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Dietary Copper Supplementation Increases Growth Performance by Increasing Feed Intake, Digestibility, 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, 3-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 5 weeks. The diet supplemented with 30 mg/kg Cu significantly increased ($P < 0.05$) the average daily feed intake (ADFI) and the average daily gain (ADG) and also the activity of serum Cu–Zn (zinc) superoxide dismutase and the digestibility of ether extract. Supplemental Cu up to 120 mg/kg did not significantly adversely affect the Zn metabolism of growing Rex rabbits. 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.

Keywords Rex rabbit · Dietary copper supplement · Growth performance · Blood biochemistry · Tissue mineral retention

Introduction

Copper (Cu) is an indispensable trace element for mammals, including Rex rabbits [1]. Copper is usually involved in enzymatic reactions in the form of Cu proteins (e.g., ceruloplasmin) or Cu-containing enzymes [2–4]. 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 [5–9]. A set of sound regulatory mechanisms maintains Cu homeostasis in monogastric animals [10–12]. However, excessive intake of Cu may lead to oxidative stress and cell damage [13].

The Rex rabbit is a variety raised for meat and fur production [14]. Rex rabbit meat contains high levels of essential amino acids and has a lower fat content than other meats [15]. The fluff is smooth and upright, full of luster, and soft and comfortable, with fine fluff. It has been reported that dietary Cu supplementation promotes growth and improves fur quality in the carnivorous mink and blue fox [16, 17]. Obviously, copper is a key trace mineral in Rex rabbit nutrition. The Cu content in rabbit diet recommended by the NRC [18] is 5 to 10 mg/kg. However, according to Schlolaut [19], the growth performance of fur rabbits improves when the Cu content in the diet is 25 mg/kg. Meanwhile, studies conducted by Zhang et al. [20] observed that the highest ADG was obtained when the copper supplemental level was 80 mg/kg, and the lowest F/G was obtained when the copper supplemental level was 40 mg/kg in the long hairy rabbits.

To summarize, although Cu is an essential nutrient for animals, excessive Cu supplementation may be harmful. 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 for Rex rabbits; additionally, the influence of dietary copper on serum biochemical, nutrient utilization, and tissue mineral retention of Rex rabbits was investigated and to determining their nutritional copper requirements.

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Materials and Methods

This study was conducted in the Rabbit Nutrition Laboratory of Shandong Agricultural University (Tai'an, China). The local experimental animal care committee and the institutional ethics committee of the Nutrition and Clinical Nutrition Department (Shandong Agricultural University) approved all protocols of this study.

Rabbit Diets, Management, and Experimental Design

In this experiment, 160 90-day-old healthy Rex rabbits (80 males, 80 females) with an average body weight of 1914.09 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). 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 fed twice a day, at 8:00 and 16:00. The rabbits could drink water freely. Before the experiment, the experimental environment was thoroughly cleaned and disinfected and fumigated with potassium permanganate and formaldehyde. Natural lighting and ventilation were maintained throughout the experimental period. The cages were sterilized once a week. The trial lasted for 6 weeks (including a 1-week adaptation period and a 5-week experimental period). The diets were designed to meet the requirements of growing Rex rabbits and according to Chen et al. [21]. The composition and chemical analysis of the basal diet are shown in Table 1.

Sample Collection and Preparation

The body weight of rabbits was recorded weekly. The amount of food given and that leftover were recorded daily throughout the trial period. A week before the end of the feeding trial, eight rabbits (4 males, 4 females) from each treatment were randomly selected and transferred to one sterilized metabolic cage. The metabolic trial lasted for 1 week (including a 3-day adaptation period and a 4-day experimental period), and the corresponding experimental diets were supplied. The total feces of each rabbit was collected and recorded for the 4 days of the experimental period. Nitrogen loss was prevented according to the method of Chen et al. [21]. Sulfuric acid, 10%, was added to feces at 5% of the fresh weight, which was stored at − 20 °C. After the metabolic experiment, all of the feces from the same rabbit were mixed together. Then, the fecal and feed samples were dried to a constant weight at 65 °C and ground to pass through a 40-mesh sieve. The total weight of manure was recorded before and after drying.

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

Ingredients	Percent	Chemical analysis[2]	Percent
Corn	10.50	DE (MJ/kg)	10.37
Soybean meal	6.00	DM	89.16
Corn germ meal	20.00	CP	16.87
Wheat bran	18.00	Ash	6.83
Husk powder	11.00	EE	4.39
Sunflower meal	12.00	CF	15.79
Alfalfa	6.00	Ca	0.70
Soya bean stem meal	12.00	P	0.54
Artemisia apiacea flour	3.00	Lys	0.53
Premix[1]	1.50	Met	0.89
Total	100.00		

[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; $CaHPO_4$, 1600 mg; NaCl, 4800 mg; lysine, 1000 mg; methionine, 2000 mg; stone powder, 1600 mg

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

Measurements

Growth Performance

The body weight (BW) of rabbits was recorded weekly. The total feed intake of each rabbit during the entire experiment was recorded. The average daily feed intake (ADFI), average daily weight gain (ADG), and feed/gain ratio (F/G) were calculated as follows.

$$\mathrm{ADFI} = \text{total feed intake per rabbit during the entire experimental period}/35$$

$$\mathrm{ADG} = \{\text{final body weight (FBW)} - \text{initial body weight (IBW)}\}/35$$

$$\mathrm{F/G} = \mathrm{ADFI}/\mathrm{ADG}$$

Carcass Trait Measurements

Eight rabbits (four males and four females) per group with an average BW equal to that of the whole treatment group were randomly selected for slaughter after 12 h of free access to water but no food. Full bore rate (%), semi-bore rate (%), spleen (%), liver (%), thymus (%), and total fat (%) were measured according to Blasco et al. [22].

Digestibility Trial

At the end of the experiment, eight rabbits (4 males, 4 females) per group were randomly selected and transferred to a

metabolic cage for digestibility trials. The quantity of feed intake and the excreted feces were recorded daily. The preparation and analysis of the feed and feces samples were conducted in accordance with the description of the AOAC [23].

The dry matter (DM), crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ether extract (EE) contents of feed and feces were determined according to the AOAC [23]. To acquire experimental samples, a part of the feed or feces was selected by the quadrature method and fully crushed to pass through a 40-mesh sieve.

Dry matter (DM) was measured by transferring feed or fecal samples (0.2 g) into a half-open weighing bottle. After oven drying at 105 ± 2 °C to constant weight, the sample was cooled in a dryer for 30 min and weighed (method 2001. 12, AOAC). The weight after cooling was the weight of dry matter, which was used to calculate the DM content of the sample.

The nitrogen (N) in a sample was determined according to Chen [20]. Feed or fecal samples (0.2 g) were transferred into digestion tubes, and catalysts (($CuSO_4$. $5H_2O$, 0.4 g; anhydrous potassium sulfate, 6 g) and concentrated sulfuric acid (10 ml)) were added simultaneously. Digestion occurred in an automatic digestion furnace (DI-210, Opsis, Skytteskogsvägen, Sweden) for 4 to 6 h until the liquid cleared. The digested sample was transferred into an automatic Kjeldahl apparatus (KjeltecTM 8000, Foss, Hilleroed, Denmark), and crude protein (CP) was calculated as N × 6.25 (method 988.05, AOAC).

The ether extract (EE) content of the sample was determined by Soxhlet extraction. Feed or fecal samples (2.0 g) were weighed, packaged in filter paper, and marked with pencil. The samples were oven-dried at 105 ± 2 °C for 6 h. The samples were cooled in a dryer for 30 min and weighed. The dried filter paper bags were placed into a Soxhlet extractor, and ethyl ether was added. The bags and ethyl ether were heated in a water bath at 60 to 75 °C for 10 h. Then, the filter paper bags were transferred to a ventilated place at room temperature to volatilize the ethyl ether. The samples were oven-dried at 105 ± 2 °C for 6 h. The samples were cooled in a dryer for 30 min and weighed.

The Cu and Zn contents in samples were measured by atomic absorption spectrophotometry (Analytik Jena novAA 400P, Jena, Germany), and all results are expressed in fresh weight. Samples (2.0 g) were weighed and transferred to a crucible pre-dried to constant weight. Samples were carefully carbonized on a high temperature (300 °C) electric heating plate for 20 min. Then, the carbonized samples were ashed in a muffle furnace at 550 °C until no carbon particles remained. The ashed samples were carefully heated on an electric heating plate, and 10 ml of 6.0 mol/l hydrochloric acid was added drop by drop until there were no bubbles (carbon dioxide might be produced). All the remaining hydrochloric acid was then added, and the samples were heated until nearly dry. After dissolving the residues with 5 ml of 6 mol/l hydrochloric acid, the sample solutions were transferred to 50-ml volumetric flasks with 5 ml of water. After cooling, the solutions were diluted to 50 ml with water and filtered three times with filter paper. Simultaneously, blank solution was prepared according to the above experimental steps. By subtracting the absorbance of the blank solution from the absorbance of the measured samples, the corrected absorbance of samples was obtained. The measured wavelengths of copper and zinc were 324.8 nm and 213.8 nm, respectively. Simultaneously, standard solution was prepared by diluting the standard solution of copper and zinc with 0.6 mol/l hydrochloric acid. Simultaneous determination of absorbance of 0.6 mol/l hydrochloric acid and standard solution. Subtract the absorbance of 0.6 mol/l hydrochloric acid from the absorbance of the standard solution and the corrected absorbance of standard solution was obtained, and the standard curves were prepared and used to calculate the Cu or Zn content.

Analyses of Serum Samples

At the end of the trial, eight rabbits (4 males, 4 females) per group were electrically stunned (70 V, pulsed direct current, 50 Hz for 5 s), and 10 ml of blood sample was collected by cardiac puncture applying a serum separation tube (BD-Pharmingen, San Diego, CA, USA). The blood samples were centrifuged at 3000 rpm for 10 min, and the upper serum was transferred to 1.5-ml centrifuge tubes and stored at − 20 °C until analysis.

Serum alanine transaminase (ALT) and aspartate transaminase (AST) were analyzed using kits (supplied by the College of Animal Science and Technology, Shandong Agricultural University, Tai'an, People's Republic of China). The concentrations were measured using an automatic biochemical analyzer (Hitachi 7020, Hitachi High Technologies, Inc., Ibaraki, Japan).

The contents of Zinc (E011-1-1) in serum were measured spectrophotometrically by Zincon colorimetric method using zinc ion detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, People's Republic of China). The contents of Copper (E010-1-1) in serum were measured spectrophotometrically by complexation colorimetry method using Copper ion detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, People's Republic of China). The concentrations of total superoxide dismutase (T-SOD) and Cu–Zn superoxide dismutase (Cu–Zn SOD) and the activity of ceruloplasmin (CER) were determined using Shanghai Enzyme-linked Biotechnology kits (Shanghai Enzyme-Linked Biotechnology Co., Ltd., Shanghai, People's Republic of China).

Analysis of Liver Samples

After slaughter, the livers were removed and weighed, and samples were taken and placed in 2.0-ml cryopreservation tubes and stored in liquid nitrogen until analysis. *SOD-1* encodes Cu–Zn superoxide dismutase, and *ATP7B* encodes the

copper transporter P-type ATPase. Total RNA was extracted by the Trizol (Thermo Fisher, Carlsbad, CA, USA) method. The RNA concentration was measured on a DU 640 nucleic acid spectrophotometer (Beckman Coulter, Inc., 250 S. Kraemer Boulevard Brea, CA, USA). The quality of extracted RNA was detected by agarose gel electrophoresis. Reverse transcription reactions (20 μl) contained 1000 ng of total RNA, 4 μl 5×*Evo M-MLV*RT Master Mix (supplied by the Accurate Biotechnology Co., Ltd., Hunan, People's Republic of China). Real-time PCR analysis was carried out with an Applied Biosystems 7500 real-time PCR system (Applied Biosystems, Foster, CA, USA). Each RT reaction served as a template in a 20-μl PCR containing 0.2 μmol/l of each primer and SYBR Green master mix (Takara, Dalian, People's Republic of China). Real-time PCRs were performed at 95 °C for 10 s of pre-denaturation, followed by 40 cycles of denaturation at 95 °C for 5 s and annealing and extension at 60 °C for 40 s. A standard curve was plotted to calculate the efficiency of real-time PCR primers. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as the normalization gene, and the results of the relative mRNA quantification was verified using β-actin levels [24, 25], and mRNA expression was analyzed using the $2^{-\Delta\Delta CT}$ method [26]. The primer sequences are shown in Table 2.

Statistical Analyses

The data are expressed as the mean ± standard error of the mean (SEM). 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 *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. The sample sizes for different analyses were the following: growth performance, $n = 40$; carcass traits, $n = 8$; blood biochemistry, $n = 8$; tissue mineral retention, $n = 8$; nutrient utilization, $n = 8$; and mRNA level, $n = 8$. Differences at $P < 0.05$ were considered to be significant, and *P* values between 0.05 and 0.1 were considered as a trend.

Results

Growth Performance

The effects of dietary Cu supplementation on the growth performance of growing Rex rabbits are shown in Table 3. Average daily gain and ADFI were significantly affected by Cu supplementation ($P < 0.05$). In the entire trial period, there was no significant difference in BW or F/G ratio among the treatment groups ($P > 0.05$), although the FBW tended to increase with the addition of dietary Cu ($0.05 < P < 0.1$).

Blood Biochemistry

The effects of Cu supplementation on serum biochemistry of Rex rabbits are shown in Table 4. The Cu level did not significantly affect the serum levels of ALT, AST, Cu, Zn, CER, or T-SOD ($P > 0.05$). However, serum Cu–Zn SOD increased significantly with supplemented Cu ($P < 0.05$).

The effects of the dietary Cu supplementation levels on gene expression in the liver tissue of growing Rex rabbits are shown in Fig. 1. Supplemental dietary Cu significantly increased the expression of the *SOD-1* gene ($P < 0.05$) but had no significant effect on the expression of *ATP7B* ($P > 0.05$). Although the expression of *SOD-1* increased significantly compared with the control group, there were no significant differences among the treatment groups.

Nutrient Utilization

The effects of dietary Cu supplementation on the digestibility of nutrients in growing Rex rabbits are shown in Table 5. Although the digestibility of DM, CP, crude fiber (CF), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were not affected by dietary Cu supplementation ($P > 0.05$), the diet supplemented with 30 mg/kg Cu significantly increased ($P < 0.05$) the digestibility of ether extract.

Table 2 Primer sequences of related genes

Genes	GenBank accession no.	Primer sequences (5′-3′)	Product size (bp)
SOD-1	NM_001082627	F: 5′-GACGCATAACAGGACTGACCG-3′ R: 5′-AACACATCAGCGACACCATTG-3′	196
ATP7B	XM_008273868	F: 5′-CTCTGTGCTGGTTGCCTTGATGG-3′ R:5′-TGTCGCCGTCTGAGCCTGTG-3′	146
GAPDH	NM_001082253.1	F: 5′-CACCAGGGCTGCTTTTAACTCT-3′ R:5′- CTTCCCGTTCTCAGCCTTGACC-3′	163
β-actin	XM_002722894.3	F: 5′- CGCAGAAACGAGACGAGATT-3′ R:5′-GCAGAACTTTGGGGACTTTG-3′	123

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

Items	Level of dietary copper supplement (mg/kg)				*P* value
	0	30	60	120	
IBW (g)	1916.03±32.98	1964.44±30.63	1926.03±40.00	1930.66±37.83	0.7905
FBW (g)	2662.14±35.76	2778.75±35.67	2712.50±27.72	2682.11±33.81	0.0782
ADG (g/day)	21.32±0.48^{b}	23.27±0.53^{a}	22.47±0.67 ^{ab}	21.47±0.55^{b}	0.0470
ADFI (g/day)	164.79±0.43^{b}	166.19±0.61^{a}	166.13±0.32 ^{a}	165.29±0.34^{ab}	0.0089
F/G	7.89±0.23	7.27±0.16	7.60±0.22	7.90±0.23	0.1089

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

IBW initial body weight, *FBW* final body weight, *ADG* average daily weight gain, *ADFI* average daily feed intake, *F/G* feed/gain

Carcass Traits

The effects of dietary Cu supplementation on carcass traits of growing Rex rabbits are shown in Table 6. Carcass traits were not significantly different ($P > 0.05$) among the treatment groups.

Tissue Mineral Contents

The effects of dietary Cu addition on tissue Cu and Zn contents in growing Rex rabbits are shown in Tables 7 and 8, respectively. Copper supplementation significantly increased the concentration of Cu in the liver ($P < 0.05$) and in feces (Cu excretion, $P < 0.05$; Table 7). The concentration of Cu in hand muscle, leg muscle, and loin muscle were not affected by dietary Cu supplementation ($P > 0.05$). Copper supplementation did significantly affect tissue Zn contents in the different treatments ($P > 0.05$; Table 8).

Discussion

Results obtained in our experiment suggest that supplementation of copper has beneficial effects on the growth of Rex rabbits. Ayyat and Bassuny [27, 28] reported that diets supplemented with 100 to 400 mg/kg copper sulfate significantly improve rabbit growth performance. Sui et al. [25] suggested that dietary Cu significantly increases ADG and ADFI in growing Rex rabbits. Similar results were obtained in this research, dietary Cu significantly increased ADG at 30 mg/kg Cu and ADFI at 30 and 60 mg/kg Cu (Table 3). Brandao et al. [29] found that high blood Cu levels can promote basal growth hormone secretion in normal adults. However, in this study, dietary Cu supplement had no effect on serum Cu contents (Table 4). Therefore, the increase in ADG in this study might be due to the increase in ADFI. In addition, Cu supplementation can significantly affect the F/G ratio in weanling pigs [7], blue foxes [16], and poultry [9]. However, in this study, the addition of dietary Cu had no

Table 4 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)	74.88 ± 2.55	74.63 ± 2.85	76.25 ± 2.58	71.25 ± 4.51	0.1249
AST (U/l)	47.25 ± 2.68	46.00 ± 2.78	45.13 ± 3.01	49.00 ± 1.39	0.5327
CER (U/l)	15.70 ± 0.66	18.14 ± 0.78	19.05 ± 0.60	17.89 ± 1.62	0.1528
Cu (μmol/l)	9.75 ± 0.49	9.65 ± 0.17	10.24 ± 0.25	10.48 ± 0.30	0.2505
Zn (μmol/l)	17.39 ± 0.32	18.26 ± 0.68	17.67 ± 0.35	16.83 ± 0.23	0.1587
Cu–Zn SOD (U/ml)	62.62 ± 2.24^{a}	81.11 ± 1.62^{b}	82.34 ± 2.14^{b}	78.65 ± 1.08^{b}	<0.001
T-SOD (U/ml)	152.90 ± 3.11	162.12 ± 3.65	161.72 ± 4.23	163.23 ± 3.22	0.1917

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

ALT alanine transaminase, *AST* aspartate transaminase, *CER*: ceruloplasmin, *Cu* copper, *Zn* zinc, *Cu–Zn SOD* Cu–Zn superoxide dismutase, *T-SOD* total superoxide dismutase

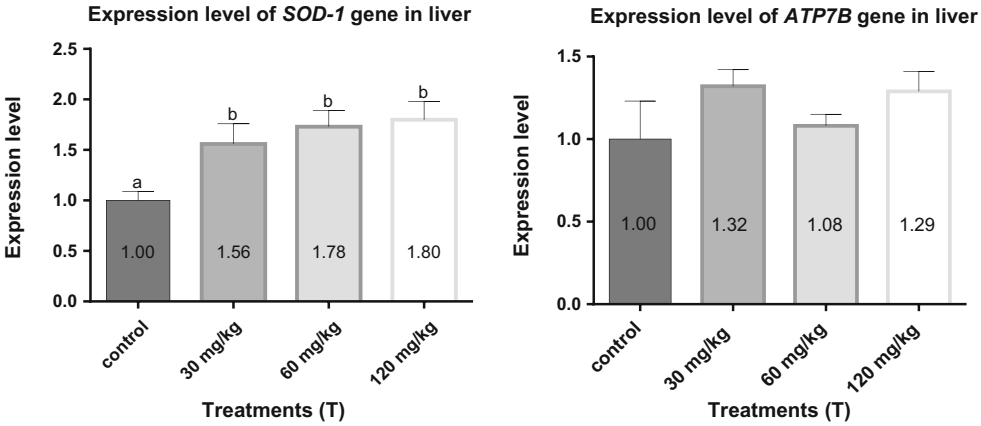

Fig. 1 Liver *SOD-1* and *ATP7B* gene expression levels of Rex rabbits in response to dietary treatments including a basal diet (control) and a basal diet supplemented with 30.60 or 120 mg/kg copper. Date reflect mean ± SD from 8 rabbit in 3 independent experiments. Bars with different letters differ significantly at $P < 0.05$

effect on the F/G ratio, which might be because the Rex rabbits were at the end of the growing period, when animals have slower growth. Although Cu can promote lipolysis in vitro [30], the addition of Cu to the diet in this study had no significant effect on the total fat percentage of Rex rabbits (Table 6). Meanwhile, in this study, dietary Cu had no significant effects on the carcass traits, including full bore rate, semi-bore rate, spleen, liver, and thymus (Table 6). Similar results were observed in finishing pigs [31]. Interestingly, the addition of 60 mg/kg Cu to the diet increased ADFI but had no effect on ADG remains unexplained. Therefore, whether the supplemental levels of Cu in the diet affected the digestibility of nutrients was examined.

Copper significantly increased the ADFI and ADG of Rex rabbits in the experiment. This result might be due to differences in nutrient digestibility and absorption rate among the different groups. Our results showed that there was a significant difference in EE digestibility between the control group and the 30 mg/kg Cu group (Table 5), and no significant differences were detected among treatment groups in the digestibility of DM, ADF, NDF, CF, and CP (Table 5). This result is consistent with that in blue foxes [18]. Similarly, Dave [32] found that Cu (250 mg Cu/kg diet) at pharmacological concentrations increases the apparent digestibility of EE in weanling pigs. Therefore, the absence of an effect on EE digestibility might explain why the increase in ADFI had no effect on ADG in the 60 mg/kg Cu group.

Dietary supplementation with Cu did not affect serum ALT, AST, Cu content, Zn content, CER, or T-SOD in the current study (Table 4). In a clinical examination of liver function, the activities of AST and ALT in serum are usually regarded as biomarkers of liver function [33, 34]. Generally speaking, an increase in serum transaminase is positively correlated with the severity of liver disease [35]. Hwang et al. [34] found toxic effects of Cu on growing rats when the dose in the diet exceeded 300 ppm, with significant increases in the contents of serum ALT and AST. However, our results showed that dietary supplementation with Cu did not affect serum Cu content, and the content of Cu in the liver increased (Tables 4 and 7). According to Hedemann [10], the mechanisms that control Cu homeostasis are usually very effective in preventing Cu poisoning in monogastric animals. Roberts et al. [36] found that most (85%) of dietary copper is secreted by bile and excreted in feces; this is consistent with our experimental results (Table 7). Although the maximum allowable level of Cu in Rex rabbits remains unknown, the results

Table 5 Effects of level of dietary copper supplementation on the digestibility of nutrients in growing Rex rabbits

Items	Level of dietary copper supplement (mg/kg)				*P* value
	0	30	60	120	
DM	60.90 ± 1.01	61.52 ± 0.50	60.89 ± 0.96	62.68 ± 1.00	0.4912
CP	71.54 ± 1.58	70.50 ± 1.95	71.31 ± 2.41	72.17 ± 2.46	0.9563
EE	87.53 ± 0.66^{b}	90.53 ± 0.64^{a}	89.03 ± 0.62^{ab}	88.89 ± 0.33^{ab}	0.0106
CF	15.73 ± 0.63	14.37 ± 0.78	16.50 ± 0.38	15.88 ± 1.07	0.2651
ADF	13.04 ± 0.55	12.66 ± 1.04	13.84 ± 0.63	12.81 ± 0.53	0.6936
NDF	34.48 ± 2.25	30.28 ± 1.79	33.83 ± 1.83	36.55 ± 1.37	0.7205

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

DM dry matter, *CP* crude protein, *EE* ether extract, CF crude fiber, *ADF* acid detergent fiber, *NDF* neutral detergent fiber

Table 6 Effects of level of dietary copper supplementation on carcass traits of growing Rex rabbits

Items	Level of dietary copper supplement (mg/kg)				*P* value
	0	30	60	120	
Live weight (g)	2650.71 ± 19.73	2672.50 ± 18.54	2678.75 ± 15.89	2676.88 ± 19.86	0.6778
Full bore ratio (%)	49.11 ± 0.69	48.81 ± 1.03	49.21 ± 0.72	50.21 ± 0.76	0.6500
Semi-bore ratio (%)	52.89 ± 0.67	52.91 ± 0.95	53.40 ± 0.66	53.96 ± 0.76	0.7274
Spleen (%)	0.04 ± 0.002	0.04 ± 0.005	0.04 ± 0.004	0.04 ± 0.003	0.6046
Liver (%)	2.57 ± 0.09	2.66 ± 0.09	2.72 ± 0.12	2.64 ± 0.12	0.7907
Thymus (%)	0.15 ± 0.01	0.16 ± 0.02	0.16 ± 0.02	0.13 ± 0.01	0.3552
Total fat (%)	0.57 ± 0.11	0.82 ± 0.15	0.87 ± 0.09	0.77 ± 0.17	0.4238

Data are expressed as least squares means with pooled SEM; $n = 8$ per treatment

of this experiment indicate that Rex rabbits can at least tolerate 120 mg/kg Cu without negative effects on their health. Ceruloplasmin, a Cu-containing glycoprotein with oxidase activity, is biosynthesized by the liver and can be regarded as a nontoxic metabolic pool of Cu in blood circulation [37]. Ceruloplasmin can effectively protect the body from the oxidative stress caused by free radicals [37]. In this study, dietary Cu supplementation had no significant effect on serum CER. This result is consistent with those found in rats [38] and silver foxes [39]. To explain why the addition of Cu to the diet had no significant effect on serum CER in Rex rabbits, the expression of the copper transporter gene *ATP7B* in the liver was examined. The gene *ATP7B* encodes a P-type ATP enzyme involved in Cu transport [40], which functions in the transfer of Cu in liver cells to serum and liver CER [38, 41, 42]. In this study, dietary Cu supplementation had no significant effect on *ATP7B* gene expression (Fig. 1). Therefore, when the content of Cu in the liver increased, CER biosynthesis apparently was not affected, or the excess Cu in the liver was not transported to CER; thus, there was no significant effect on serum CER. 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 [18] and functions in the scavenging of superoxide anion free radicals. In this experiment, the activity of Cu–Zn SOD increased significantly with Cu supplementation. This result is consistent with those in blue foxes [16] and broiler chickens [43]. In Holstein cows [37] and rats [38], dietary Cu supplementation significantly increases the expression level of the *Cu–Zn SOD* gene in the liver, which may explain the increase in serum Cu–Zn SOD activity in this study.

The liver is the main storage organ for Cu. When too much Cu is consumed, it accumulates in the liver and not in muscle tissue [16], and therefore, Cu has little effect on the oxidative stability of rabbit meat [11]. In most species, stored Cu mostly binds to metallothionein [44] to strictly control the absorption, distribution, and elimination of Cu [45, 46]. Danzeisen et al. [47] found that the Cu content in animal plasma is strictly controlled, and the Cu content in the liver is usually used as an indicator of excessive Cu in the animal body [48]. In rats, pigs, blue foxes, and poultry, Cu in the liver increases with an increase in dietary Cu content [49–51]. Those results are similar to those in this study, in which dietary Cu content was positively correlated with liver and fecal Cu content (Table 7). Valenzuela et al. [52] reported the following Cu and Zn contents in various tissues of rabbits fed diets without excess Cu and Zn: Cu: liver (mg/kg fresh) 38.9 ± 8.9, hand (mg/kg fresh) 0.8 ± 0.2, loin (mg/kg fresh) 0.7 ± 0.2, leg (mg/kg fresh) 0.8 ± 0.2, Zn: liver (mg/kg fresh) 25.5 ± 2.9, hand (mg/kg fresh)

Table 7 Effects of levels of dietary copper supplementation on tissue concentrations of copper in growing Rex rabbits

Items	Level of dietary copper supplement (mg/kg)				*P* value
	0	30	60	120	
Liver (mg/kg)	44.73 ± 2.00^{d}	125.42 ± 2.24^{c}	135.96 ± 3.97^{b}	216.97 ± 4.23^{a}	<0.0001
Loin (mg/kg)	0.91 ± 0.06	1.33 ± 0.04	0.91 ± 0.03	0.90 ± 0.20	0.1034
Hand (mg/kg)	0.98 ± 0.06	0.97 ± 0.05	0.98 ± 0.06	1.07 ± 0.08	0.6419
Leg (mg/kg)	1.00 ± 0.06	1.09 ± 0.05	1.02 ± 0.08	1.01 ± 0.06	0.7817
Cu excretion (mg/kg)	20.65 ± 1.41^{d}	67.92 ± 2.48^{c}	111.24 ± 2.99^{b}	214.63 ± 3.92^{a}	<0.0001

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

Table 8 Effects of level of dietary copper supplementation on tissue concentrations of zinc in growing Rex rabbits

Items	Level of dietary copper supplement (mg/kg)				*P* value
	0	30	60	120	
Liver (mg/kg)	179.47 ± 4.31	185.24 ± 3.31	178.40 ± 2.08	180.89 ± 6.22	0.6626
Loin (mg/kg)	6.17 ± 0.35	6.27 ± 0.40	7.07 ± 0.23	6.24 ± 0.40	0.2503
Hand (mg/kg)	6.81 ± 0.28	7.68 ± 0.41	5.57 ± 0.43	7.04 ± 0.50	0.2473
Leg (mg/kg)	6.70 ± 0.29	6.86 ± 0.32	6.57 ± 0.42	7.14 ± 0.47	0.7477
Zn excretion (mg/kg)	87.86 ± 2.22	85.95 ± 1.08	84.02 ± 1.49	85.13 ± 0.82	0.3732

Data are expressed as least squares means with pooled SEM; $n = 8$ per treatment

13.3 ± 1.2, loin (mg/kg fresh) 6.1 ± 0.4, and leg (mg/kg fresh) 9.1 ± 1.7. However, in this study, the Cu contents in loin, hand, and leg muscles were not affected by dietary Cu levels (Table 7). In addition, as shown in Table 8, dietary Cu supplementation had no significant effect on the Zn content in the liver or loin, hand, and leg muscles or feces. An interaction between Cu and Zn is generally assumed [53]. For example, Zn supplements decrease the serum and hepatic concentrations of Cu in blue foxes [16]. However, in this study, the addition of high Cu levels had no significant effect on the Zn content in serum and liver. Therefore, Cu may not have an antagonistic effect on Zn in Rex rabbits.

In summary, the overall results of this study suggest that 30 mg/kg Cu is the optimum amount of Cu to supplement in the diet of Rex rabbits.

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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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with Ethical Standards

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

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