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EFFECT OF DIETARY COPPER SUPPLEMENTATION ON GROWTH PERFORMANCE AND ANTIOXIDANT ACTIVITY IN REX RABBITS

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ABSTRACT

Copper is often used as a growth promoter, at the same time copper is one of the most important essential trace elements for fur animals, especially Rex rabbits. However, too much copper added to the diet may harm animal health, and copper excreted in feces can pollute the environment. In this study, three-month-old Rex rabbits were randomly divided into four groups and fed a basal diet containing 0, 30, 60, or 120 mg/kg Cu for five weeks. The diet supplemented with 30 mg/kg Cu significantly increased ($P < 0.05$) the average daily feed intake (ADFI) (+0.8%), the average daily gain (ADG) (+9.1%), the activity of serum Cu–Zn (zinc) superoxide dismutase (+29.5%) and the digestibility of ether extract (+3.4%). Overall, the data in this study indicate that 30 mg/kg is the optimal level of Cu supplementation in the diet of growing Rex rabbits. The results will provide a reference to improve the breeding of Rex rabbits and possibly other animals. In follow-up studies, the amount of copper in the diet should be reduced as much as possible from the baseline of 30 mg/kg copper.

Key words: Rex rabbit, Dietary copper supplement, Growth performance, Blood biochemistry

INTRODUCTION

Copper (Cu) is an indispensable trace element for mammals, including Rex rabbits. Copper is usually involved in enzymatic reactions in the form of Cu proteins (e.g., ceruloplasmin) or Cu-containing enzymes (Harris, 2003). Copper is widely used as a growth promoter in livestock and poultry production, and Cu deficiency is characterized by retarded growth, gray hair, bone abnormalities, and anemia (Wang *et al.*, 2015). A set of sound regulatory mechanisms maintains Cu homeostasis in monogastric animals (Hedemann *et al.*, 2016). However, excessive intake of Cu may lead to oxidative stress and cell damage (Kumar *et al.*, 2016).
The Cu content in rabbit diet recommended by the (NRC, 1977) is 5 to 10 mg/kg. However, according to Schlolaut (Schlolaut, 1987), the growth performance of fur rabbits improves when the Cu content in the diet is 25 mg/kg. Meanwhile, studies conducted by Zhang (Zhang *et al.*, 2019) observed that the highest ADG was obtained when the copper supplemental level was 80 mg/kg, and the lowest feed conversion rate (FCR) was obtained when the copper supplemental level was 40 mg/kg in the long hairy rabbits. At present, the dietary Cu requirement of Rex rabbits has not been studied, including in the later growing period, and it is necessary to determine the appropriate Cu concentration in rabbit feed. Therefore, the main objective of this study was to evaluate the effects of dietary copper on growth performance, serum biochemical, nutrient utilization, and tissue mineral retention of Rex rabbits.

MATERIALS AND METHODS

Animals and experimental design

In this experiment, 160 90-days-old healthy Rex rabbits (80 males, 80 females) with an average body weight of 1914±18.34 g were divided into four treatments, with 40 replicates (20 males, 20 females) in each treatment (1 rabbit per replicate). Copper was added as copper sulfate pentahydrate (CAS: 7758-99-8; Shanghai aladdin Biochemical Technology Co., Ltd., Shanghai, People's Republic of China). All the diets were pressed into particles with a diameter of 4-6 mm and stored in ventilation, cool, dark place. The treatments were a basal diet (control: measured Cu content 8.4 mg/kg) or a basal diet supplemented with 30, 60, or 120 mg/kg Cu (measured Cu content 39.1, 67.5, and 127.7 mg/kg, respectively). The rabbits were housed in single cages and rabbits were given *ad libitum* access to food and water. Natural lighting and ventilation were maintained throughout the experimental period. The cages were sterilized once a week. The trial lasted for six weeks (including a one-week adaptation period and a five-week experimental period). The diets were designed to meet the requirements of growing Rex rabbits and according to NRC (NRC, 1977). The composition and chemical analysis of the basal diet are shown in Table 1.

Table 1: Composition and nutrient levels of the basal diet (air-dry basis) fed to Rex rabbits.

Ingredients	%	Chemical analysis[2]	%
Corn	10.5	DE (MJ/kg)	10.4
Soybean meal	6.0	DM	89.2
Corn germ meal	20.0	CP	16.9
Wheat bran	18.0	Ash	6.83
Husk powder	11.0	EE	4.39
Sunflower meal	12.0	CF	15.8
Alfalfa	6.00	Ca	0.70
Soya bean stem meal	12.0	P	0.54
Artemisia apiacea flour	3.00	Lys	0.53
Premix[1]	1.50	Met	0.89

[1] The premix provided the following per kilogram of diet: vitamin A, 10,000 IU; vitamin D4, 100 IU; vitamin E, 60 mg; vitamin K3, 2 mg; vitamin B1, 5 mg; vitamin B2, 10 mg, vitamin B11, 2.5 mg; vitamin B12, 0.01 mg; choline chloride, 600 mg; iron (as ferrous sulfate), 50 mg; zinc, 50 mg; selenium, 4 mg; iodine, 0.6 mg; manganese, 4 mg; $CaHPO_4$, 1,600 mg; NaCl, 4,800 mg; lysine, 1,000 mg; methionine, 2,000 mg; stone powder, 1,600 mg.

[2] Digestive energy is theoretically calculated, and other nutritional indicators are measured values.

RNA isolation and analysis

Total RNA extraction and quantitative reverse transcription-PCR (**qRT-PCR**) of mucosa samples were performed as described previously. The relative amount of mRNA of a gene was calculated according to the 2 delta delta CT method (Livak and Schmittgen, 2001) using glyceraldehyde 3-phosphate dehydrogenase (***GAPDH***) as normalizing gene and the control group as calibrator group.

Table 2: Primers of the houskeeping GAPDH gene and transporter genes for Real Time PCR

Gene	Primer sequence		Product size
GAPDH	F: CACCAGGGCTGCTTTTAACTCT	R: CTTCCCGTTCTCAGCCTTGACC	163bp
β-actin	F: CGCAGAAACGAGACGAGATT	R: GCAGAACTTTGGGGACTTTG	123bp
SOD-1	F: GACGCATAACAGGACTGACCG	R: AACACATCAGCGACACCATTG	196bp

Statistical analysis

The data are expressed as the mean ± standard error of the mean. The analysis of variance (ANOVA) was performed on the data from more than two groups, followed by Dunnett's multiple comparisons or Tukey's HSD. A Student's t-test was performed on the data from two groups. All statistical analyses were conducted using JMP Pro software (SAS Institute, Cary, NC, USA). Before ANOVA or t-test, the variance homogeneity of the data was analyzed.

RESULTS AND DISCUSSION

Results obtained in our experiment suggest that supplementation of copper has beneficial effects on the growth performance of Rex rabbits (Table 3). Previous studies have reported that diets supplemented with 100 to 400 mg/kg copper sulfate significantly improved rabbit growth performance (Ayyat *et al.*, 1995; Bassuny, 1991).

Table 3: Effects of the level of dietary copper on growth performance of growing Rex rabbits

Items	Level of dietary copper supplement (mg/kg)				*P*-value
	0	30	60	120	
Initial Body Weight (g)	1916±32.9	1964±30.6	1926±40.00	1931±37.8	0.79
Final Body Weight (g)	2662±35.8	2779±35.7	2712±27.72	2682±33.8	0.078
Average Daily Gain (g/d)	21.3±0.48[b]	23.3±0.53[a]	22.5±0.67[ab]	21.5±0.55[b]	0.047
Average Daily Feed Intake (g/d)	164.8±0.43[b]	166.2±0.6 [a]	166.1±0.32[a]	165.2±0.34[ab]	0.009
Feed Conversion Rate (feed/gain)	7.9±0.23	7.3±0.16	7.6±0.22	7.9±0.23	0.10

Means with different letters within a row differ significantly ($P < 0.05$); data are expressed as least squares means (n=40).

Our results showed that there was a significant difference in EE digestibility between the control group and the 30 mg/kg Cu group (Table 4), and no significant differences were detected among treatment groups in the digestibility of DM, ADF, NDF, CF, and CP (Table 4). This result is consistent with that in blue foxes (Wu *et al.*, 2015). Similarly, Dove (Dove, 1995) found that Cu (250 mg Cu/kg diet) at pharmacological concentrations increases the apparent digestibility of EE in weanling pigs.

Table 4: Effects of level of dietary copper on the digestibility of nutrients in growing Rex rabbits

Items	Level of dietary copper supplement (mg/kg)				P-value
	0	30	60	120	
Dry Matter	60.9±1.01	61.5±0.50	60.9±0.96	62.7±1.00	0.49
Crude Protein	71.5±1.58	70.5±1.95	71.3±2.41	72.2±2.46	0.95
Ether Extract	87.5±0.66[b]	90.5±0.64[a]	89.0±0.62[ab]	88.9±0.33[ab]	0.011
Crude Fibre	15.7±0.63	14.4±0.78	16.5±0.38	15.9±1.07	0.26
Acid Detergent Fibre	13.0±0.55	12.7±1.04	13.8±0.63	12.8±0.53	0.69
Neutral Detergent Fibre	34.5±2.25	30.3±1.79	33.8±1.83	36.5±1.37	0.72

Means with different letters within a row differ significantly (P＜0.05); data are expressed as least squares means (n=8).

Table 5: Effects of level of dietary copper supplementation on blood biochemistry of growing Rex rabbits

Items	Level of dietary copper supplement (mg/kg)				P-value
	0	30	60	120	
ALT (U/l)	74.9±2.55	74.6±2.85	76.2±2.58	71.2±4.51	0.12
AST (U/l)	47.2±2.68	46.02.78	45.1±3.01	49.0±1.39	0.53
CER (U/l)	15.7±0.66	18.14±0.78	19.0±0.60	17.9±1.62	0.15
Cu (µmol/l)	9.75±0.49	9.65±0.17	10.2±0.25	10.5±0.30	0.25
Zn (µmol/l)	17.4±0.32	18.3±0.68	17.7±0.35	16.8±0.23	0.15
Cu-Zn SOD (U/ml)	62.6±2.24[a]	81.1±1.62[b]	82.3±2.14[b]	78.6±1.08[b]	＜0.001
T-SOD (U/ml)	153±3.11	162±3.65	161±4.23	163±3.22	0.19

ALT alanine transaminase; AST aspartate transaminase; CER: ceruloplasmin; Cu copper; Zn zinc; Cu-Zn SOD Cu–Zn superoxide dismutase; T-SOD total superoxide dismutase. Means with different letters within a row differ significantly (P＜0.05); data are expressed as least squares means (n=8).

Composed of two subunits (one with a Cu atom and one with a zinc atom), Cu-Zn SOD is the most common SOD in eukaryotes and functions in the scavenging of superoxide anion free radicals. In this experiment, the activity of Cu-Zn SOD increased significantly with Cu supplementation (Table 5). This result is consistent with those in broiler chickens (Jorsoz *et al.*, 2017). In Holstein cows (Fu *et al.*, 2020) and rats (Zhang *et al.*, 2020), dietary Cu supplementation significantly increases the expression level of the Cu–Zn SOD gene in the liver, which might explain the increase in serum Cu–Zn SOD activity in this study (Figure 1).

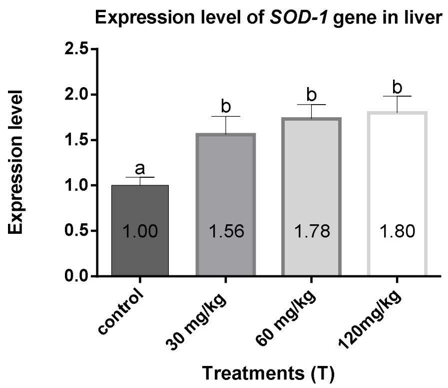

Figure 1: Liver SOD-1 gene expression levels of Rex rabbits in response to dietary treatments. Date reflect mean±sd from 8 rabbit in 3 independent experiments. Bars with different letters differ significantly at P < 0.05.

CONCLUSIONS

Dietary Cu had a positive effect on the growth performance of Rex rabbits. The digestibility of crude fat and the activity of serum Cu–Zn SOD increased with Cu supplemented to the diet. To summarize, the addition of 30 mg/kg Cu to the diet is the most suitable supplement to improve the growth performance of Rex rabbits.

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