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Effect of catalase on the growth performance, antioxidation, and microbial metabolism of weaned rabbits

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Abstract: The present study aimed to investigate the effect of catalase (CAT) on the growth performance, antioxidation, and microbial metabolism of weaned rabbits. Dietary treatments consisted of control and CAT supplementation at 100 (T1), 150 (T2), and 200 IU/kg of diet (T3). A total of 240 weaned rabbits were randomly assigned to 4 groups with 6 replicates of 10 rabbits each. The feeding trial lasted for 28 days. Results showed that T2 and T3 increased ($P < 0.05$) body weight gain and gain/feed ratio, and decreased ($P < 0.05$) diarrhoea rate, compared to the control. Also, serum antioxidative parameters and oxidative stress products were beneficially regulated ($P < 0.05$) by the dietary CAT administration. Faecal microbiota including *Bacteroidetes, Prevotella,* and *Bifidobacterium* in T2 or T3 was increased ($P < 0.05$). Dietary CAT with changed microbiota further influenced the metabolites from carbohydrates and proteins, evidenced by increased lactic acid, acetate, branched-chain fatty acids, and short-chain fatty acids, and decreased valerate, isovalerate, methylamine, tryptamine, putrescine, cadaverine, spermidine, and total amines. It is concluded that CAT can be used to improve growth performance by beneficially regulating the antioxidation, microbiota, and metabolites in weaned rabbits.

Keywords: amine; gut microbe; short-chain fatty acid; oxidant injury product

Catalase (CAT), an enzyme that catalyzes the breakdown of hydrogen peroxide into oxygen and water, is present in various tissues of all known animals, especially in the liver. CAT is a marker enzyme in the peroxisome system, accounting for about 40% of the total peroxisome enzymes (Alomar et al. 2016; Sepasi Tehrani and Moosavi-Movahedi 2018; Baker et al. 2023). Initially, CAT is used in the food industry to remove hydrogen peroxide from milk for cheese production and it is also used in food packaging to prevent food from being oxidized. Recently, rodent or clinical research has shown that CAT was found capable of relieving oxidative stress-associated degenerative diseases and nonalcoholic fatty liver disease (Shin et al. 2018; Nandi et al. 2019). Curiously, how about the excellent antioxidant function of CAT in the scenario of farm animals? Especially, with the ban on growth-promoting antibiotics, oxidative stress induces the frequency of subclinical or clinical diseases in farm animals. However, the literature about the effect of CAT on farm animals is very limited. Li et al. (2020a, b) reported that dietary supplementation of 120 IU/kg of exogenous CAT improved

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growth performance, antioxidant capacity, intestinal development, and gut microbiota, but reduced oxidative stress and liver damage of piglets.

Weaned animals are sensitive to varied conditions in the management, the feed, and the environment. To deal with these changes or stresses, the most common method is to enhance their antioxidant capacity. Meat-type rabbits, belonging to monogastric herbivores, are increasingly popular in some countries, especially in those with grain shortage, and the information about CAT on rabbits is unavailable. Based on the action theory of CAT and its current reports in antioxidation, the present study aimed to test the hypothesis that CAT can be used as an antioxidant in weaned rabbits by investigating growth performance, serum antioxidation, and gut microbiota.

MATERIAL AND METHODS

Animal ethics approval

Research on animals was conducted according to the rules of the committee on animal use at Henan University of Science and Technology (No. 2021016).

Diets, animals, and samples

Commercial CAT has an activity of 60 IU/g (Liaoning Vetland Bio-Technology Co., Ltd, Liaoning, China). There were four dietary treatments including control (a basal diet) and CAT at 100 (T1), 150 (T2), and 200 (T3) IU/kg of diet. The determined values of CAT in T1, T2, and T3 were 92, 155, and 201 IU/kg. The basal diet was formulated referring to the Nutritional Requirement of Chinese Growing Rabbits (Standards in Agricultural Industries in China, NY/T 4049-2021), and its ingredients and chemical compositions were listed in Table 1.

A total of 240 weaned male rabbits (IRA) at approximately 32 days of age (0.935 ± 0.010 kg, BW ± SD) were randomly distributed into four groups with six replicates of 10 rabbits each. The feeding trial after a 3-day adjustment period lasted for 28 days. The animal house had a controlled room temperature of 22 °C and automatic mechanical ventilation. All rabbits were given *ad libitum* access to diets and water throughout the feeding trial (Wang et al. 2019). Rabbits and feed were weighed weekly, and feed efficiency was adjusted for mortality on a replicate basis. The general health of rabbits was monitored twice a day.

Feed intake, body weight gain, feed/gain ratio, survival rate, and diarrhoea rate per replicate were calculated throughout the feeding trial according to the formulas: average daily body weight gain (ADG, g/day) = (final weight – initial weight)/(days on test × rabbits on test), average daily feed intake (ADFI, g/day) = total feed intake/(days on test × rabbits on test), feed/gain ratio = ADFI/ADG, survival rate (%) = surviving rabbits/rabbits on

Table 1. Ingredient and nutritional contents of basal diets (air-dry basis)

Ingredient	Content (%)
Corn	15.7
Soy meal	10.5
Brewers dried grain	9.7
Corn DDGS	8.3
Peanut vine	36.0
Wheat bran	13.0
Wheat middling	5.0
Calcium hydrogen phosphate	0.7
Choline	0.1
Premix[1]	1.0
Nutritional level[2]	
Crude protein	16.51
Digestible energy (MJ/kg)	10.80
Ether extract	2.50
Starch	13.22
Crude fiber	16.37
Lysine	0.81
Methionine + cysteine	0.62
Ca	0.83
P	0.42

DDGS = distillers dried grains with solubles

[1]The premix provided the following per kg of diet: vitamin A 6 000 IU, vitamin D_3 1 000 IU, vitamin E 50 mg, vitamin K 1 mg, vitamin B_1 1 mg, vitamin B_2 3 mg, niacin 30 mg, pantothenic acid 10 mg, folic acid 0.2 mg, vitamin B_{12} 10 µg, Fe 50 mg, Zn 50 mg, Cu 5 mg, Mn 8 mg, I 0.5 mg, Se 0.1 mg, NaCl 5 g, Lys 1.5 g, Met 1.5 g

[2]Digestible energy was calculated using the Chinese Feed Database (v31, 2020) and others were determined values

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test) × 100, and diarrhoea rate (%) = (rabbits with diarrhoea/rabbits on test) × 100. Mushy, yellow to brown, bloody, or mucus-covered faeces were identified as diarrhoea.

On the last day of the feeding trial, four fasted rabbits per replicate were randomly selected and blood samples were collected from the left ear vein and prepared for quantifying serum immunoglobulins and whole blood lymphocytes (Wang et al. 2011). After blood sampling, the faecal samples of the four rabbits were collected for determining microbiota and metabolites.

Chemical analysis

The contents of nutrients in the diets were determined according to the method by AOAC (2007), including dry matter (930.15), crude protein (990.03), crude fibre (962.09), ether extract (920.39), total starch (996.11), Ca (968.08), total P (964.06), and amino acids (982.30E). Amino acids were determined using an AA analyzer (Beckman 6300; Beckman Coulter, Inc., Fullerton, CA, USA).

The activity of CAT in diets and samples was determined according to the Determination of Hydrogen Peroxidase Activity of Grains and Oils (GB/T 5522-2008, China Standard). The activity of CAT (IU/kg) was expressed as grams of hydrogen peroxide consumed by the interaction of CAT with hydrogen peroxide in a kilogram sample. Briefly, at pH 7.7, CAT was extracted from the sample, a certain amount of hydrogen peroxide was added to the extract solution to decompose hydrogen peroxide under the action of CAT, and then the excess hydrogen peroxide was titrated with potassium permanganate solution. The activity of CAT in the sample was calculated from the consumption of potassium permanganate solution.

Commercial kits from Nanjing Jiancheng Biological Institutes (Nanjing, China) were used for determining superoxide dismutase (SOD, A001-0302), glutathione peroxidase (GPx, A005-102), malondialdehyde (MDA, A003-1-2), protein carbonyl (PCO, A-087-1-2), 8-hydroxydeoxyguanosine (8-OHdG, H165-1-1), lactic acid (A019-1-1). Faecal short-chain fatty acids were determined using gas chromatography (7890A, Agilent Technologies, Inc., Santa Clara, CA, USA) according to the method by Yu et al. (2017). Ammonia nitrogen (NH_3-N) was assayed with HACH® kits using spectrophotometer absorbance at the wavelength of 655 nm (Hach Method 10031; range: 0.4–50.0 mg/l). Biogenic amines were determined by High-Performance Liquid Chromatography (1100 series, Agilent Technologies, Inc., Santa Clara, CA, USA).

Bacterial quantification

Approximately 0.18–0.22 g of solid faeces (without contamination) were weighed and put in a centrifuge tube, isolated, and purified for total DNA using commercial kits from Sangon Biotech (Shanghai, China). The concentration and purity were assayed by NanoDrop One (Thermo Scientific, Waltham, MO, USA) with the ranges of optical density at 1.80–2.00 (260/280) and approximately 2.00 (260/230). Total bacteria, *Firmicutes*, *Bacteroidetes*, *Lactobacillus*, *Escherichia coli*, *Clostridium* cluster IV, *Ruminococcus*, *Prevotella*, and *Bifidobacterium* were quantified by measuring 16S rRNA copies using real-time PCR (CFX96, Bio-Rad, Hercules, CA, USA). The primers were synthesized by Sangon Biotech. The information on primers and real-time PCR systems was referred to the reports by Yu et al. (2017). All samples were determined in triplicate.

Statistical analysis

Values were expressed as means and SEM using one-way ANOVA of SPSS v23.0 software (SPSS, Inc., Chicago, IL, USA). Differences between treatment means were determined by Tukey's test at $P < 0.05$. A statistical unit for growth performance applied to all rabbits, whereas statistical units for serum antioxidation and microbial metabolism were the average values of four sampled rabbits per replicate.

RESULTS

Growth performance and diarrhoea rate

Compared to the control (Table 2), diets T1 and T2 increased ($P < 0.05$) the final body weight; the three CAT doses increased ($P < 0.05$) ADG and decreased ($P < 0.05$) the feed/gain and diarrhoea rate of rabbits. Furthermore, T3 showed a more pronounced ($P < 0.05$) effect on feed/gain than T1.

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Table 2. Effect of catalase on the growth performance and diarrhoea incidence of weaned rabbits

Item	Control	Dietary catalase (IU/kg)			SEM	*P*-value
		T1 (100)	T2 (150)	T3 (200)		
Initial BW (kg/rabbit)	0.94	0.93	0.93	0.94	0.004	0.816
Final BW (kg/rabbit)	1.98^{b}	2.11^{ab}	2.16^{a}	2.13^{a}	0.061	0.047
ADG (g/day)	38.6^{b}	42.1^{a}	43.8^{a}	42.6^{a}	1.010	0.046
ADFI (g/day)	159	159	162	153	3.182	0.109
Feed/gain ratio	4.12^{a}	3.78^{b}	3.70^{bc}	3.59^{c}	0.070	0.034
Survival rate (%)	96.0	98.0	98.0	98.0	1.851	0.852
Diarrhea rate (%)	4.44^{a}	2.91^{b}	2.17^{b}	2.53^{b}	1.325	< 0.001

ADFI = average daily feed intake; ADG = average daily body weight gain; BW = body weight

$^{a–c}$Means among treatments without the same superscripts are significantly different ($P < 0.05$)

Serum antioxidation

As shown in Table 3, in contrast with the control, dietary CAT supplementation increased ($P < 0.05$) the activity of CAT and SOD in the serum of rabbits, but there were no CAT dose effects on these antioxidative enzymes. Meanwhile, CAT decreased ($P < 0.05$) the serum oxidative stress products including MDA, PCO, and 8-OHdG. There were no differences in the serum oxidative stress products between the three CAT doses.

Faecal microbiota

The amounts of total bacteria, *Firmicutes*, *Ruminococcus*, *Clostridium* cluster IV, and *Escherichia coli* in the faeces of rabbits were unaffected by the addition of CAT, compared to the control (Table 4). For *Lactobacillus*, only T2 was higher ($P < 0.05$) than T3. Also, the populations of *Bacteroidetes* in T2 were greater ($P < 0.05$) than in the control and T3. *Prevotella* in T2 was the highest ($P < 0.05$) among treatments. The *Bifidobacterium* amount in T2 and T3 was greater ($P < 0.05$) than in the control.

Microbial metabolites

For carbohydrate metabolites, compared to the control (Table 5), the contents of lactic acid, acetate, butyrate, isobutyrate, BCFA, and total SCFA in CAT treatments were increased ($P < 0.05$) whereas valerate and isovalerate were decreased ($P < 0.05$); propionate was unaffected by the dietary treatments, and the dose effects were found for T2 or T3 on lactic acid and BCFA.

Table 3. Effect of catalase on the serum antioxidation of weaned rabbits

Item	Control	Dietary catalase (IU/kg)			SEM	*P*-value
		T1 (100)	T2 (150)	T3 (200)		
Serum antioxidative enzymes						
Catalase (IU/I)	2.23^{a}	3.08^{b}	3.17^{b}	2.96^{b}	0.130	< 0.001
SOD (IU/ml)	26.6^{a}	30.8^{b}	32.9^{b}	31.5^{b}	0.879	0.001
GPx (IU/ml)	431	465	476	481	25.17	0.752
Serum oxidative injury products						
MDA (nmol/ml)	2.67^{a}	1.90^{b}	2.11^{b}	2.10^{b}	0.064	< 0.001
PCO (nmol/ml)	0.42^{a}	0.33^{b}	0.31^{b}	0.31^{b}	0.016	< 0.001
8-OHdG (pg/ml)	27.4^{a}	23.1^{b}	21.2^{b}	23.0^{b}	1.427	0.017

8-OHdG = 8-hydroxydeoxyguanosine; GPx = glutathione peroxidase; MDA = malondialdehyde; PCO = protein carbonyl; SOD = superoxide dismutase

^{a,b}Means among treatments without the same superscripts are significantly different ($P < 0.05$)

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Table 4. Effect of dietary catalase supplementation on the faecal microbiota of weaned rabbits [Lg(16S rRNA gene copies/g of faecal content)]

Item	Control	Dietary catalase (IU/kg)			SEM	*P*-value
		T1 (100)	T2 (150)	T3 (200)		
Total bacteria	12.8	12.6	12.7	12.2	0.801	0.602
Firmicutes	10.8	10.5	12.2	10.6	0.317	0.115
Ruminococcus	9.57	10.0	10.2	9.58	0.198	0.233
Lactobacillus	9.41^{ab}	9.46^{ab}	10.2^{a}	9.14^{b}	0.256	0.014
Bacteroidetes	8.91^{b}	9.63^{ab}	10.1^{a}	8.89^{b}	0.196	< 0.001
Clostridium cluster IV	7.44	7.27	7.16	7.18	0.208	0.309
Prevotella	7.00^{b}	7.05^{b}	8.10^{a}	6.92^{b}	0.240	< 0.001
Bifidobacterium	7.86^{b}	8.46^{ab}	9.05^{a}	8.82^{a}	0.227	< 0.001
Escherichia coli	7.19	6.74	6.87	7.03	0.232	0.362

^{a,b}Means among treatments without the same superscripts are significantly different ($P < 0.05$)

Table 5. Effect of dietary catalase on faecal metabolites in weaned rabbits

Item	Control	Dietary catalase (IU/kg)			SEM	*P*-value
		T1 (100)	T2 (150)	T3 (200)		
Fatty acids						
Lactic acid (umol/g)	5.21^{c}	7.68^{b}	8.35^{a}	8.12^{ab}	0.117	< 0.001
Acetate (umol/g)	45.3^{b}	52.6^{a}	53.4^{a}	55.1^{a}	1.120	< 0.001
Propionate (umol/g)	19.5	20.4	21.0	21.9	1.024	0.052
Butyrate (umol/g)	13.4^{b}	17.3^{a}	17.5^{a}	17.7^{a}	1.035	0.035
Valerate (umol/g)	6.75^{a}	4.08^{b}	4.36^{b}	4.29^{b}	0.211	< 0.001
Isobutyrate (umol/g)	3.72^{b}	3.96^{ab}	4.11^{a}	4.21^{a}	0.202	0.022
Isovalerate (umol/g)	6.11^{a}	5.62^{ab}	4.85^{b}	4.85^{b}	0.200	0.001
BCFA (umol/g)	7.93^{c}	8.52^{bc}	11.9^{a}	9.12^{b}	0.405	< 0.001
Total SCFA (umol/g)	89.5^{b}	97.2^{ab}	105^{a}	109^{a}	3.002	0.001
Amines						
Methylamine (μg/g)	8.82^{a}	6.61^{b}	5.72^{b}	5.49^{b}	0.256	< 0.001
Tryptamine (μg/g)	17.5^{a}	12.2^{b}	10.4^{b}	9.93^{b}	0.406	< 0.001
Putrescine (μg/g)	206^{a}	162^{b}	120^{c}	112^{c}	9.108	< 0.001
Cadaverine (μg/g)	506^{a}	353^{b}	295^{c}	324^{bc}	12.11	< 0.001
Tyramine (μg/g)	19.5	15.3	15.2	16.1	1.002	0.230
Spermidine (μg/g)	32.8^{a}	28.7^{a}	21.4^{b}	24.5^{b}	1.001	< 0.001
Spermine (μg/g)	24.7^{a}	21.2^{a}	17.9^{b}	17.2^{b}	0.652	0.032
Total amines (μg/g)	815^{a}	599^{c}	486^{d}	509^{d}	12.01	< 0.001
NH_3-N (mg/g)	2.01	1.92	1.96	1.89	0.036	0.219

BCFA = branched-chain fatty acids; SCFA = short-chain fatty acids

$^{a–c}$Means among treatments without the same superscripts are significantly different ($P < 0.05$)

For amines, compared to the control, all CAT treatments decreased ($P < 0.05$) methylamine, tryptamine, putrescine, cadaverine, spermidine, spermine, and total amines; but there were no effects ($P < 0.05$) on tyramine and NH_3-N. More pronounced ($P < 0.05$) effects of T2 or T3 on putrescine, cadaverine, spermidine, spermine, and total amines were observed in comparison with T1.

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DISCUSSION

In living systems, hydrogen peroxide is generated from superoxide ions during the oxidative decomposition of the substance. A small amount of hydrogen peroxide has a disinfecting effect, whereas its excess amount is toxic to cells. Normally, these hydrogen peroxides can be decomposed by endogenous CAT (Gebicka and Krych-Madej 2019). However, numerous kinds of stress from the environment, management, and feed expose animals to a dilemma with excess reactive oxygen species and hydrogen peroxide, which erodes the health, reduces production performance, and even causes mortality of farm animals, in particular of susceptible animals including weaned rabbits. Based on these assumptions, exogenous CAT has been currently explored to alleviate or eliminate the toxicity of excess hydrogen peroxide in the body. Indeed, in the present study, dietary supplementation of CAT increased final BW and ADG, and decreased feed/gain and diarrhoea of weaned rabbits. Information about the effect of CAT on the growth and health in rabbits is unclear. In pigs, dietary supplementation of 120 IU/kg exogenous CAT improved gain/feed ratio, but it had no statistical differences in ADFI and ADG (Li et al. 2020a); also, lactating sows in the CAT group produced more milk and improved the ADG of piglets (Zhou et al. 2022). Additionally, the decreased diarrhoea rate in CAT treatments indicates that CAT can improve intestinal health, but more studies are needed.

In the present study, the antioxidative capacity of rabbits was also improved by dietary CAT, as evidenced by the increased activity of antioxidative enzymes including endogenous CAT, SOD, and GPx in the serum, and the decreased profiles of oxidative stress products of carbohydrates, proteins, and nucleic acids. The antioxidative capacity of CAT has been well documented. In a lipopolysaccharide-induced pig model, dietary exogenous CAT supplementation increased endogenous CAT, GPx, and SOD activities in the serum, liver, or intestinal mucosa, whereas it lowered the concentration or mRNA expression of MDA, caspase-3, and caspase-9 of piglets (Li et al. 2020a; Chen et al. 2021). Similarly, maternal CAT administration increased peroxidase and GPx activity, decreased total antioxidative capacity and endogenous CAT, and changed the gene expression levels of lactating sows and their offspring (Zhou et al. 2022). The study of Wang et al. (2022) further revealed that dietary CAT blocked liver enlargement, increased jejunal CAT and GPx, and decreased hepatic reactive oxide species and 8-OHdG contents, and caspase-9 expression in deoxynivalenol-induced broilers. Additionally, administration of CAT alone or mixed with glucose oxidase alleviated intestinal oxidative stress induced by diquat in weaned pigs (Sun et al. 2021).

Dietary CAT further affected faecal microbiota, reflecting the increased populations of *Lactobacillus*, *Bacteroidetes*, *Prevotella*, and *Bifidobacterium* of weaned rabbits. *Lactobacillus* has the ability to generate lactic acid, regulate intestinal flora and enhance body health (Zhao et al. 2020). *Bacteroidetes* are primary degraders of complex carbohydrate-based biomass and the genus ubiquitously exists in all ecosystems investigated to date, being particularly dominant in soils and human and animal guts. The genus *Prevotella* helps break down protein and carbohydrate foods, and it is also a conditional pathogenic bacterium, causing intestinal inflammation (Tett et al. 2021). *Bifidobacteria* are a group of bacteria called probiotics that normally live in the intestine and stomach, are helpful for digestion and stave off harmful bacteria (Schopping et al. 2022). It has been well documented that dietary antioxidants can affect the gut microbiota, but reports about the effect of CAT on microbiota are very limited. Chen et al. (2021) observed that exogenous CAT altered bacterial community diversity and richness, when it increased the abundance of *Succinivibrio* and reduced *Streptococcus*, *Faecalibacterium*, *Subdoligranulum*, and *Escherichia-Shigella* in weaned pigs. Wang et al. (2022) found that dietary CAT decreased some harmful bacteria including *Proteobacteria*, *Gammaproteobacteria*, *Enterobacteriales*, *Enterobacteriaceae*, and *Escherichia-Shigella*, but enriched the bacterial community with certain beneficial bacteria such as *Acidobacteriota*, *Anaerofustis*, and *Anaerotruncus* in broilers. Literature about dietary CAT administration in rabbits is unavailable, which needs further study.

The altered microbiota compositions by dietary CAT in the present study consequently affected the metabolites of carbohydrates and proteins, including increased BCFA and total SCFA, and decreased total amines. This indicated that dietary CAT can affect the degradation of microbiota

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to carbohydrates and proteins. Zhou et al. (2022) found that the utilization of CAT as a supplement for mothers from late pregnancy to the lactation period increased alanine transaminase, cholesterol, low-density lipoprotein level, C22:0, the n-6/n-3 PUFA ratio in the plasma or liver, and lipid catabolic genes in the jejunum, but it decreased medium- and long-chain fatty acids, C18:3n3, and lipid transporters. Perez-Estrada et al. (2019) reported that mice lacking CAT showed lower body weight, blood glucose levels, liver fat accumulation, and subsequently a shorter lifespan than wild-type mice. Also, CAT-deficient mice induced aging faster through lysosomal dysfunction (Dutta et al. 2022). Furthermore, the relationship between CAT and plasma lipid metabolism can predict the antioxidant status of tissues that affect meat quality (Skaperda et al. 2022). Additionally, literature about the effect of CAT on amine contents is unavailable. Therefore, protein metabolism and meat quality related to CAT may be curiosities for the future.

CONCLUSION

The diets containing CAT increased weight gain, feed efficiency, and antioxidative capacity, but they decreased the diarrhoea rate and oxidative stress products of weaned rabbits. Meanwhile, gut microbiota and their metabolites to carbohydrates and proteins were also beneficially regulated. The results suggest that CAT can be used as an antioxidant to improve the growth performance and health status of farm animals.

Conflict of interest

The authors declare no conflict of interest.

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