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Effects of Bamboo Leaf Extract on the Growth Performance, Blood Biochemistry, and Cecal Microbiota of Growing Rabbits

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Key words
Bamboo leaf extract, Natural additives, Ira growing rabbits, Antioxidant traits, Cecal microbial structure, Growth performance

ABSTRACT

Nowadays, there is an increasing concern among consumers regarding food safety, which has led to a growing interest in natural additives. This study investigated the impacts of bamboo leaf extract (BLE), a commercial product, on the growth performance and cecal microbiota of growing rabbits. One hundred and twenty-eight 6-week-old Ira rabbits were randomly divided into four groups. The control group was fed a basal diet, while the other groups received the basal diet added with 2.00/4.00/6.00 g BLE/kg. In relative to the control group, the rabbits fed a diet with 4.00 g BLE/kg showed significant improvement in average daily weight gain ($p < 0.001$) and a reduction in feed conversion ratio ($p = 0.001$). BLE supplementation in the diet significantly increased activities of superoxide dismutase in plasma, and catalase and superoxide dismutase in the liver ($p < 0.001$). The rabbits that consumed BLE-supplemented diets exhibited decreased abundances of Firmicutes ($p = 0.042$) and increased abundances of Bacteroidetes ($p = 0.018$), *Coprococcus,* and *Anaeroplasma* ($p < 0.001$). In summary, the inclusion of BLE in the feed diet enhances the growth performance, antioxidant properties, as well as cecal microbial composition of growing rabbits. Based on the results, it is recommended to add 4.00 g/kg of BLE to the diet.

INTRODUCTION

Rabbits are considered excellent sources of meat due to their shorter life cycle, reduced gestation period, high productivity, and efficient feed-to-meat conversion (Lebas *et al*., 1997). With the growing emphasis on maintaining a healthy diet, there has been a growing interest in rabbit meat because of its low cholesterol content and high protein richness (Dalle Zotte and Szendrő, 2011). In extensive rabbit production, the growth phase is particularly critical as rabbits become more vulnerable to pathogenic microorganisms associated with various digestive diseases. This susceptibility can result in significant economic losses for the rabbit meat industry. Therefore, improving the gastrointestinal microbiota of rabbits has become an attractive approach for enhancing the profitability of rabbit farming. Nowadays, natural feed additives, including prebiotics, probiotics, plant extracts, and organic acids, have gained popularity for their ability to improve meat quantity and quality, meeting the growing demands of consumers (Seidavi *et al*., 2021).

Bamboo leaf has long been applied in Traditional Chinese Medicine for the treatment of conditions containing atherosclerosis, diabetes, and nervous system diseases (Cheng *et al*., 2023). Bamboo leaf extract (BLE) contains various compounds, including flavone glycosides,

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phenolic acids, anthraquinones, coumarin lactones, polysaccharides, and amino acids (Ma *et al*., 2012). BLE possesses significant biological and therapeutic properties, including antioxidant and anti-inflammatory effects (Kimura *et al*., 2022), cholesterol-lowering activity (Shen *et al*., 2019a), free radical scavenging ability (Rajendran *et al*., 2004), and immune-enhancing properties. Currently, BLE finds applications in animal husbandry and medicine. As a dietary supplement, BLE has been shown to enhance antioxidant and immune activities, as well as lipid metabolism, in weaned piglets (Zhang *et al*., 2013). In addition, BLE has been found to improve rumen fermentation and production performance in cows experiencing heat stress (Li *et al*., 2021). Furthermore, BLE has demonstrated the ability to ameliorate diabetic nephropathy in diabetic rats, potentially by hindering oxidative stress via the activation of the ser/thr protein kinase pathway (Ying *et al*., 2017). The inclusion of 4.00 g/kg BLE in broiler diets has been found to be beneficial for energy metabolism and antioxidant status (Xie *et al*., 2023). Moreover, the use of bamboo leaf flavonoids (BLF) has been shown to promote immunity and modulate the structure of the gut microbiota in broilers (Shu *et al*., 2020).

Nevertheless, there is a lack of studies focusing on the impact of BLE on rabbits. Our research hypothesis posits that BLE, as a phytogenic feed additive, can positively influence the growth performance and antioxidant capacity of growing rabbits. Thus, the primary aim of the current work was to examine the impacts of BLE supplementation on the growth performance, antioxidant activity, immune characteristics, and cecal microbiota composition of growing rabbits. Specifically, we sought to explore the influence of BLE on rabbits during their growth stage, thereby establishing a fundation for the potential use of BLE in rabbit diets.

MATERIALS AND METHODS

Animals and experimental design

Zhejiang XinHuang Biotechnology Co., Ltd. (Zhejiang, China) prepared the bamboo leaf extract, which had a flavonoid concentration of 70.0 mg per gram of BLE and a polyphenol concentration of 50.4 mg per gram of BLE. The extraction process utilized an enzyme-assisted extraction method. In details, the bamboo leaves were crushed to 1-2 mm, and mixed with ethyl acetate in a 1:8.5-10 ratio (W/W). The mixture was extracted by ultrasonic at 50-70°C to obtain the polyphenol. Then leaf residue was abstracted ultrasound at 50-70°C, adding anhydrous ethanol, and filtrated by X-5 macroporous resin to obtain the flavonoid. To obtain bamboo leaf extract, the extracted polyphenol and flavonoid were mixed in a 18:25 ratio. A total of one hundred and twenty-eight healthy weaned Ira rabbits, aged 6 weeks and weighing 978 ± 7.03 g each were assigned randomly to 4 groups: basal diet without BLE supplementation (control diet, CON); CON + 2.00 g BLE/kg diet (BLE2); CON + 4.00 g BLE/kg diet (BLE4); and CON + 6.00 g BLE/kg diet (BLE6). The basal diet, formulated in accordance with the nutritional requirements for rabbits recommended by De Blas and Mateos (2021). was provided to all groups. Table I presents the composition of the basal diet. Each treatment consisted of four replicates, with eight rabbits housed in four cages (two rabbits per cage). The dimensions of the cages were 50.0 cm × 40.0 cm × 60.0 cm. Based on the experiment, the rabbits had *ad libitum* access to food and water. Using exhaust fans and thermostatically-controlled heaters, the experimental temperature was kept at 18.0 ±1.0°C. The study duration was 45 days, including a 3-day adaptation period.

Table I. Basal diet composition (dry matter basis).

Ingredients[1]	
Alfalfa meal	34.0%
Corn	25.5%
Soybean meal	13.0%
Rapeseed dregs	3.0%
Wheat bran	20.0%
Salt	0.2%
Mountain flour	0.3%
4% Premix	4.0%
Total	100%
Nutrient composition[2]	
ME, MJ/kg	9.2%
Crude protein	16.2%
Crude fiber	13.6%
Lys	0.84%
Thr	0.60%
Ca	0.68%
P	0.42%

[1]Premix offered per kg of diet: Vitamin A, 10000 IU; Vitamin D_3, 1500 IU; Vitamin E, 80 mg; Fe ($FeSO_4 7H_2O$), 100 mg; Zn ($ZnSO_4 H_2O$), 70 mg; Mn ($MnSO_4 H_2O$), 15 mg; Cu ($CuSO_4 H_2O$), 24 mg; Se (Na_2SeO_3), 0.3 mg; I ($Ca(IO_3)_2$), 0.5 mg; Methionine, 0.40%; Threonine, 0.40%. [2]ME (metabolic energy) represented the calculated value, whereas other nutrient levels stood for measurements.

Sample collection

At the conclusion of the experiment, the rabbits underwent a 12-h fasting period, after which two rabbits

from each replicate were randomly chosen. Blood samples were gathered from the posterior auricular vein. The collected blood was allowed to stand at 4.0°C for 1 h, followed by centrifugation at 3000 rpm for 10 min. The resulting supernatant was preserved at –80.0°C for subsequent analysis. For anesthesia, sodium pentobarbital was administered intravenously. Liver tissue samples were taken from the middle of the right lobule in the anatomical location and preserved at –80.0°C for analysis of antioxidant enzymes.

Growth performance

The initial body weights (BWs) of the rabbits were recorded before the morning feeding on both day 4 and day 46. The daily feed intake in each cage was measured, allowing for the calculation of average daily body weight gain (ADG; total body weight gain divided by the duration of the experiment), average daily feed intake (ADFI; total feed intake divided by the duration of the experiment), and feed conversion ratio (FCR; feed intake divided by body weight gain).

Plasma and liver antioxidant assays

The activity of various antioxidant enzymes were assessed using dedicated kits and following the provided protocols (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China). In details, glutathione peroxidase (GSH-PX) assay kit (colorimetric method, A005-1-2), superoxide Dismutase (SOD) assay kit (hydroxylamine method, A001-2-2), malondialdehyde (MDA) assay kit (TBA method, A003-1-2), catalase (CAT) assay kit (visible light, A007-1-1), and total antioxidant capacity assay kit (colorimetric method, A015-1-2) were used in our experiment.

Blood biochemical parameters and immunological assays

The levels of plasma immunoglobulin A/G/M (IgA/G/M) were quantified with a commercial ELISA kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) following the provided protocols. The concentrations of plasma globulin (GLB), albumin (Alb), triglycerides (TG), and total cholesterol (TC) were measured using a biochemical analyzer (model: Sorvall ST 40).

Analysis of cecal microflora composition

In line with the provided instructions, the total genomic DNA (gDNA) of the cecal bacteria was extracted using the stool DNA kit (Omega, M5636–02). High-throughput sequencing (HTS) of the 16S rRNA gene was adopted for exploring the bacterial diversity and composition in the cecum. Specific primers targeting the V3-V4 regions of the 16S rRNA gene (forward: 5′-ACTCCTACGGGAGGCAGCA-3′; reverse: 5′-GGACTACHVGGGTWTCTAAT-3′) were applied to enable sample multiplexing during sequencing. The microbiome bioinformatics analysis was carried out with QIIME2 2019.4, following specified protocols with minor modifications. The sequencing data were analyzed using the R package (v3.2.0) and QIIME2. Alpha diversity indices at the operational taxonomic unit (OTU) level, containing observed species, Chao1 richness estimator, Simpson index, Shannon diversity index, Good's coverage, Pielou's evenness, and Faith's PD, were calculated with the OTU table in QIIME2. The results were visualized using box plots.

Statistical analysis

The obtained data were explored with SAS 9.4, and a one-way ANOVA was carried out. Tukey's multiple comparison test was adopted for determining the differences among the data. The results are represented as mean ± SEM, with a significance level of $p < 0.05$, suggesting statistical significance.

RESULTS

Growth performance

There existed no significant differences ($p > 0.10$) in ADFI and initial body weight (BW) among the four groups of growing rabbits (Table II). However, in relative to the CON group, rabbits in the BLE4 group presented a significant elevation in final weight ($p = 0.048$), average daily weight gain (ADG) ($p = 0.003$), and a decrease in feed conversion ratio (FCR) ($p = 0.001$).

Table II. Role of BLE as a diet supplement in the growth performance of growing rabbits.

Items	Treatments[1]				SEM[2]	*p* value
	CON	BLE2	BLE4	BLE6		
Initial weight (g)	975	977	977	975	12.3	0.999
Final weight (g)	2027^{a}	2089^{ab}	2144^{b}	2064^{ab}	34.2	0.048
ADG (g)	25.0^{a}	26.5^{ab}	27.9^{b}	25.9^{a}	0.49	0.003
ADFI (g)	104	104.5	105	104	0.23	0.190
FCR	4.18^{b}	3.96^{ab}	3.77^{a}	4.02^{ab}	0.07	0.001

The ^{a,b} values in each row that have different superscripts suggest significant differences ($p<0.05$). BLE: bamboo leaf extract; ADG, average daily weight gain; ADFI, average daily feed intake; FCR, feed conversion ratio. [1]CON: control (basal diet + 0 g BLE/kg diet); BLE2/BLE4/BLE6: basal diet + 2.0 g/4.0 g/6.0 g BLE/kg diets, separately. [2]Standard error of the means.

Antioxidant analysis of the plasma and liver

No significant differences existed among the groups

regarding GSH-Px and T-AOC activity in both plasma and liver (Table III; $p > 0.05$). However, rabbits fed with BLE4 or BLE6 diets had lower levels of MDA in both plasma and liver in relative to those fed with the CON diet ($p < 0.001$). BLE supplementation in the diet notably elevated the activities of SOD in the liver and plasma compared to the control diet ($p < 0.001$). The rabbits fed with the BLE6 diet showed higher CAT activity in plasma when compared with the other three groups ($p < 0.001$). Additionally, as the dietary BLE levels increased, the liver CAT activity of the rabbits also increased ($p < 0.001$). The rabbits in the BLE4 and BLE6 groups exhibited higher liver GSH-Px activity compared to those in the CON and BLE2 groups ($p < 0.001$).

Table III. The effects of BLE as a diet supplement on the plasma and liver anti-oxidation indices of growing rabbits.

Items	CON	BLE2	BLE4	BLE6	SEM[2]	*p* value
Plasma antioxidant index[1]						
MDA (nmol/mL)	70.8^{b}	68.0^{b}	48.1^{a}	46.5^{a}	1.93	< 0.001
GSH-Px (U/mL)	346	353	345	351	8.84	0.9
SOD (U/mL)	30.3^{a}	35.6^{c}	38.4^{d}	33.1^{b}	0.66	< 0.001
CAT (U/mL)	58.4^{a}	58.9^{a}	59.6^{a}	69.3^{b}	1	< 0.001
T-AOC (U/mL)	21.6	21.9	22.8	21.6	0.75	0.68
Liver antioxidant ındex[1]						
MDA (U/mg prot)	56.0^{c}	53.5^{bc}	49.5^{b}	40.4^{a}	1.24	< 0.001
GSH-Px (U/mg prot)	852^{a}	860^{a}	948^{b}	1073^{c}	18.5	< 0.001
SOD (U/mg prot)	207^{a}	233^{b}	243^{b}	330^{c}	5.99	< 0.001
CAT (U/mg prot)	1360^{a}	1479^{b}	1579^{c}	1677^{d}	32.1	< 0.001
T-AOC (U/mg prot)	27.1	26.7	27	26.8	0.56	0.098

$^{a–d}$Values in each row that have different superscripts indicate significant differences (p<0.05)). BLE, bamboo leaf extract; MDA, malondialdehyde; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; T-AOC, total antioxidant capacity. [1]CON, control (basal diet + 0 g BLE/kg diet); BLE2/BLE4/BLE6, basal diet + 2.0 g/4.0 g/6.0 g BLE/kg diets, separately. [2]Standard error of the means.

Plasma biochemical parameters

The results shown in Table IV indicated that no significant differences existed in plasma levels of Alb, IgA, and IgG among the four rabbit groups ($p > 0.100$). However, the supplementation of BLE increased the levels of GLB ($p = 0.002$), with the BLE6 group having the highest GLB levels. When compared with the CON group, the BLE4 and BLE6 groups had lower levels of TC ($p < 0.001$) and TG ($p = 0.004$), as well as higher levels of IgM in the plasma ($p < 0.001$).

Table IV. The effects of BLE as a diet supplement on the plasma biochemical parameters of growing rabbits.

Items	Treatments[1]				SEM[2]	*p* value
	CON	BLE2	BLE4	BLE6		
ALB (g/L)	40.7	39.7	39.5	39.7	0.83	0.261
GLB (g/L)	21.4^{a}	26.8^{b}	27.9^{b}	30.0^{c}	1.02	0.002
TC (mmol/L)	5.51^{c}	5.19^{bc}	4.67^{b}	3.84^{a}	0.17	< 0.001
TG (mmol/L)	1.44^{b}	1.27^{b}	0.92^{a}	0.75^{a}	0.10	0.004
IgA (ug/mL)	2977	2946	3100	3124	103	0.108
IgG (g/L)	21.9	23.1	22.7	23.6	0.68	0.217
IgM (ug/mL)	546^{a}	577^{a}	717^{b}	708^{b}	26.3	< 0.001

$^{a–c}$Values in each row that have different superscripts indicate significant differences (p<0.05). BLE, bamboo leaf extract; ALB, albumin; GLB, globulin; TC, total cholesterol; TG, triglycerides; IgA/G/M, immunoglobulin A/G/M. [1]CON, control (basal diet + 0 g BLE/kg diet); BLE2/BLE4/BLE6, basal diet + 2.0 g/4.0 g/6.0 g BLE/kg diets, separately. [2]Standard error of the means.

Cecal microflora composition analysis

Venn diagrams were utilized to compare the similarities and differences in microbial communities among the groups. Figure 1A demonstrates that the number of OTUs for the CON, BLE2, BLE4, and BLE6 groups were 4572, 5457, 6204, and 6781, respectively. Additionally, the numbers of unique OTUs for the CON, BLE2, BLE4, and BLE6 groups were 2833, 3689, 4240, and 4846, respectively. The cecal microbial communities of the rabbits in all four groups shared 502 common OTUs. Figure 1B displays an increase in the Chao 1 index in rabbits with BLE supplementation compared to the CON group. Observed species in the cecal microbiota of the BLE4 and BLE6 groups was higher compared to the CON group ($p = 0.007$). However, there existed no obvious differences among the groups regarding the Shannon, Simpson, Faith_pd, and Pielou_e indices ($p > 0.05$).

At the phylum level (Fig. 1C), the predominant microbial phyla in the cecum were Firmicutes, Bacteroidetes, Verrucomicrobia, and Tenericutes. BLE supplementation caused a notable reduction in the abundance of Firmicutes ($p = 0.044$) while increasing the abundance of Bacteroidetes in the cecal microbiota of the rabbits ($p = 0.018$). At the genus level (Fig. 1D), the most abundant genera were *Ruminococcus*, *Oscillospira*, *Akkermansia*, and *Coprococcus*. As depicted in Figure 1E, the group supplemented with 6.00 g BLE/kg showed an increased abundance of *Oscillospira* in the cecum ($p = 0.031$) compared to the other groups. Furthermore, an increase in dietary BLE levels enhanced the abundance of *Coprococcus* in the rabbit cecum ($p \leq 0.001$). The addition of BLE notably elevated the abundance of *Anaeroplasma*

in the cecum of the rabbits ($p < 0.001$), while the BLE2 group exhibited a decreased abundance of *Bilophila* in the cecum compared to the CON group ($p = 0.015$).

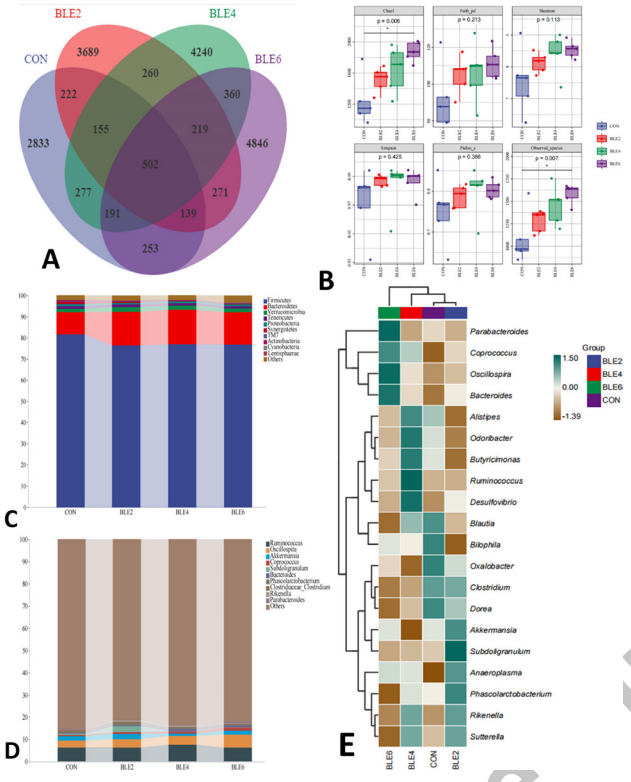

Fig. 1. The impacts of dietary supplementation with BLE on the bacterial community in the cecum of growing rabbits. (A) Venn diagram presenting the unique and shared microbial genera among the four groups. The 97% sequence identity level was selected for the determination of the OTUs. (B) The microbiota diversity in the cecum samples was estimated based on the Chao 1, Faith_pd, Simpson, Shannon, Observed species, and Pielou_e indices. *$p < 0.05$. (C) Gut microbiota at the phylum level in the growing rabbits of the four groups along with their relative abundances (top 10 for abundance). (D) Gut microbiota at the genus level in the growing rabbits of the 4 groups, along with their relative abundances (top 10 for abundance). (E) Heatmap analysis of the bacterial communities according to the 20 most significantly clustered genera. The diverse colors imply the diverse abundances of the genera among the four groups. BLE, bamboo leaf extract; CON, basal diet + 0 g BLE/kg diet; BLE2/BLE4/BLE6, basal diet + 2.0/4.0/6.0 g BLE/kg diets. n = 20 per group.

DISCUSSION

In our study, the group of growing rabbits that received dietary supplementation with BLE4 exhibited improved ADG and final weight, as well as FCR. While there is limited literature on the impacts of BLE on rabbit growth, previous studies have indicated the positive effects of BLE or BLF on broiler growth. For instance, Shen *et al.* (2019b) showed that adding 2.00 g/kg of BLE to broiler diets improved ADG and reduced FCR. The underlying mechanisms for these growth-promoting effects may be attributed to flavonoids acting as estrogen-like compounds, facilitating rabbit growth and promoting anabolism (Havsteen, 2002). However, it is important to note that a previous study involving piglets did not observe any changes in growth performance with BLE supplementation (Yu *et al.*, 2022; Zhang *et al.*, 2013). These contrasting findings may be attributed to differences in BLE doses and the use of different animal species across studies.

MDA is a by product of peroxidation, and antioxidant enzymes containing CAT, SOD, and GSH-Px play a vital role in protecting the cell structure from damage caused by excessive oxygen radicals (Yu *et al.*, 2018). T-AOC reflects the overall level of antioxidant enzymes. In our study, rabbits in the BLE4 and BLE6 groups exhibited lower MDA levels in the plasma and liver compared to the CON group. Similar findings have been shown in previous studies involving other animals, where broilers showed a decrease in liver tissue MDA concentration with BLE supplementation (Shen *et al.*, 2019b), and pigs exhibited a decrease in plasma MDA concentration with BLE supplementation (Shen *et al.*, 2019b). Our results also demonstrated that BLE supplementation increased liver GSH-Px activity (4.00/6.00 g BLE/kg), plasma and liver SOD activity (2.00/4.00/6.00 g BLE/kg), and plasma CAT activity (6.00 g BLE/kg) as well as liver CAT activity (2.00/4.00/6.00 g BLE/kg). These findings suggest that dietary supplementation with BLE enhances the antioxidant capacity in growing rabbits. Limited information is available on the role of BLE in antioxidant enzyme activity in growing rabbits. However, previous studies have shown that dietary supplementation with BLE promotes GSH-Px enzyme activity in both the serum and liver of broilers (Shen *et al.*, 2019b). Additionally, BLE has been reported to exhibit antioxidant effects through free radical scavenging, including the scavenging of DPPH and peroxyl radicals (Hu *et al.*, 2000). This evidence supports the conclusion that BLE possesses the ability to scavenge free radicals (Ni *et al.*, 2013), there by positively affecting the antioxidant capacity of growing rabbits.

The analysis of plasma biochemical parameters provides valuable insights into the health and clinical status of animals. Albumin and globulin serve as informative inflammatory-nutritional biomarkers, while immunoglobulins, including IgA, IgG, and IgM, are crucial indicators of the body's immune capacity. In our study, the

plasma GLB levels increased in growing rabbits fed with BLE-supplemented diets. Furthermore, supplementation with 4.00 or 6.00 g BLE/kg resulted in decreased levels of TC and TG, accompanied by an increase in plasma IgM levels. Based on our knowledge, there have been no previous reports on the impacts of BLE on the plasma biochemical parameters of rabbits. However, studies have suggested that dietary supplementation with BLE can increase IgG levels compared to the CON, while IgA levels remained unchanged in weaned piglets (Zhang *et al*., 2013). The discrepancies between these findings could be caused by the differences in the animal models and BLE dosage levels used in the respective experiments. Certain studies have demonstrated that BLE can decrease serum TC and TG levels in various animals (Nirmala *et al*., 2018; Park and Jhon, 2009). In our study, supplementation with BLE4 and BLE6 diets led to decreased TC and TG levels in the plasma of rabbits compared to the CON group. However, the underlying mechanisms responsible for these impacts of BLE on blood biochemical parameters remain to be elucidated and should be considered in future research.

The diversity of the intestinal microbiota exerts a vital role in the health and nutrition of the host. The increased alpha-diversity indices (Shannon, ACE, Chao 1, and Simpson) suggest that supplementation with BLE may be beneficial for enhancing cecal microbial diversity. Our findings revealed higher Chao 1 values in the BLE groups and higher Observed species values in the BLE4 and BLE6 groups when compared with the CON group. Nevertheless, no significant differences were found in the Shannon, Simpson, Faith pd, and Pielou e indices among the four groups. Similar results were shown by Li *et al.* (2021), who discovered increased Chao 1 (p = 0.06) and Shannon (p= 0.07) indices in the rumen of dairy cows following BLE supplementation. The disparities in these findings could be attributed to variations in BLE dosages and the animal species used across different studies.

Consistent with previous research (Bäuerl *et al*., 2014), our study identified Firmicutes, Bacteroidetes, and Verrucomicrobia as the predominant phyla in the cecal microbiota of rabbits. However, in another study, the cecal microbiota of growing Rex rabbits was found to be dominated by Firmicutes, followed by Bacteroidetes, Proteobacteria, as well as Verrucomicrobia (Wang *et al*., 2017). At the genus level, *Ruminococcus* was the most abundant genus, followed by *Oscillospira*, *Akkermansia*, and *Coprococcus*, according to our study. Conversely, other studies reported dominant genera as *Ruminococcus* and *Oscillospira,* followed by *Coprococcus, Bacteroides*, and *Blautia* in the cecal microbiota of Rex rabbits (Velasco-Galilea *et al*., 2018; Zou *et al*., 2016). These discrepancies are likely due to the use of different animal species in these studies.

A lower Firmicutes/Bacteroidetes ratio is related to more efficient lignocellulose digestion (Güllert *et al*., 2016). In our study, BLE supplementation elevated the abundance of Bacteroidetes, leading to a decreased Firmicutes/Bacteroidetes ratio in the cecum of rabbits. This finding is consistent with a previous study (Li *et al*., 2021). *Oscillospira* has been linked to positive effects on certain diseases, such as obesity-related metabolic disorders (Yang *et al*., 2021). *Coprococcus* significantly affects host health via the production of short-chain fatty acids (SCFAs) and vitamin B (Nogal *et al*., 2021). *Anaeroplasma* has been reported to alleviate chronic gut inflammation (Beller *et al*., 2019). Our results showed that rabbits fed with the 6.00 g BLE/kg diet had a higher abundance of *Oscillospira*, while all BLE supplementation groups exhibited increased abundances of *Coprococcus* and *Anaeroplasma* in the cecum. These findings suggest the beneficial effects of BLE on intestinal health. The *Bilophila* genus is associated with inflammation, bile acid metabolic disorders, and intestinal barrier dysfunction (Natividad *et al*., 2018). Since bile acids are cholesterol metabolites, the lower relative abundance of *Bilophila* in the cecum of rabbits fed the BLE2 diet may be associated with an improved profile of intestinal microbial function and reduced plasma TC.

CONCLUSION

To conclude, the inclusion of BLE in the diet of growing rabbits caused notable improvements in final body weight, ADG, and a reduction in FCR. Furthermore, BLE supplementation demonstrated enhanced antioxidant activity in these rabbits, as indicated by the decreased concentration of MDA and increased activities of CAT, SOD, and GSH-Px in both plasma and the liver. Additionally, rabbits receiving BLE supplementation exhibited favorable changes in their cecal microbiota, including increased microbial diversity and elevated abundances of *Oscillospira, Coprococcus*, and *Anaeroplasma*, as well as a decreased Firmicutes/Bacteroidetes ratio and *Bilophila* abundance. However, the precise mechanisms underlying these effects of BLE remain unclear and warrant further investigation in future studies.

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Ethical approval

The Xiangbiao Warren (Yibin, Sichuan, China) served as the location for each animal feeding trial. The experimental analysis was conducted at the Laboratory for Bio-feed and Molecular Nutrition of Yibin Vocational and Technical College. The experimental protocols were approved by the Animal Care and Use Committee of Yibin Vocational and Technical College.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Statement of conflict of interest

The authors have declared no conflict of interest.

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