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Original Research Article

Dietary *Litsea cubeba* essential oil supplementation improves growth performance and intestinal health of weaned piglets

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A B S T R A C T

This paper was to determine the effects of dietary *Litsea cubeba* essential oil (LEO) supplementation on growth performance, immune function, antioxidant level, intestinal morphology and microbial composition in weaned piglets. One hundred and ninety-two piglets (Duroc × [Large White × Landrace]) with 6.85 ± 0.22 kg mean body weight weaned at 21 d of age were randomly assigned to 4 treatment groups with 8 replicates and were fed with a basal diet (CON) or CON diet containing 100 (LLEO), 200 (MLEO) and 400 (HLEO) mg/kg LEO. The results revealed that HLEO supplementation ($P < 0.05$) increased the average daily gain on d 28 compared with CON. MLEO and HLEO supplementation decreased ($P < 0.05$) feed conversion ratio. LEO-containing diets had a lower ($P < 0.05$) diarrhea rate. Supplementation with HLEO increased ($P < 0.05$) total antioxidant capacity (T-AOC) both in the serum and liver. Meanwhile, the supplementation of MLEO and HLEO resulted in higher ($P < 0.05$) glutathione peroxidase (GPx) activities both in serum and liver. Supplementation of HLEO increased ($P < 0.05$) serum immunoglobulin A, immunoglobulin G and interleukin-10, whereas supplementation with MLEO and HLEO decreased ($P < 0.05$) tumor necrosis factor-α. Villus height in the duodenum or jejunum was increased ($P < 0.05$) in the HLEO group, and the villus height to crypt depth ratio in the jejunum was also improved ($P < 0.05$) in the MLEO group. The addition of LEO increased ($P < 0.05$) the richness and diversity of the microbial community in the cecum, which mainly increased the relative abundance of *Oscillospiraceae_UCG-005, Faecalibacterium, Blautia* and *Coprococcus*. Piglets supplemented with HLEO increased ($P < 0.05$) the concentration of short chain fatty acids (SCFA), including acetic acid in the cecum and propionic acid in the colon. In conclusion, these findings indicated that LEO supplementation improved growth performance and intestinal health in weaned piglets.

1. Introduction

Weaning stress, including nutritional, psychological and environmental pressures, affects growth performance, physiological and metabolic activities, intestinal immunity, and increases diarrhea rate (Campbell et al., 2013; Gabler et al., 2019). Antibiotic growth promoters (AGP) have become a mainstay for improving growth, maintaining feed efficiency and preventing pathogenic infections in the livestock and poultry industry (Van den Bogaard and Stobberingh, 1996). However, the wide use of AGP has given rise to the emergence of antibiotic resistant bacteria, and has raised concerns about food safety as well as environmental and public health issues (He et al., 2020). In January 2006, AGP were forbidden as animal feeds in the European Union to avoid adverse effects on animals and the environment. In recent years, AGP have been banned as medicinal feed additives in China (Wang et al., 2021). Some alternatives have advantages as substitutions for AGP, such as

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immune-modulating agents, essential oils (EO), bacteriophages, enzymes, probiotics, and anti-microbial peptides (Cheng et al., 2014). Thus, these products which are safe and green alternatives to AGP have received a worldwide appeal to attention (Anonye, 2016).

Litsea cubeba, widely distributed in tropical or subtropical areas (Kamle et al., 2019), is the most valuable medicinal plant in the Lauraceae family (Wang et al., 2016). The main pharmacological activities of *L. cubeba* essential oil (LEO) include acting as an anti-inflammatory mediator (Liao et al., 2015), anti-oxidative properties (Huang et al., 2013), sedative effects (Sattayakhom et al., 2021), and the alleviation of Alzheimer's disease (Lee et al., 2021) and rheumatoid arthritis (Zhao et al., 2020). In addition, the superior anti-microbial activities of LEO suggest that it may have potential as an AGP substitute (Gogoi et al., 2018). In a recent study, LEO could inhibit methicillin-resistant *Staphylococcus aureus*, thus mitigating leakage of intracellular macromolecules (Hu et al., 2019). Meanwhile, LEO inhibited *Acinetobacter baumannii* by suppressing the metastasis of ketone bodies and some amino acids, suggesting that LEO possesses antibacterial activity (Yang et al., 2021). Furthermore, *L. cubeba* supplementation improved carp's growth performance, feed efficiency and nonspecific immunity (Nguyen et al., 2016). However, there is little available literature on LEO utilization in farm animals. Based on the above, this study was designed to evaluate the protective effects of dietary LEO in piglets suffering from weaning stress.

2. Materials and methods

2.1. Ethics statement and preparation of LEO

The experiment and all animal procedures followed the animal welfare and experimental protocols by the Committee of Animal Care at Hunan Agricultural University (Permit Number: CACAHU, 2021-00269). Encapsulated *L. cubeba* essential oil was acquired by Nuoz Biological Technology (YiYang, Hunan, China). The main components of LEO determined by GC–MS are shown in Supplementary Table S1.

2.2. Experimental design, animals and diets

One hundred and ninety-two 21-d-old weaned piglets (initial weight: 6.85 ± 0.22 kg) were randomly allotted into 4 treatment groups fed with a basal diet (CON) or CON diet containing 100 (LLEO), 200 (MLEO) and 400 (HLEO) mg/kg LEO. Each treatment group was divided into 8 pens containing 6 pigs per pen. Supplementary Table S2 shows the composition and nutrients of the basal diet performed by National Research Council nutrient requirements (National Research Council, 2012). All piglets were given experimental diets with ad libitum feeding in the 28-day trial. After overnight fasting, each piglet was weighed on d 1 and 28 on an empty stomach. We recorded the average daily feed intake (ADFI) and average daily gain (ADG), and calculated the feed conversion ratio by ADFI/ADG. According to a previous study, the diarrhea rate (%) was monitored daily (Hung et al., 2019).

2.3. Preparation and sample collection

On d 28, after weighing all the piglets, one pig with average body weight from each pen was chosen for slaughter. All selected piglets were anesthetized by pentobarbital sodium. Serum was centrifuged after blood sample collection by anterior vena cava puncture. A piece of liver was cut from the left lobe. The duodenum, jejunum and ileum in each piglet were sectioned at the middle, and their gut digesta were also taken from this site. All samples were promptly collected from live piglets and transferred to suitable temperatures for storage.

2.4. Antioxidant capacity

One gram of liver was ground and dissolved in 10 mL exclusive extract solution. The supernatant was used for antioxidant capacity assays after centrifugation. The antioxidant-related indices in serum and liver, including total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), catalase, glutathione peroxidase (GPx), and malondialdehyde (MDA), were measured in accordance with specific reagent kit protocols (Beijing Boxbio Science & Technology, Beijing, China).

2.5. Immune responses

The levels of tumor necrosis factor–α (TNF-α), immunoglobulin (Ig) A, IgG and IgM, and the cytokines interleukin-1β (IL-1β), IL-6, and IL-10 in serum, were detected using a pig-specific enzyme-linked immunosorbent assay (ELISA) kit (Cusabio, Wuhan, China). All indicators were conducted according to the ELISA protocol in triplicate.

2.6. Morphological measurements

After slaughter, the mid-duodenum, mid-jejunum, and mid-ileum samples were quickly fixed in fixative solution. After 72 h of standing, the small intestinal slices were embedded in paraffin wax. The transverse section of wax was cut into 8 μm sections and placed on glass slides. Hematoxylin and eosin (H&E) staining was performed after dewaxing. The villus height, crypt depth and height to crypt (V:C) ratio were defined as previously reported (Wang et al., 2021). Briefly, 10 well-oriented villi per slide were calculated using a Zeiss Axio Scope 5 positive fluorescence microscope.

2.7. Microbial composition of the cecal digesta

The microbial community in cecal digesta was extracted using QIAamp Fast DNA Stool mini kits (Qiagen, Hilden, Germany). Previous studies referred to the primers for the 16S rRNA genes (Xu et al., 2016) and the conditions of PCR amplification (Yang et al., 2020). The Circos diagram reflects the composition and distribution between taxonomic levels and treatment groups (Arias-Giraldo et al., 2020). Principal co-ordinates analysis (PCoA) indicates the distribution of microbes between CON and LEO groups using the Qiime pipeline (Vazquez-Baeza et al., 2013). The linear discriminant analysis effect size (LEfSe) figure is a visual algorithm for probing biomarkers that identifies enrichment of different taxonomic levels (i.e., phylum to genus) simultaneously between CON and LEO groups (Solano-Aguilar et al., 2018).

2.8. Short chain fatty acids

Short chain fatty acids (SCFA) include acetic, propionic, isobutyric, butyric, isovaleric and pentanoic acids. The measurement of these SCFA followed a previously described method (Yin et al., 2022). One gram cecum and colon digesta were homogenized into 6-fold ultra-pure water. After centrifugation, 9-fold clear supernatant and 1-fold 25% HPO_3 were mixed in a new tube for the analysis of SCFA by gas chromatography (Agilent 7890, CA, USA).

Table 1
Effects of dietary *Litsea cubeba* essential oil (LEO) supplementation on growth performance in weaned piglets.

Item	Treatments[1]				SEM	*P*-value
	CON	LLEO	MLEO	HLEO		
Body weight on d 1, kg	6.98	6.84	6.81	6.79	0.216	0.931
Body weight on d 28, kg	14.6[b]	16.2[ab]	16.7[ab]	17.6[a]	0.643	0.022
Average daily gain, g/d	271[b]	333[ab]	354[a]	385[a]	20.7	0.005
Average daily feed intake, g/d	523	572	583	645	32.7	0.112
Feed conversion ratio	1.96[a]	1.72[ab]	1.67[b]	1.67[b]	0.062	0.007
Diarrhea rate, %	5.81[a]	2.64[b]	2.31[b]	1.64[b]	0.514	<0.001

Feed conversion ratio = average daily feed intake/average daily gain.
[a, b] Different superscript letters in each indicator represent a significant difference (P-value < 0.05).
[1] CON means piglets fed basal diet without LEO; LLEO means CON diet with 100 mg/kg LEO; MLEO means CON diet with 200 mg/kg LEO; HLEO means CON diet with 400 mg/kg LEO.

2.9. Statistical analysis

The individual pen was used as a statistical unit for the diarrhea rate, and the individual pig was used as a statistical unit for other data. The results were analyzed using v20.0 Statistical Product Service Solutions (SPSS) software. The difference in diarrhea rate was analyzed by chi-square contingency test. Differences in microbiota abundance in cecal digesta were analyzed by LEfSe using Kruskal–Wallis rank sum test. The differences in other data were analyzed via Tukey's multiple comparisons test. Significant differences among CON, LLEO, MLEO and HLEO groups were defined as $*P < 0.05$.

Table 4
Effects of dietary *L. cubeba* essential oil (LEO) supplementation on intestinal morphology in weaned piglets.

Item	Treatments[1]				SEM	*P*-value
	CON	LLEO	MLEO	HLEO		
Villus height (V)						
Duodenum, μm	317[b]	330[ab]	359[ab]	391[a]	16.1	0.017
Jejunum, μm	275[b]	304[ab]	331[ab]	357[a]	19.2	0.035
Ileum, μm	250	260	281	289	13.6	0.197
Crypt depth (C)						
Duodenum, μm	161	164	154	155	9.38	0.873
Jejunum, μm	183	175	165	170	8.42	0.523
Ileum, μm	145	151	143	140	8.01	0.742
V:C ratio						
Duodenum, μm/μm	1.98[b]	2.06[ab]	2.37[ab]	2.56[a]	0.107	0.002
Jejunum, μm/μm	1.53[b]	1.76[ab]	2.02[a]	2.12[a]	0.115	0.006
Ileum, μm/μm	1.75	1.75	2.00	2.09	0.116	0.117

[a, b] Different superscript letters in each indicator represent a significant difference (P-value < 0.05).
[1] CON means piglets fed basal diet without LEO; LLEO means CON diet with 100 mg/kg LEO; MLEO means CON diet with 200 mg/kg LEO; HLEO means CON diet with 400 mg/kg LEO.

3. Results

3.1. Growth performance

The effect of LEO supplementation on growth performance is shown in Table 1. HLEO supplementation increased ($P < 0.05$) the final body weight relative to CON piglets. Piglets fed the MLEO and HLEO diet improved ($P < 0.05$) ADG and decreased feed conversion

Table 2
Effects of dietary *L. cubeba* essential oil (LEO) supplementation on antioxidant status in weaned piglets.

Item	Treatments[1]				SEM	*P*-value
	CON	LLEO	MLEO	HLEO		
Serum						
Total antioxidant capacity, U/mL	10.7[b]	12.3[ab]	14.3[ab]	15.0[a]	0.884	0.012
Total superoxide dismutase, U/mL	43.2	45.9	48.6	49.2	3.99	0.717
Catalase, U/mL	79.4	78.6	83.0	83.3	2.88	0.58
Glutathione peroxidase, U/mL	159[b]	167[ab]	198[a]	200[a]	9.58	0.008
Malondialdehyde, nmol/mL	9.03	9.08	7.90	7.56	0.647	0.261
Liver						
Total antioxidant capacity, U/mL	19.3[b]	21.6[ab]	24.4[ab]	27.1[a]	1.90	0.042
Total superoxide dismutase, U/mL	53.0	52.5	56.2	59.3	5.03	0.779
Catalase, U/mL	45.3	48.4	52.9	55.8	3.69	0.216
Glutathione peroxidase, U/mL	99.6[b]	138[a]	144[a]	156[a]	8.76	<0.001
Malondialdehyde, nmol/mg prot	2.99	2.56	2.57	2.48	0.235	0.434

[a, b] Different superscript letters in each indicator represent a significant difference (P-value < 0.05).
[1] CON means piglets fed basal diet without LEO; LLEO means CON diet with 100 mg/kg LEO; MLEO means CON diet with 200 mg/kg LEO; HLEO means CON diet with 400 mg/kg LEO.

Table 3
Effects of dietary *L. cubeba* essential oil (LEO) supplementation on immune function and inflammatory cytokines in weaned piglets.

Item	Treatments[1]				SEM	*P*-value
	CON	LLEO	MLEO	HLEO		
Immune function						
Immunoglobulin A, μg/mL	1.15[b]	1.34[ab]	1.27[ab]	1.54[a]	0.076	0.022
Immunoglobulin G, μg/mL	3.43[b]	3.89[ab]	4.13[ab]	4.42[a]	0.219	0.043
Immunoglobulin M, μg/mL	1.95	2.05	2.22	2.57	0.249	0.479
Inflammatory cytokine						
Tumor necrosis factor-α, ng/mL	346[a]	287[ab]	185[b]	186[b]	35.6	0.01
Interleukin-1β, pg/mL	128	120	115	124	7.59	0.657
Interleukin-6, pg/mL	28.0	23.0	26.1	24.5	2.22	0.43
Interleukin-10, pg/mL	79.5[b]	88.5[ab]	90.7[ab]	110[a]	7.11	0.038

[a, b] Different superscript letters in each indicator represent a significant difference (P-value < 0.05).
[1] CON means piglets fed basal diet without LEO; LLEO means CON diet with 100 mg/kg LEO; MLEO means CON diet with 200 mg/kg LEO; HLEO means CON diet with 400 mg/kg LEO.

ratio when compared to the CON diet, and LEO-containing diets had a lower ($P < 0.05$) diarrhea rate.

3.2. Antioxidant capacity

Table 2 shows the antioxidant indicators in serum and liver between the CON and LEO treatment groups. HLEO improved ($P < 0.05$) T-AOC bioactivity in both serum and liver. Dietary MLEO and HLEO supplementation had higher ($P < 0.05$) GPx activities in serum, and the LEO groups had greater ($P < 0.05$) GPx activities in the liver in comparison with the CON group.

3.3. Immune function and inflammatory cytokines

The effects of LEO on immune function and inflammatory cytokines are illustrated in Table 3. HLEO treatment increased ($P < 0.05$) the contents of IgA and IgG. With respect to inflammation-related cytokines, weaned piglets in the HLEO group displayed lower TNF-α ($P < 0.05$) and higher IL-10 ($P < 0.05$) levels than the CON treatment ($P < 0.05$). In addition, piglets supplemented with MLEO also decreased ($P < 0.05$) TNF-α levels when confronted with the CON diet.

3.4. Intestinal morphology

Table 4 shows the H&E staining results of the duodenum, jejunum and ileum. Compared with the CON group, the villus height in the HLEO group was increased ($P < 0.05$) both in the duodenum and jejunum. In the duodenum, the V:C ratio in the HLEO group was improved ($P < 0.05$) versus the CON group. Especially in the jejunum, the V:C ratio in piglets fed with MLEO and HLEO supplementation was also improved ($P < 0.05$) in comparison with the CON diet.

3.5. Microbial composition in the cecum

At the assigned 97% similarity level, 1,103 operational taxonomic units were clustered from 1,657,789 high-quality sequences. As shown in Supplementary Figure S1, the mean-based rarefaction curves demonstrated adequate sequences in the CON and LEO groups for subsequent experiments. Figure 1 shows the richness and diversity between the CON and LEO treatment groups. HLEO supplementation improved ($P < 0.05$) the ACE, Sobs and Chao indices compared to the CON diet. Similarly, MLEO supplementation increased ($P < 0.05$) the Shannon, ACE and Chao indices. Therefore, it is probable to ascertain that LEO-containing diets increased the cecal microbial diversity.

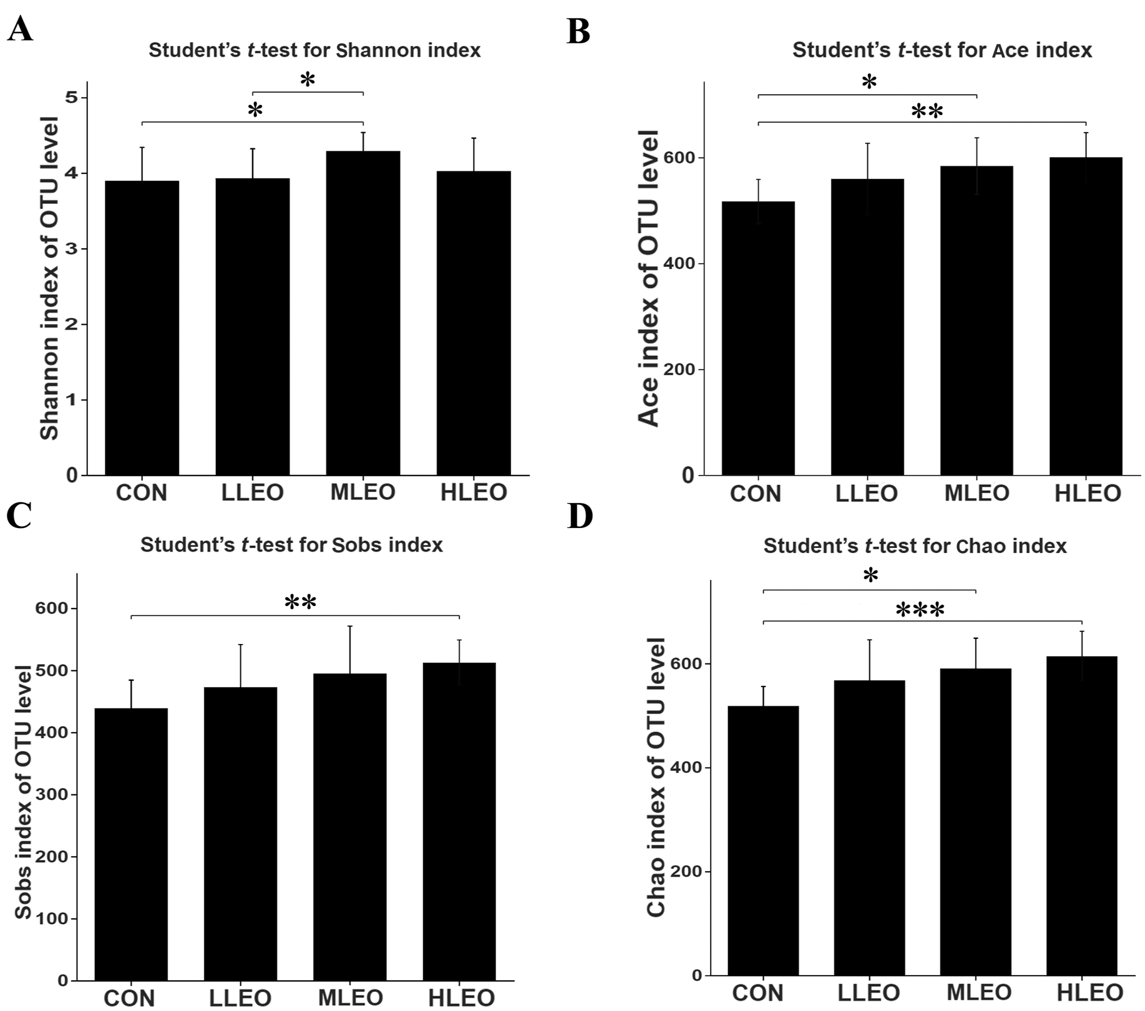

Fig. 1. The richness and diversity index of species at 97% similarity level: Shannon (A), Ace index (B), Sobs index (C) and Chao index (D). CON = pigs fed basal diet; LLEO = pigs fed basal diet + 100 mg/kg *L. cubeba* essential oil (LEO); MLEO = pigs fed basal diet + 200 mg/kg LEO; HLEO = pigs fed basal diet + 400 mg/kg LEO. Asterisks indicate statistical differences between different groups: * 0.01 ≤ $P < 0.05$; ** 0.001 ≤ $P < 0.01$; *** $P < 0.001$.

As shown in Fig. 2, the Circos plots visually express microbial communities' relative abundance and distribution in cecal digesta. Firmicutes (69.64% to 80.63%), Proteobacteria (6.43% to 13.31%), Bacteroidota (3.87% to 10.23%), Spirochaetota (0.39% to 4.31%) and Actinobacteriota (1.05% to 3.11%) were the top 5 dominant phyla in all groups. Although these aforementioned phyla were the most significantly different, the abundances of other phyla were also diverse among the CON and LEO supplementary diets. Based on the Bray–Curtis distance (Fig. 3), PCoA demonstrated that the microbial community was isolated ($P < 0.05$) between the CON and other groups with principal components of 18.73% PC1 and 13.35% PC2 variation, respectively. The results showed that piglets supplemented with CON and HLEO had significant changes in microflora structure.

LEfSe analysis (Fig. 4) displayed the most representative microbes from the phylum to genus level in the CON and LEO groups. Notably, the HLEO group expanded ($P < 0.05$) the abundance of 2 orders, 5 families and 9 genus levels. However, the CON treatment significantly heightened ($P < 0.05$) the relative abundance of 1 order, 2 families and 9 genus levels. The results were determined by the linear discriminant analysis (LDA) score, which is shown in Fig. 5. Key phylotypes of microbiota communities in cecal digesta of weaned pigs fed basal diet as CON (noted in red), basal diet with LLEO (noted in blue), basal diet with MLEO (noted in green), and basal diet with HLEO (noted in pink) were shown using the Kruskal–Wallis rank sum test.

3.6. SCFA

Table 5 shows the concentration of a total of 6 SCFA levels in piglet intestines. In the cecum, dietary HLEO supplementation increased ($P < 0.05$) acetic acid and a total of 6 SCFA when confronted with the CON group. In the colon, the HLEO diet also advanced ($P < 0.05$) propionic acid and a total of 6 SCFA compared with the CON group.

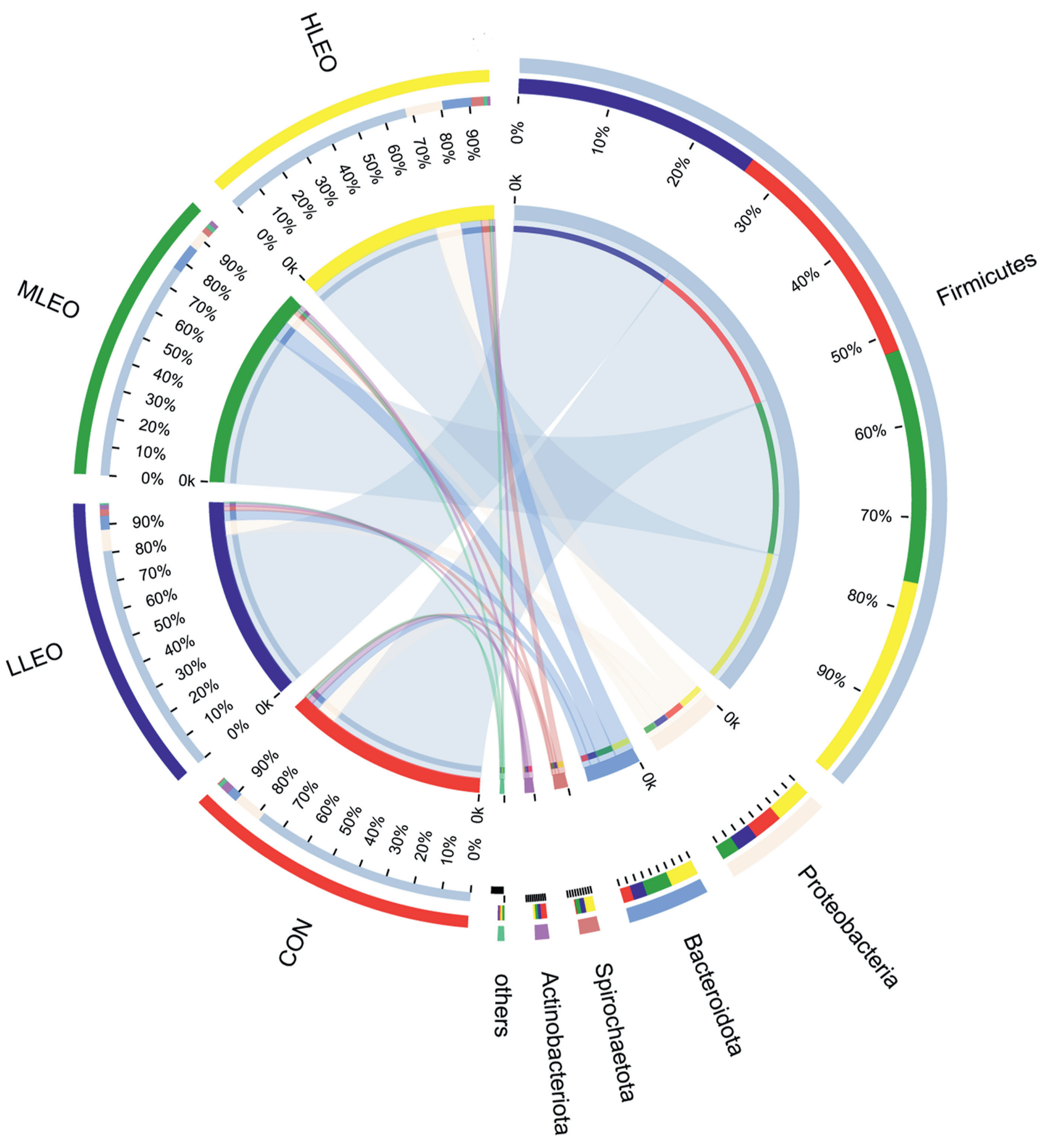

Fig. 2. The Circos graph visualizes microbial community distribution on phylum level from the cecal digesta between CON and LEO groups. The width of the bars from treatment groups indicates the relative abundance at the phylum level. CON means piglets fed basal diet without *L. cubeba* essential oil (LEO); LLEO means CON diet with 100 mg/kg LEO; MLEO means CON diet with 200 mg/kg LEO; HLEO means CON diet with 400 mg/kg LEO.

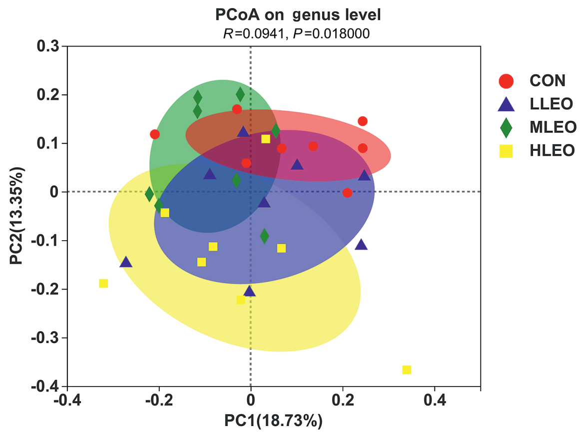

Fig. 3. Principal co-ordinates analysis (PCoA) of microbial structure from the cecal digesta between CON and LEO groups (based on the Bray–Curtis distance). CON means piglets fed basal diet without *Litsea cubeba* essential oil (LEO); LLEO means CON diet with 100 mg/kg LEO; MLEO means CON diet with 200 mg/kg LEO; HLEO means CON diet with 400 mg/kg LEO.

4. Discussion

After weaning, piglets commonly suffer from weaning stress due to sudden changes in physiology and psychology, which may result in diarrhea and growth retardation (Pluske et al., 1997). Various studies have reported that EO supplementation may mitigate the detrimental effects of weaning stress by improving antioxidant and anti-inflammatory performance and maintaining gut health in weaned piglets (Gois et al., 2016). The European Food Safety Authority approved the safety and efficacy of LEO so that it could be added to most animal feed (Bampidis et al., 2021). In the present paper, LEO supplementation increased the final body weight on d 28 and ADG, and decreased the feed conversion ratio in weaned pigs. The precedent study found that dietary supplementation of *L. cubeba* leaf powder improved the growth performance and feed efficiency by stimulating nonspecific immunity in carp (Nguyen et al., 2016). The possible underlying mechanism of this finding might be that EO enhances flavor and odor which increases feed palatability (Franz et al., 2010). In addition, some researchers have revealed that EO has been used as an AGP alternative for livestock production (Zhai et al., 2018; Girard et al., 2020).

Furthermore, the diarrhea rate has been used as a representative index of gut health (Pierce et al., 2005). In this study, the piglets supplemented with LEO had a lower diarrhea rate than the CON group, irrespective of dose. Li et al. (2012) found that encapsulated EO supplementation reduced the frequency of diarrhea in piglets. This may be due to the possibility that *L. cubeba* has a long history of application in the traditional therapy of diarrhea, suggesting the protective function of *L. cubeba* for gut health (Xie and Liang, 1996). Another reason may be due to another positive effect of *L. cubeba*, which can enhance health by eliminating oxidative stress (Hwang et al., 2005), indicating that LEO supplementation could be used

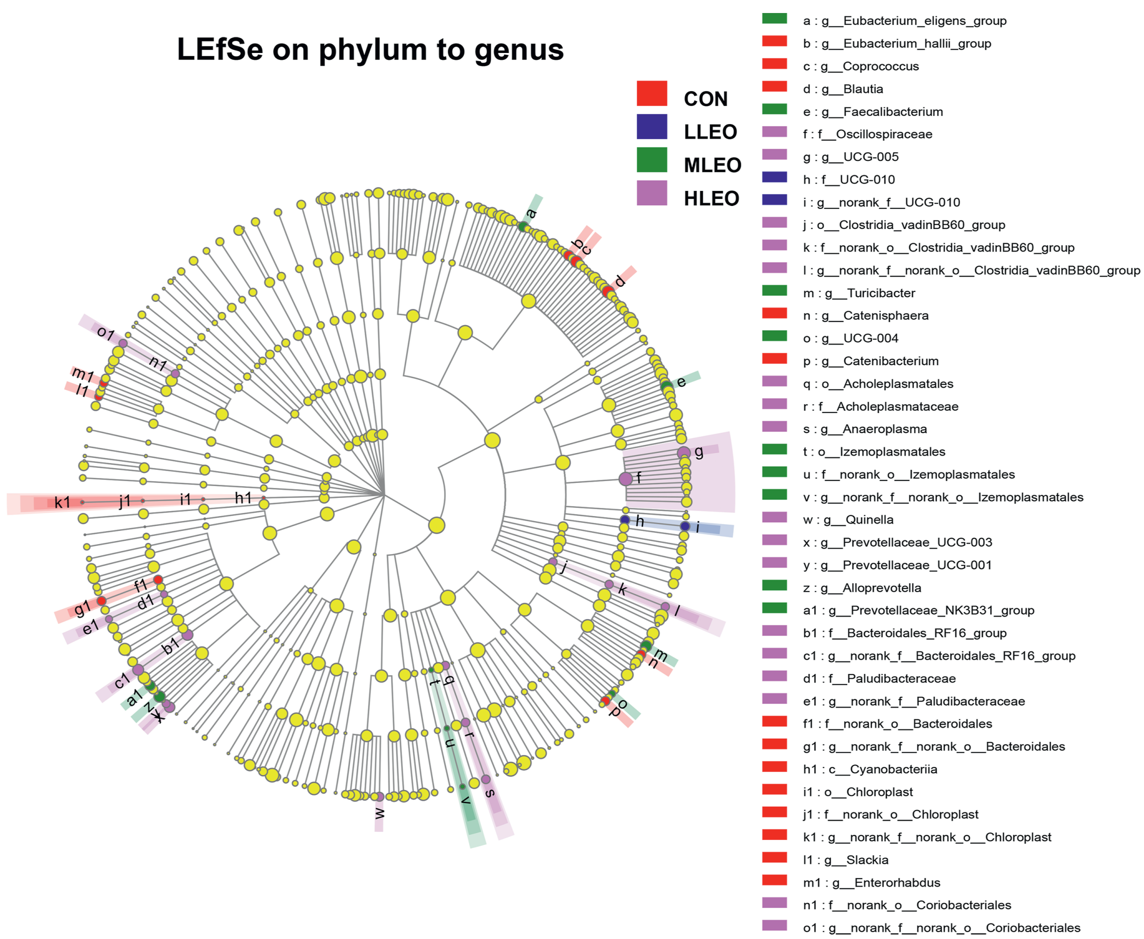

Fig. 4. Linear discriminant analysis effect size (LEfSe) cladogram illustrating discriminatively dominant taxa in piglet cecal digesta microbiota (based on all-against-all strategy). CON means piglets fed basal diet without *L. cubeba* essential oil (LEO); LLEO means CON diet with 100 mg/kg LEO; MLEO means CON diet with 200 mg/kg LEO; HLEO means CON diet with 400 mg/kg LEO.

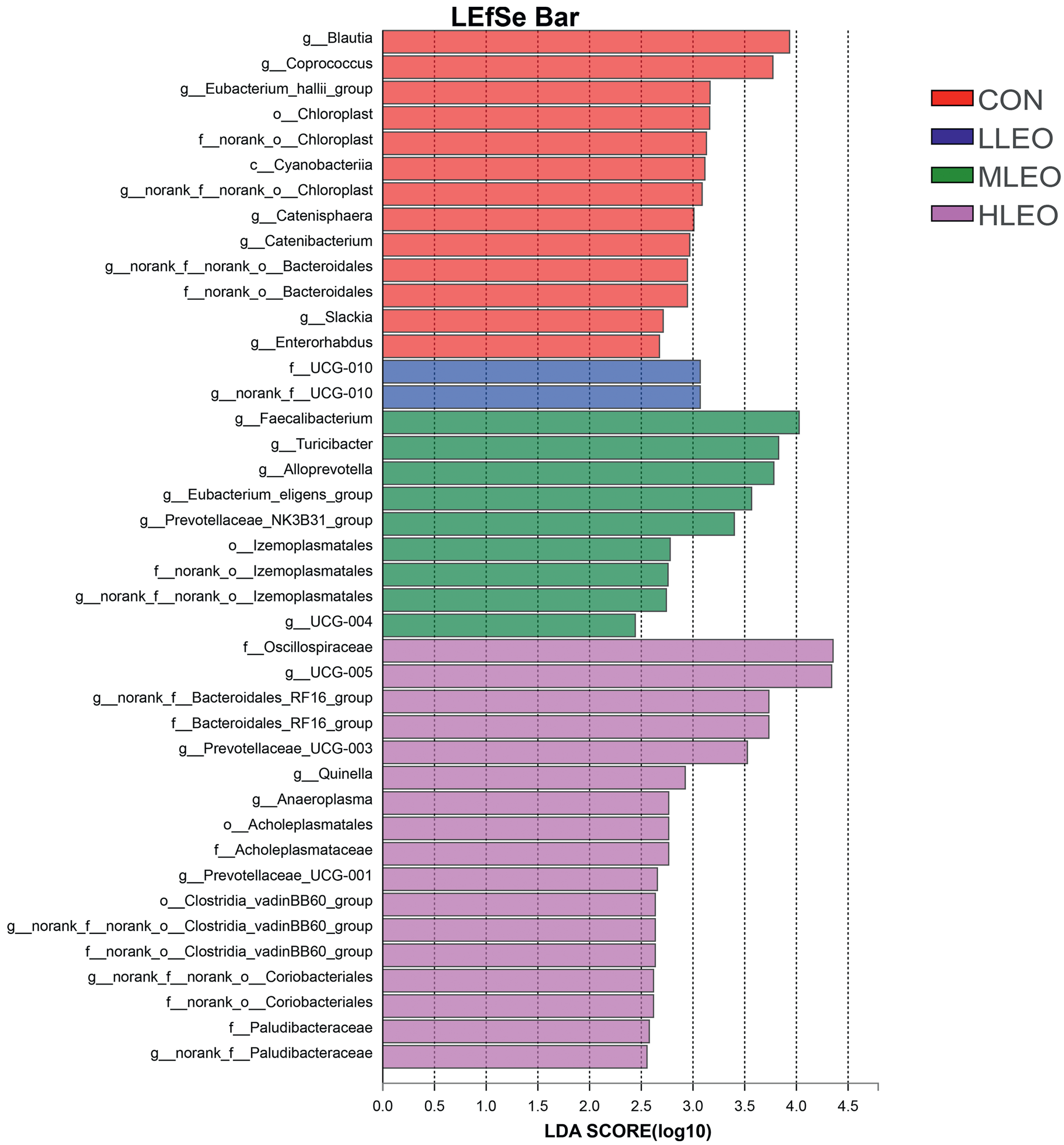

Fig. 5. Linear discriminant analysis (LDA) score distribution histogram for Linear discriminant analysis effect size (LEfSe) on phylum to genus level. Only taxa with LDA scores >2 are presented. CON = pigs fed basal diet; LLEO = pigs fed basal diet + 100 mg/kg *L. cubeba* essential oil (LEO); MLEO = pigs fed basal diet + 200 mg/kg LEO; HLEO = pigs fed basal diet + 400 mg/kg LEO.

in weaned piglets to reduce the antibiotic burden. Thus, the promoted efficacy in holistic health in piglets may contribute to improved growth performance.

For swine production, inflammation around weaning may result in unsatisfactory growth performance (Ajuwon, 2014). IgA, IgG, and IgM are the key immunologic factors representing the immune levels of weaned piglets (Yuan et al., 2015). These Igs are constituents of humoral immunity in most mammals, as they enhance phagocytosis by mononuclear macrophages and inhibit the reproduction of pathogenic viruses and microorganisms (Planchais et al., 2020). Our study found that piglets fed LEO had heightened IgA and IgG levels in serum. *L. cubeba* has been used in herbal anti-inflammatories to alleviate gastroenteropathy, edema, and rheumatoid arthritis by facilitating immune responses (Huang, 1997). Furthermore, local immunity is usually activated between d 0 and 2 post-weaning, while TNF-α, IL-1β, and IL-6, are considered three major pro-inflammatory cytokines involved in weaning stress (Pie et al., 2004). In our study, the LEO-containing diets down-regulated the concentration of TNF-α in serum. Our results showed a lower concentration of IL-10 in the CON group, which is in agreement with Lin et al. (2013), who found that both the water and ethanol extract of *L. cubeba* attenuated adjuvant arthritis in a

Table 5
Effects of dietary *L. cubeba* essential oil (LEO) supplementation on short chain fatty acids (SCFA) in weaned piglets.

Item	Treatments[1]				SEM	*P*-value
	CON	LLEO	MLEO	HLEO		
Cecum						
Acetic acid, mg/g	4.68[b]	5.01[ab]	5.11[ab]	5.39[a]	0.161	0.042
Propionic acid, mg/g	2.19	2.24	2.32	2.36	0.165	0.739
Isobutyric acid, μg/g	171.1	159.8	190.3	217.4	25.26	0.426
Butyric acid, mg/g	0.977	0.98	1.07	1.11	0.149	0.912
Isopentoic acid, μg/g	244.6	251.5	312.4	359.0	41.08	0.292
Valeric acid, μg/g	275.7	294.2	328.9	312.9	34.82	0.742
Total SCFA, mg/g	8.54[b]	8.94[ab]	9.33[ab]	9.75[a]	0.374	0.04
Colon						
Acetic acid, mg/g	5.67	5.99	6.03	6.25	0.268	0.538
Propionic acid, mg/g	2.56[b]	2.81[ab]	2.73[ab]	3.35[a]	0.188	0.041
Isobutyric acid, μg/g	299.6	362.2	310.0	371.9	31.78	0.295
Butyric acid, mg/g	1.81	1.85	1.82	2.04	0.245	0.897
Isopentoic acid, μg/g	544.9	707.7	585.4	715.7	67.13	0.205
Valeric acid, μg/g	520.5	691.0	559.7	756.4	72.02	0.101
Total SCFA, mg/g	11.4[b]	12.4[ab]	12.0[ab]	13.5[a]	0.510	0.058

[a, b] Different superscript letters in each indicator represent a significant difference (*P*-value < 0.05).
[1] CON means piglets fed basal diet without LEO; LLEO means CON diet with 100 mg/kg LEO; MLEO means CON diet with 200 mg/kg LEO; HLEO means CON diet with 400 mg/kg LEO.

rodent model. However, excessive immune function may lead to energy and nutrients intended for growth to be used for the immune response (Niu et al., 2022). It is possible that EO supplementation primarily alleviates intestinal immune defense stress, which may contribute to nutrient allocation towards growth rather than immune defense (Zeng et al., 2015). In addition, EO from the Lauraceae family had anti-inflammatory activities that interacted with signaling cascades involving cytokines and pro-inflammatory genes (Damasceno et al., 2019). Thus, the better immune capacity in piglets fed LEO diets may lead to a moderate level of immunoglobulins and anti-inflammatory cytokines, which inhibits adverse reactions after weaning.

Oxidative stress is a common problem in swine production and seriously impacts animal welfare and profitability (Yin et al., 2014). Changes in oxidative stress markers, including T-SOD, catalase, GPx and T-AOC, were observed. MDA level reflects an imbalanced status of the antioxidant defense system in piglets (Zhu et al., 2012). The reactive oxygen species (ROS) scavenging enzymes, such as T-SOD and catalase, downgrade superoxide radicals and hydrogen peroxide in piglets (Xu et al., 2014). The reduction of lipid peroxides is catalyzed by GPx, which catalyzes glutathione disulfide into two glutathione molecules by reducing equivalent NADPH (Brigelius-Flohe et al., 2013). Moreover, the content of MDA is generally considered a representative index of lipid peroxidation (Jiang et al., 2016). T-AOC (defined as the ferric ion-reducing antioxidant power method) represents the redox state in the body (Benzie and Strain, 1996). In the current study, dietary LEO supplementation activated the levels of GPx and T-AOC in the serum and liver. LEO is a well-known novel food preservative due to its potential for antioxidant activity (Wang et al., 2012). We speculated that citral, a significant active volatile oil in LEO, may inhibit oxidative stress and apoptosis in mice with focal segmental glomerulosclerosis (Yang et al., 2013). These findings suggested that dietary LEO supplementation improved antioxidant capacity by counteracting oxidative stress in weaned piglets.

Integrated intestinal morphology is essential to nutrient absorption capacity, gut health and growth during the growing phase (Chen et al., 2019). Intestinal villi are positively correlated with nutrient absorption rates, as the more extensive the villus height, the larger the absorption area (Chen et al., 2018). However, the crypt depth indicates the degree of gut injury. Gut epithelial tissue is key to a healthy mucosal structure that responds to inflammation through cytokines and tolerates feeding antigens (Pitman and Blumberg, 2000). After weaning, the shortened villus height and extended crypt depth deplete nutrient absorption capacity due to the changes in feed composition and nutrient levels (van der Peet-Schwering et al., 2007). A previous investigation showed that EO improved the production performance of laying hens by decreasing crypt depth in the ileum (Cheng et al., 2022). Recently, LEO tended to extend villus height and V:C ratio, which may be attributed to citral in LEO playing an imperative role in porcine intestinal epithelial cells (Li et al., 2022). Thus, weaning pigs had better growth performance and feed efficiency due to the protective function of LEO on intestinal barrier function.

The gut microbiota maintains the health of piglets through nutrient metabolism, mucosal barrier function, and pathogen invasion resistance (Azad et al., 2020). In our research, we sequenced and analyzed the cecal digesta, reflecting the species richness of the gut microbiota. In this paper, the Shannon, ACE, Sobs and Chao indices are qualitative alpha diversity measures (Rogers et al., 2016). In a recent article, similar observations by Cremonesi et al. (2022) demonstrated that a mixture of EO added to piglet diets increased alpha diversity indices. We found that Firmicutes/Bacteroidetes (F:B) ratio was decreased by LEO supplementation in a dose-related manner. It is speculated that a lower F:B ratio is beneficial for digesting polysaccharide-rich feed and resisting inflammatory diseases in the intestine (Ni et al., 2021). A related study indicated that piglets suffering from diarrhea had a diminished abundance of *Bacteroidota* and *Spirochaetota* in the fecal microbial community (Qi et al., 2021). Some EOs selectively activate the growth capacity of specific bacteria essential to gut health (Ruzauskas et al., 2020). The bacteria enriched in HLEO were *Oscillospiraceae_UCG-005*, which had a strong relationship with acetic acid and a total of 6 SCFA, according to the SCFA results (Li et al., 2021). Several studies demonstrated that *Faecalibacterium* is considered a probiotic as it protects the digestive system from intestinal pathogens in the MLEO group (Michalak et al., 2020). By contrast, excessive *Blautia* might result in subnormal absorption of grain components in pig feed (Moen et al., 2016). In addition, increased *Coprococcus* levels might be affiliated with an increased diarrhea rate by regulating the expression of swine leukocyte antigen gene (Huang et al., 2016). It has been shown that supplementing weaned piglet diets with LEO suppressed the majority of genera related to anti-nutritional factors or potential pathogens, which were replaced by beneficial bacteria to maintain the intestinal microflora balance.

The SCFA are the primary fermentation products of microbial metabolites in the cecum and colon, promoting gut health by inhibiting inflammation and maintaining gut integrity (Liu et al., 2020). The intestinal epithelium can rapidly absorb acetic acid into peripheral blood and provide energy for the host; propionic acid is absorbed by the epithelium and transferred to the liver, which is used for glycogen synthesis (den Besten et al., 2013). In addition, total SCFA could decrease pH in the intestine, increase beneficial bacteria reproduction, and constrain potential pathogens (Fukuda et al., 2011). Moreover, Yang et al. (2019) reported that the mixture of EO and organic acids improved fecal SCFA, and modulated the microflora community in weaned piglets. The current study demonstrated that acetic acid and propionic acid production increased total SCFA with dietary supplementation of LEO. These results suggested that piglets fed the LEO diet positively modulated SCFA production in the cecum and colon, which may partially explain the mechanism of gut microbiota in improving energy efficiency and the bacteriostatic effect, and reducing inflammation.

5. Conclusions

The present research demonstrated that dietary LEO could improve growth performance and intestinal health in weaned piglets. The beneficial effects may be explained by improving antioxidant capacity, immune response, intestinal morphology and microbial community in the cecum. The results attained in this research could apply to developing AGP alternatives for weaned piglets. Moreover, LEO supplemented at a recommended dose of 400 mg/kg diet were found to be beneficial.

Author contributions

Zhe Yang: Conceptualization, Formal analysis, Writing - Original Draft; **Fang Wang**: Methodology, Validation, Writing - Review & Editing; **Yexin Yin**: Data Curation, Software; **Zhimou Liu**: Resources; **Peng Huang**: Methodology; **Qian jiang**: Writing - Review & Editing. **Yulong Yin**: Funding acquisition, Project administration; **Jiashun Chen**: Investigation, Supervision, Writing - Review & Editing. All authors have read and approved the final manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2022.11.004.

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