Copper Sources and Levels on Serum Indices of Growing Blue Foxes The effects of different copper sources and levels on serum indices of growing blue foxes are shown in Table 4 and Table 5 . As shown in Table 4, dietary copper level had a highly significant effect on serum urea nitrogen (UN) content ($P<0.01$), with the 50 and 100 mg/kg copper supplementation groups showing significantly higher serum UN content than the control group ($P<0.01$). Dietary copper source also had a highly significant effect on serum UN content ($P<0.01$), with the copper methionine group showing significantly higher serum UN content than the copper sulfate group ($P<0.01$). Dietary copper source and level had no significant effects on serum total protein, albumin, globulin, copper, or iron contents, nor on alanine aminotransferase or aspartate aminotransferase activities ($P>0.05$). No interaction between copper source and copper level was observed for serum total protein, albumin, globulin, UN, copper, or iron contents, or for alanine aminotransferase or aspartate aminotransferase activities ($P>0.05$).

As shown in Table 5, dietary copper source and level had no significant effects on serum ceruloplasmin (Cp) or copper-zinc superoxide dismutase (Cu-Zn SOD) activities ($P>0.05$). No interaction between copper source and copper level was observed for serum Cp or Cu-Zn SOD activities ($P>0.05$).

Discussion

3.1 Effects of Different Copper Sources and Levels on Growth Performance of Growing Blue Foxes Studies have shown that dietary supplementation with appropriate copper levels has obvious growth-promoting effects on young monogastric animals such as weaned piglets. In this experiment, dietary supplementation with exogenous copper improved the growth performance of growing blue foxes, consistent with the findings of Liu et al. [3] on growing blue foxes. Bai et al. [14] reported that using copper methionine as a copper source in diets improved the growth rate and body length of raccoon dogs during the winter fur growth period. Li [15] found that high-dose copper (40–80 mg/kg) had growth-promoting effects on rex rabbits, with copper methionine showing significantly better effects than copper sulfate. This study found that copper methionine had slightly better growth-promoting effects than copper sulfate at the same supplementation level, consistent with the above reports. Amino acid copper is a chelated product of amino acids and copper that can be absorbed through amino acid pinocytosis, alleviating competitive antagonism among trace elements, improving copper ion absorption and utilization, and reducing copper' s oxidative damage to vitamins and fats in feed. The growth-promoting mechanism of copper in blue foxes is currently unclear but may be related to improved digestive enzyme activities and increased secretion of growth-related hormones. Du [16] reported that appropriate copper ion concentrations can improve the activities of various digestive enzymes, thereby enhancing nutrient absorption from feed. Luo et al. [17] demonstrated that high copper can increase lipase activity in weaned piglets. A key characteristic of fur animal diets is their high fat content, making the effect of copper on improving fat digestibility particularly prominent in blue fox diets. Luo et al. [18] reported that copper can promote growth in young pigs by stimulating growth hormone gene expression in the pituitary, with copper glycinate more effectively increasing growth hormone mRNA synthesis in weaned piglets than copper sulfate. The effects of high-copper diets on growth-related hormone levels in growing blue foxes remain unclear and require further investigation.

3.2 Effects of Different Copper Sources and Levels on Nutrient Digestibility Studies have shown that high-dose copper can improve nutrient utilization by enhancing intestinal mucosal morphology, increasing digestive enzyme activities, and improving intestinal microflora structure [19]. Radecki et al. [20] reported that high copper can reduce the energy requirement for tissue maintenance by thinning the jejunum, shortening duodenal villi, and slowing tissue turnover rates in the jejunum, thereby increasing energy available for production. Shurson et al. [21] reported that dietary supplementation with high copper can increase crypt depth in the small intestine. Dove [22] found that adding high copper to piglet diets significantly improved digestibility of dry matter, protein, and fat. Luo et al. [17] further revealed the effects of high copper on digestive enzymes, showing that high copper can promote intestinal lipase and phospholipase A activities, and in vitro experiments confirmed that high copper can increase pancreatic lipase activity. Wu [9] reported that dietary copper supplementation can promote bile secretion in mink, leading to better emulsification of dietary fat and improved fat digestibility. Kirchgessner et al. [23] reported that appropriate copper ion concentrations can activate pepsinogen and increase pepsin activity, promoting protein breakdown and absorption. High copper can increase gastrin levels in piglet blood, which stimulates secretion of hydrochloric acid, pancreatic juice, and bile, and also stimulates chief cells to secrete pepsinogen, promoting protein digestion. In this study, the digestibility of dry matter, crude protein, and ether extract significantly increased with increasing dietary copper levels, possibly because copper supplementation improved digestive enzyme activities and intestinal mucosal morphology, making dietary nutrients more easily absorbable. Turnlund et al. [24] used stable isotope tracing to study the relationship between dietary copper level and absorption rate, finding that copper absorption rate gradually decreased with increasing dietary copper levels. This study found that blue foxes fed high-copper diets had significantly higher fecal copper content than those fed low-copper diets. Copper methionine can be absorbed through amino acid pinocytosis, resulting in higher absorption efficiency than copper sulfate. At consistent copper supplementation levels, blue foxes fed copper methionine had lower fecal copper content.

3.3 Effects of Different Copper Sources and Levels on Serum Biochemical Indices of Growing Blue Foxes Serum ALT and AST are important indicators for detecting liver function in humans and animals. Studies have shown that high copper can cause liver damage and hepatocyte destruction, leading to increased serum AST and ALT activities [25-26]. This experiment showed that dietary supplementation with 100 mg Cu/kg as copper sulfate or copper methionine did not cause obvious liver damage in blue foxes. Serum proteins are important substances for maintaining osmotic pressure and water balance, mainly composed of ALB and GLO. ALB plays an important role in copper transport in the body, as copper ions absorbed into the portal vein first bind to ALB or histones in serum before reaching the liver through blood circulation. With increasing dietary copper levels, more copper ions are absorbed by the intestine, requiring more ALB for transport, which may explain the increasing trend in serum ALB with higher dietary copper levels. Li [15] found that serum UN content in rex rabbits decreased with increasing dietary copper levels, which is inconsistent with the results of this experiment. The discrepancy may be due to different physiological characteristics between rex rabbits and blue foxes. Copper content in animal plasma is strictly regulated by the body; however, when copper content exceeds the regulatory range, plasma copper content increases significantly, potentially leading to hypercupremia [27]. Copper can improve iron utilization, so serum iron content in blue foxes tends to increase with higher dietary copper levels.

3.4 Effects of Different Copper Sources and Levels on Serum Cp and Cu-Zn SOD Activities of Growing Blue Foxes Cu-Zn SOD and Cp are two important copper-containing enzymes in animals that play crucial antioxidant roles in the body, effectively protecting against oxidative stress caused by free radicals. Feng et al. [28] found that dietary supplementation with 250 mg/kg copper sulfate and 100 mg/kg copper proteinate increased serum Cp activity in weaned piglets, and 100 mg/kg copper proteinate also increased Cu-Zn SOD activity in red blood cells. Wu [9] reported that dietary copper supplementation increased serum Cp and Cu-Zn SOD activities in mink. In this study, although differences in serum Cu-Zn SOD activity among groups were not significant, serum Cp activity increased with increasing dietary copper levels, consistent with studies on mink and pigs.

Conclusion

Supplementing 50 mg/kg copper as either copper methionine or copper sulfate to a basal diet containing 14.44 mg/kg copper can achieve good growth-promoting effects in growing blue foxes. Furthermore, copper methionine can significantly reduce fecal copper excretion, representing a more efficient and environmentally friendly copper source.

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Note: Figure translations are in progress. See original paper for figures.