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Research Article

Effects of microencapsulated *Enterococcus faecalis* on the growth performance, immune function, and cecal microflora of weaned piglets

L. Zhang[1,2], Y. Qi[1], X. Shang[1,2] and Q. Li[1*]

[1]Hebei Key Laboratory of Animal Diversity, Langfang Normal University, Langfang- 065 000, P. R. China;
[2]Langfang Key Laboratory of Food Nutrition and Safety, Langfang Normal University, Langfang- 065 000, P. R. China

Abstract

Probiotics are offered as dietary supplements in animal husbandry, and microencapsulation protects probiotic activity. Here, feed microencapsulated *Enterococcus faecalis* of weaned piglets on the growth performance, immune function, and cecal microbiota were investigated. A control diet (NC), a 0.05% Aureomycin (PC) diet or a microencapsulated *E. faecalis*–supplemented (1×10^9 cfu/kg of diet; ME) diet was fed to 90 weaned piglets for 28 days. Compared with the piglets fed the NC diet, those fed the PC and ME diets showed a significant increase (P<0.001) in final weight, ADG, and ADFI but a significant decrease in the F : G ratio (P<0.01) during the 28-day experimental period. Compared with the NC group, the PC and ME groups showed significant (P<0.05) decreases in serum TNF-α, IL-6, IL-1, IgG, IgM, and IgA concentrations. High-throughput sequencing revealed that the diet modulated cecal bacterial diversity, including an increase in *Lactobacillus* abundance. Thus, microencapsulated *E. faecalis* promotes the growth performance, immunity and optimal intestinal flora of weaned piglets.

Keywords: Health, Intestinal flora, Microencapsulation, Piglets, Probiotics

Highlights

- Internal gelation and emulsion were used as an encapsulation method to microencapsulate *Enterococcus faecalis* with a final cell density of ~1.0×10^{10} cfu/g.
- Microencapsulated *Enterococcus faecalis* can improve ADG, IgA, IgM, IgG and reduce FCR, TNF-α, IL-6 and IL-1 of weaned piglets.
- High-throughput sequencing revealed that Microencapsulated *Enterococcus faecalis* can enrich the diversity of bacterial flora of weaned piglets.

INTRODUCTION

Antimicrobial usage in animal husbandry is a concern owing to the presence of antimicrobial drug residues in animal-based food products and the increase in the abundance of antibiotic-resistant bacteria (Diraviyam *et al*., 2014). For example, the detection of antibiotic growth promoters (AGPs) in the environment and in animal products has led to a ban on in-feed AGPs in many countries (Hulst *et al*., 2013); hence, there is a need for viable alternatives that enhance the natural defense mechanisms of animals (Verstegen and Williams, 2002). In recent years, the popularity of probiotic health-based products has increased rapidly, and the addition of probiotics to diets has been shown to improve gut microflora populations, growth performance, immune function, and intestinal health (Georgeta *et al*., 2020; Zhaxi *et al*., 2020). Probiotics must remain viable during their use, *i.e*., they must be capable of being re-cultured on solid agar to provide their health benefits (Fuller, 1991). Due to the sensitivity of probiotics to environmental factors including stomach acid, bile salts, and high temperature, their effects in feed can be diminished substantially. Several methods that increase the resistance of these sensitive microorganisms to adverse environmental factors have been reported and include the appropriate selection of bile-and acid- resistant strains, stress adaptation (Liu *et al*., 2020) and microencapsulation (Pupa *et al*., 2021a).

The use of microencapsulation technology has been suggested as an effective approach to protecting probiotic activity that could also help control the release of probiotics throughout the gastrointestinal tract in a cost-effective manner (Gerez *et al*., 2012). The application of microcapsules to living cells can be divided into two categories: pro-encapsulation and pre-encapsulation. The focus of the present study is

*Corresponding Author, E-mail: liqiuling2000@126.com, zhanglin19880719@163.com

pre-encapsulation, in which a small volume of cells is mixed with the encapsulation materials, and the microencapsulated bacteria are growing in the new MRS medium (Zhang *et al*., 2015).

Enterococcus is a genus of lactic acid bacteria that normally inhabits the gastrointestinal tract of animals, and some enterococci strains are used as probiotics in animal husbandry (Cao *et al*., 2013). The dietary supplementation of these strains has been shown to significantly enhance the growth performance, immune system and gut microbiota balance of treated broilers and piglets. For example, *Enterococcus faecalis* isolated from the intestines of healthy animals reduced the incidence of diarrhea and improved growth performance in weaned piglets (Hu *et al*., 2015). In addition, microencapsulated *E. faecalis* improved the performance parameters, immune response, and intestinal microbiota composition of broiler chickens (Zhang *et al*., 2015).

Although encapsulation has been shown to protect bacteria and improve their survival in the host environment, the effects of a diet with reduced antibiotic levels and microencapsulated *E. faecalis* supplementation on the growth and fecal bacteria community structure of animals remain understudied. In the present study *E. faecalis* was encapsulated using emulsion and internal gelation and determined the growth performance, immune responses and cecal microflora of weaned piglets.

MATERIALS AND METHODS

Bacterial strains: The *E. faecalis* strain (CGMCC No. 9353) used in this research was provided by the China General Microbiological Culture Collection Center and cultured under microencapsulated and anaerobic conditions in Man–Rogosa–Sharpe (MRS) (Sinopharm Chemical Reagent Corporation, Beijing, China) broth at 37°C for 18h. Collected through centrifugal free (unencapsulated) cells and washed with sterilized water, after which they were resuspended in fresh MRS broth in the targeted numbers.

Preparation of microcapsules: The probiotics were microencapsulated using the internal gelation and emulsion method. An amount of 100 mL of sterile 1.8% (w/v) sodium alginate (Sinopharm Chemical Reagent Corporation, Beijing, China) and 5 mL of concentrated and washed probiotic bacteria (10^8 cfu/mL) were mixed with 0.45 g of calcium carbonate (Sinopharm Chemical Reagent Corporation, Beijing, China) dissolved in 300 µL of sterile water. After homogenization, the mixture was dispersed into a paraffin oil phase containing 0.2% (wt/vol) Span80 (Sinopharm Chemical Reagent Corporation, Beijing, China) and then emulsified for 5 min via stirring at 400 rpm. Subsequently, 900 µL of glacial acetic acid mixed with 10 mL of paraffin oil was added to the mixture, which was stirred for 10 min. Sterilized water was then added to the emulsification system to draw the microbeads into the water phase, and the top oil layer was collected via aspiration and centrifuged for subsequent use. After washing three times with sterile water, fresh MRS medium was added to the fermenter to enable the continuous growth of microencapsulated bacteria. Finally, the proliferated microencapsulated probiotics (final cell density: ~1.0×10^{10} cfu/g) were harvested and dried using a fluidized bed. The microcapsules were broken via chemical treatment as described by Qi *et al*. (2006), after which they were transferred to MRS agar plates to determine the number of *E. faecalis* cells. The mean particle size of the microencapsulated probiotic product, determined using a Mastersizer-2000 Laser Particle Analyzer (Malvern Instruments, Ltd., Malvern, UK), was 625µm.

Piglets, diets and experimental design: In total, 90 weaned piglets (Duroc × Landrace Large White Yorkshire) with an initial body weight of 8.55±0.10 kg were randomly assigned to three treatments, each with five replicate pens with six weaned piglets per pen. The piglets were provided water and a corn and soybean meal–based diet (Table 1) *ad libitum* through a nipple drinker and feeder, respectively. The three treatments were as follows: a control group fed a basal

Table 1. Composition and nutrient levels of the basal diet (air-dry basis; %)

	Treatment[1]		
Item	**NC**	**PC**	**ME**
Ingredients (%)			
Corn	52.00	52.00	52.00
Wheat middling	8.90	8.90	8.90
Soybean meal	10.00	10.00	10.00
Extruded soybean	8.00	8.00	8.00
Imported fish meal	8.00	8.00	8.00
Whey	7.00	7.00	7.00
Choline chloride	0.10	0.10	0.10
Phospholipid	2.00	2.00	2.00
Premix[2]	4.00	4.00	4.00
Total	100.00	100.00	100.00
Nutrient level[3] (%)			
DE	13.52	13.52	13.52

Cont. Table 1.

Table 1., Cont. ...

Item	Treatment[1]		
	NC	PC	ME
CP	20.96	20.95	20.94
Lys	0.98	0.98	0.97
Met+Cys	0.58	0.57	0.58
Thr	0.59	0.59	0.58
Ile	0.67	0.66	0.67
Ca	0.82	0.81	0.81
TP	0.60	0.59	0.59
AP	0.42	0.41	0.42

[1]NC: basal diet, PC: basal diet containing 0.05% Aureomycin, ME: basal diet with microencapsulated *Enterococcus faecalis* at a dose of 1×10^9 cfu/kg of feed; [2]Supplied with the following per kg of diet: vitamin A- 10,500 IU, vitamin D3- 450 IU, vitamin E- 10 mg, pantothenic acid- 20 mg, vitamin B_6- 2 mg, biotin- 0.3 mg, folic acid- 5 mg, vitamin B_{12}- 0.009 mg, ascorbic acid- 50 mg, Cu- 140 mg, Fe- 160 mg, Mn- 50 mg, Zn- 130 mg, Se- 0.3 mg, I- 0.5 mg; [3]Values for net energy were calculated, and the contents of ether extract, crude protein, acid-detergent fiber, neutral-detergent fiber, ash, Ca, and P were determined using routine methods.

diet (NC), an antibiotic-treated group fed a basal diet supplemented with 500 mg of Aureomycin per kg (PC), and a microencapsulated probiotics-treated group fed a basal diet supplemented with 100 mg of encapsulated probiotics per kg (ME). The piglets were raised in cages individually, and the experiment was performed over 28 days (Liu *et al*., 2017).

Growth performance: The initial body weight and final body weight of the piglets were measured on days 1 and 50 to determine their average daily weight gain (ADG). The amounts of feed residue offered were recorded every day to confirm the individual daily feed intake (ADFI), and the feed: gain (F : G) ratio was calculated as weight gain/feed intake based on the feed intake and body weight data (Zhang *et al*., 2022).

Blood characteristics: A number of weaned piglets were selected for blood collection on day 28. In total, 30 weaned piglets from each replicate pen (10 piglets per treatment) were randomly selected for blood sampling. Blood was collected from the front cavity vein using an aseptic needle and a pro-coagulation tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). The blood samples were stored on ice during collection, and serum was obtained via centrifugation at $1,610 \times g$ and 4°C for 15 min. Finally, the samples were stored at -20°C until further analyses were performed.

Serum IgG, IgM, IgA, TNF-α, IL-1, and IL-6 levels were determined using a double-antibody sandwich ELISA (Hou *et al*., 2021). Reagent kits for IgG, IgM, IgA, IL-1, IL-6, and TNF-α were measured using commercial kits supplied by the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The protocols are as follows. The standard product with the known concentration of the substance is to be measured, and the sample with unknown concentration is added to the microporous enzyme label plate for detection. First, the substance to be tested and the biotin-labeled antibody were incubated simultaneously. After washing, avidin-labeled HRP was added. After incubation and washing, the unbound enzyme binding is removed, and then substrates A and B are added to act with the enzyme binding at the same time. The depth of the color is proportional to the concentration of the substance to be measured in the sample.

Cecal microbiome: Eighteen cecal content samples (six samples per treatment) were randomly selected on day 28 and collected in sterile containers, packed carefully, frozen via immersion in liquid N_2 and stored at -80°C. Subsequently, the cecal microbiota was analyzed using high-throughput sequencing, including DNA extraction, PCR-amplified 16S rRNA analysis, amplicon sequencing, and sequence data processing.

DNA extraction: Microbial genomic DNA was extracted from the cecal content samples using a MoBio Power Soil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA) according to the manufacturer's instructions (Zhang *et al.*, 2015).

PCR-amplified 16S rRNA analysis: The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified using the universal primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) (Peris *et al*., 2015). The PCR amplification programs were set as follows: 95°C for 5 min; 28 cycles of 95°C for 45 s, 55°C for 50 s, and 72°C for 45 s; and a final extension at 72°C for 10 min. The PCR products were purified using an Agencourt AMPure XP Kit (Qiagen, Venlo, Limburg, Netherlands). Deep sequencing was performed on a Miseq platform at Allwegene Company (Beijing). After the run, base calling, error estimation, and image analysis were performed using an Illumina Analysis Pipeline Version 2.6. The sequences were removed, and draw data were screened if they (i) were shorter than 230 bp, (ii) had a quality score of ≤20, (iii) contained ambiguous bases, and/or (iv) did not exactly match the

primer sequences and barcode tags. Qualified reads were separated using the sample-specific barcode sequences and trimmed using the Illumina MiSeq platform.

Statistical analysis: Data were analyzed using ANOVA via SPSS for Windows (version 17.0; IBM, Chicago, IL). Significant differences among treatment groups were tested using Tukey multiple-range tests. P<0.05 was considered statistically significant.

RESULTS

Growth performance: Growth performance data are shown in Table 2. No differences (P≥0.95) were observed in the initial weight of the piglets among the treatment groups. Compared with the piglets fed the NC diet, those fed the PC and ME diets showed significantly higher (P<0.001) in final weight, ADFI and ADG. The F : G ratio was significantly *(*P<0.01) decreased during the 28-day experimental period.

Blood characteristics: As expected, IgG, IgM and IgA concentrations in the ME and PC groups were significantly (P<0.05) higher than those in the NC group. The IgA, IgG and IgM concentrations did not differ between the ME and PC groups (Table 3). TNF-α, IL-1 and IL-6 concentrations were significantly (P<0.05) lower in the PC and ME groups than in the NC group. Nevertheless, these concentrations did not differ significantly (P>0.05) between piglets among the ME and PC groups (Table 4).

Table 2. Effects of Aureomycin and microencapsulated *Enterococcus faecalis* on the growth performance of piglets[1]

	Treatment[1]				
Items	NC	PC	ME	SEM[2]	P-Value
Initial weight, kg	8.80	8.89	8.90	0.05	0.95
Final weight, kg	18.81[b]	20.12[a]	20.05[a]	0.66	<0.01
ADG[3], kg	0.35[b]	0.39[a]	0.39[a]	0.02	<0.01
ADFI[4], kg	0.59[b]	0.63[a]	0.63[a]	0.02	<0.01
F : G[5], g/g	1.69[a]	1.62[b]	1.62[b]	0.05	<0.01

[a,b]Means with different superscript letters in the same row are significantly different (P<0.05); [1]NC: basal diet, PC: basal diet containing 0.05% Aureomycin, ME: basal diet with microencapsulated *Enterococcus faecalis* at a dose of 1×10^9 cfu/kg of feed; [2]Each mean represents five replicate pens with six piglets per pen; [3]ADG- average daily gain; [4]ADFI- average daily feed intake; [5]F : G- feed conversion ratio

Table 3. Effects of Aureomycin and microencapsulated *Enterococcus faecalis* on immune globulins (Igs) in piglets[1] (μg/mL)

	Treatment[1]				
Items	NC	PC	ME	SEM[2]	*P*-Value
IgG[3]	126.23[b]	149.53[a]	150.42[a]	5.41	<0.01
IgM[4]	15.84[b]	24.96[a]	25.45[a]	0.65	<0.01
IgA[5]	61.12[b]	73.54[a]	76.97[a]	1.32	<0.01

[a,b]Means with different superscript letters in the same row are significantly different (P<0.05); [1]NC: basal diet, PC: basal diet containing 0.05% Aureomycin, ME: basal diet with microencapsulated *Enterococcus faecalis* at a dose of 1×10^9 cfu/kg of feed; [2]Each mean represents five replicate pens with three piglets per pen; [3]IgG: immune globulin G; [4]IgM: immune globulin M; [5]IgA: immune globulin A

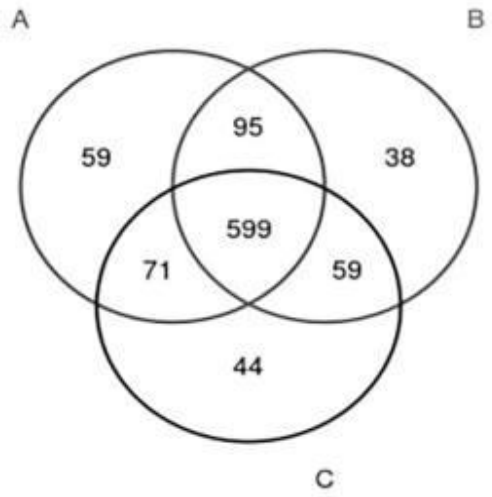
Table 4. Effects of Aureomycin and microencapsulated *Enterococcus faecalis* on interleukin (IL)-6, IL-1 and tumor necrosis factor (TNF)-α in the sera of piglets[1] (pg/mL)

	Treatment[1]				
Items	NC	PC	ME	SEM[2]	*P*-Value
TNF-α	285.23[a]	186.26[b]	190.82[b]	7.65	0.02
IL-6	60.45[a]	37.65[b]	39.54[b]	0.39	0.01
IL-1	98.52[a]	75.68[b]	74.24[b]	1.45	0.01

[a,b]Means with different superscript letters in the same row are significantly different (P<0.05); [1]NC: basal diet, PC: basal diet containing 0.05% Aureomycin, ME: basal diet with microencapsulated *Enterococcus faecalis* at a dose of 1×10^9 cfu/kg of feed; [2]Each mean represents five replicate pens with three piglets per pen.

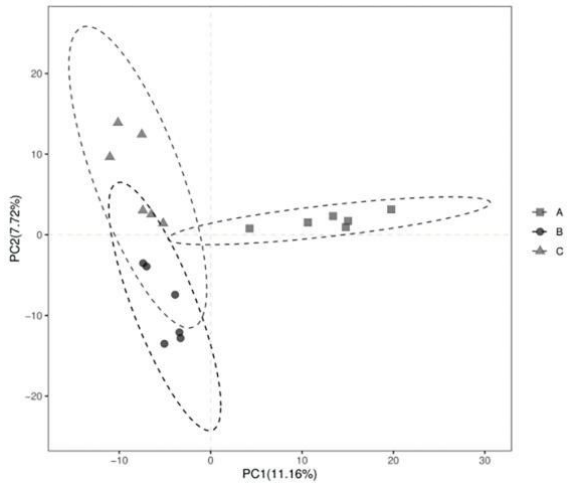
Cecal microbiome: The microbiota in the cecal content is shown in a Venn diagram (Fig. 1). A total of 965 observed taxonomic units (OTUs) were shared among the three treatment groups. The NC group piglets had 59 unique OUTs; the PC group piglets had 38 unique OUTs. Theme group piglets had 40 unique OUTs (Fig. 1). However, the ME and PC groups in an unweighted principal coordinates analysis plot had similar microflora (Fig. 2). The within-habitat or a-diversity of cecal microbiota, indicated by the diversity (Shannon index). The richness (Chao 1) among the three treatments is presented in Fig. 3. Chao 1, and Shannon index in the ME group was significantly (P>0.05) higher than those of the piglets in the NC and PC groups.

Using the Ribosome database project, each sample was analyzed for taxon abundance at the level of phylum, class, order, family, and genus. In NC group, *Bacteroidaceae*, *Veillonellaceae* and



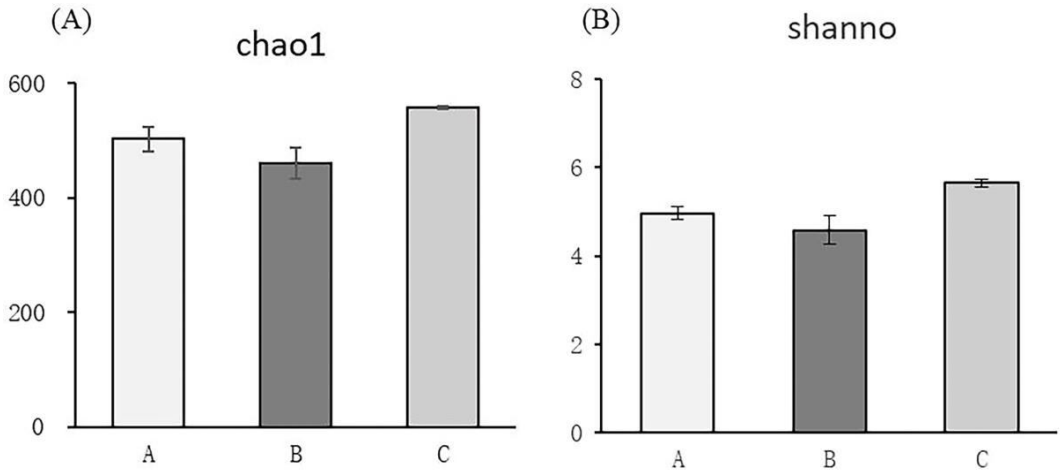
[A: basal diet (NC); B: basal diet containing 0.05% Aureomycin (PC); C: basal diet with microencapsulated *Enterococcus faecalis* at a dose of 1×10^9 cfu/kg of feed (ME)]

Fig. 1. Venn diagram showing the numbers of common and unique observed taxonomic units (OTUs) in the microflora community in the colonic contents of weaning piglets



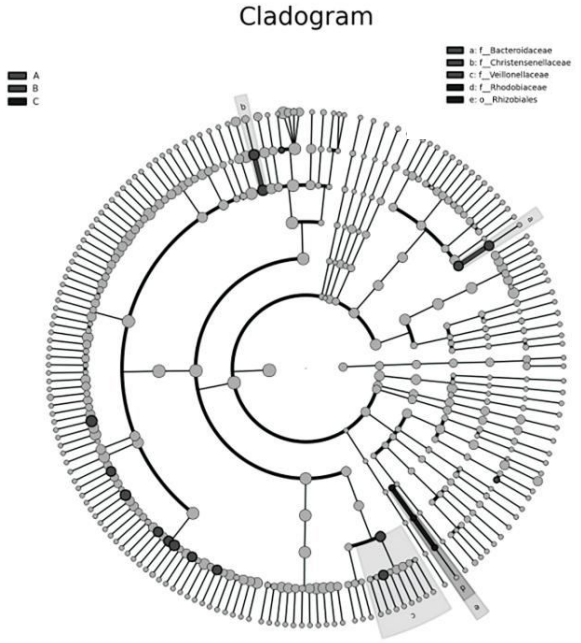
[A: basal diet (NC); B: basal diet containing 0.05% Aureomycin (PC); C: basal diet with microencapsulated *Enterococcus faecalis* at a dose of 1×10^9 cfu/kg of feed (ME)]

Fig. 2. Principal coordinate analysis of the bacterial composition similarity based on the unweighted UniFrac distances in the cecal digesta of three treatments



[A: basal diet (NC); B: basal diet containing 0.05% Aureomycin (PC); C: basal diet with microencapsulated *Enterococcus faecalis* at a dose of 1×10^9 cfu/kg of feed (ME)]

Fig. 3. Alpha diversity of ileal and cecal bacteria for the observed (A) Chao1 index and (B) Shannon index



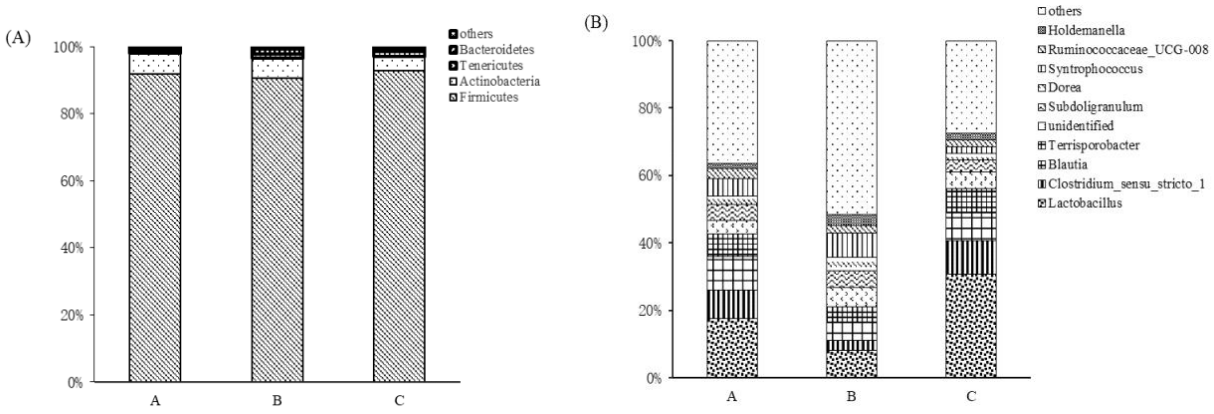
[A: basal diet (NC); B: basal diet containing 0.05% Aureomycin (PC); C: basal diet with microencapsulated *Enterococcus faecalis* at a dose of 1×10^9 cfu/kg of feed (ME)]

Fig. 4. Differential enrichment of cecal microbiota. Cladogram shows the microbial species that differed significantly between the groups in Linear effect size analysis.

Firmicutes was significantly (P<0.05) higher in PC piglets (Fig. 5). Compared with the PC group, the number of *Syntrophococcus* was significantly (P<0.05) lower and *Clostridium* and *Lactobacillus* was significantly (P<0.05) higher in the ME group (Fig. 5).

DISCUSSION

Probiotics are live microorganisms that reside in the intestine and provide health benefits to the host. As a preservation method, microencapsulation protects probiotics from potentially harmful chemical and physical reactions, also increasing mucoadhesive properties and releasing the probiotics in the intestine at a controlled rate (Yao *et al*., 2020; Ermis, 2021; Pupa *et al*., 2021b). In the current study, microencapsulated *E. faecalis* was added to the diet of weaned piglets at 1×10^9 cfu/kg. Many studies have found the positive effects of antibiotic on decreasing the diarrhea rate and improving animal growth performance of weaned piglets (Ji *et al*., 2023; Maykelly *et al*., 2023). In the present study, the Aureomycin supplementation in feed significantly decreased the F : G ratio, whereas it significantly increased the final body weight, ADG, and ADFI. Microencapsulated *E. faecalis* had similar effects to Aureomycin in terms of the growth performance of weaned piglets. Consistent with this result, dietary



[A: basal diet (NC); B: basal diet containing 0.05% Aureomycin (PC); C: basal diet with microencapsulated *Enterococcus faecalis* at a dose of 1×10^9 cfu/kg of feed (ME)]

Fig. 5. Microbiota composition determined via the 16S rRNA gene sequencing of fecal samples. The relative abundance of bacterial taxa at the phyla (A) and genera (B) levels of the fecal bacteria present in weaned piglets are shown for both the control and treatment groups.

Christensenellaceae were enriched. Whereas in ME group, *Rhizobiales* were showed the enrichment (Fig. 4). The most dominant bacterial phyla was Firmicutes, which comprised 90.69%–92.70% of the samples. Compared with the NC piglets, the number of

supplementation with microencapsulated *E. faecalis* can improve the production performance of monogastric animals in other studies. For example, dietary supplementation of microencapsulated *Lactobacillus plantarum MB001* can enhance average

daily gain and reduce feed conversion ratio of broilers throughout the 42-d trial period (Vimon *et al*., 2023). Dietary treatments with microencapsulated *Lactobacillus rhamnosus LB1* can improve the body weight gain from 25 to 31 days of age and modulate the intestinal microbiota of piglets, including an increase in *Lactobacillus* relative abundance of cecal microflora (Li *et al*., 2023).

Serum immune parameters can be used as a key parameter to reflect the immune status of weaned piglets. It is necessary to maintain relatively high levels for the health of weaning piglets (Yuan *et al*., 2015; Wang *et al*., 2018). In the present study, compared with the NC diet, dietary treatment with microencapsulated *E. faecalis* significantly increased serum IgA, IgG, and IgM levels. Several previous studies have shown that supplementation of probiotics enhances the humoral immune response by increasing the levels of immunoglobulin (Salim *et al*., 2013; Zhang and Kim, 2014). It has been reported that, compared with control piglets, supplemented with *E. faecalis* presented an increased serum IgM level on day 14 and IgA, IgG, and IgM levels on day 28 (Wang *et al*., 2019). Szabó *et al*. (2009) research showed that *E. faecium* supplementation to weaning piglets challenged with *Salmonella Typhimurium* had higher serum IgA and IgM levels. These results demonstrated that *E. faecium* plays a positive role in enhancing immune status and lymphocyte response, contributing to weaned piglet protection against pathogenic microorganisms. When weaned piglets are infected with pathogens or alteration of intestinal flora, it leads to the release of proinflammatory factors (Humphrey *et al*., 2019).

Probiotics can improve the differentiation and proliferation of intestinal immune cells, promote the expression of anti-inflammatory cytokines, and control the expression of proinflammatory cytokines. Chen *et al*. (2018), i.e. that *Clostridium butyricum* modulates the inflammatory process by reducing TNF-α levels in weaned piglets. In addition, Deng *et al*. (2017) found that the administration of *Bacillus subtilis* reduced plasma TNF-α levels in mice, increased anti-inflammatory cytokine interleukin-10 (IL-10) production and was able to decrease the secretion of the major proinflammatory cytokines IL-1and IL-6 by 70% and 80%, respectively (Sichetti *et al*., 2018).

In recent years, 16S rRNA-based next-generation sequencing has been used to successfully detect the diversity of piglet intestinal microbiota (Shang *et al*., 2018). In this study, we used the high-throughput sequencing of 16S rRNA amplicons to analyze the microbiota community of cecal contents in weaned piglets. The results showed that Firmicutes was the dominant phyla, followed by Actinobacteria and Tenericutes, accounting for 90% of all the sequences, and compared with the NC piglets, *Firmicutes* abundance was significantly increased in PC piglets. Dietary supplementation with compound probiotics on weaned piglets and analysis of fecal microbiota showed that the relative abundances of Bacteroides and Firmicutes were increased compared with the control group (Xu *et al*., 2020). This research showed that *Lactobacillus* abundance was increased in the ME group but decreased in the antibiotic group. Consistent with the findings of Mo *et al*., 2022, the results showed that *E. faecalis* supplementation induced a significant increase in *Lactobacillus* abundance and a significant decrease in the abundance of *Treponema* 2 and *Prevotellaceae* NK3B31 groups in the top ten most abundant genera of female growing-finishing pigs. Several studies have shown that *Lactobacillus plantarum* and *Lactobacillus reuteri* strains can improve performance, diarrhoea prevention, stress alleviation, immunity and microbiota modulation of piglets (Hou *et al*., 2015). In this research, the growth improvement of the ME group might have been due to enhanced immune capacity and improved intestinal health of piglets. Alpha diversity indexes calculations, such as Shannon and Chao1, we used indices to compare microbial diversity among the three groups. The results showed that the PCA of the ME group differed from that of the NC group, i.e., the Shannon and Chao1 indices of diversity changed significantly in the feces of weaned piglets after supplementation with *E. faecalis.* Similarly, Pajarillo *et al*. (2015) showed that supplementing feed with *E. faecalis* NCIMB 11181 altered bacterial diversity in the gut and feces of weaned piglets, with a significant increase in the Shannon and ACE indices observed in feces samples. Supplementation with *E. faecalis* can also alter the microbial community composition in the gut and feces of livestock (Hemarajata and Versalovic, 2013; Park *et al*., 2016).

These findings confirmed that microencapsulated *E. faecalis* as a dietary supplement could enhance the performance and immunity; that effect was similar to that of antibiotics. However, microencapsulated *E. faecalis* has more advantages in regulating weaning piglets' intestinal health than antibiotics. Therefore, based on our research, microencapsulated *E. faecalis* feed additive could be a feasible alternative to antibiotics in the weaned pigs diet for the reduction of antibiotics use.

Conflict of interests: We wish to confirm that there are no known conflicts of interest associated with this

publication, and there has been no significant financial support for this work that could have influenced its outcome.

Author contribution: LZ: Involved in the investigation, data generation, and preparing original draft; QL: Engaged in conceptualization, data curation, supervision and final editing; YQ: Involved in statistical analyses; XS: Methodology editing.

Data availability statement: Raw data of this study are available from the corresponding author.

Ethical statement: The procedures in this study were approved by the Institutional Animal Care and Use Committee of the College of Life Science, Langfang Normal University, Langfang, China.

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