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Effects of microencapsulated *Lactobacillus plantarum* and fructooligosaccharide on growth performance, blood immune parameters, and intestinal morphology in weaned piglets

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ABSTRACT

This experiment aimed to investigate the effects of a symbiotic, consisting of microencapsulated *Lactobacillus plantarum* (MLP) and fructooligosaccharide (FOS) on growth performance, blood immune parameters, and intestinal microbiota in weaned piglets. Ninety weaned piglets were assigned to three dietary groups (CON: basal diet; ANT: basal diet + aureomycin; SYN: basal diet + MLP and FOS) for a four-week trial. Compared to CON, pigs in the SYN group had higher weight gain and feed intake, and lower diarrhea rate ($P < .05$). Also, pigs in the SYN group had improved plasma IgA and IgG concentrations, and increased ($P < .05$) numbers of lactic acid bacteria in the colon compared to CON. In conclusion, feeding a synbiotic based on MLP and FOS had beneficial effects on growth performance, plasma immune parameters, and intestinal microbiota, indicating the potential of it to serve as an alternative to feed antibiotics in weaned pig diets.

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Introduction

Post-weaning diarrhea mainly relates to intestinal microbiota disorders, resulting in dehydration, fever, slow growth, and reduced feed consumption (Campbell, Crenshaw, & Polo, 2013). These disorders are frequently related to infections with enterotoxigenic *Escherichia coli* and *Salmonella typhimurium* (da Rosa et al., 2015; Zimmermann, Bauer, & Mosenthin, 2001). Feed antibiotics, therefore, have been widely used since early 1950s in the swine industry to promote growth and prevent infections (Cromwell, 2002). However, their side effects, including antimicrobial resistance and antimicrobial residues in swine products, have caused a major concern for the modern husbandry. Since 2006, the European Union has banned the use of antibiotic growth promoters in feed as additives (Steiner, 2006). As an alternative feed additive for antibiotics, probiotics claim beneficial effects by relieving intestinal microbiota disorders, decreasing intestinal

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pathogenic bacteria, promoting growth, and improving feed efficiency (Mappley et al., 2013). The mechanisms underlying these effects include (1) inhibiting pathogenic bacteria from colonizing the gut; (2) producing bacteriocins (Xu, Shen, Wu, & Li, 2017); (3) reducing intestinal pH value; and (4) regulating the immune function (Mata-Haro, Reséndiz-Sandoval, & Hernández, 2014). *Lactobacillus plantarum* has been certified as one of the most promising feed probiotics with beneficial effects on the gastrointestinal health and growth of weaning piglets (Pieper et al., 2009).

Emulsion and internal gelation is an efficient way to produce microcapsules of living cells with hydrocolloids, for example, alginate, pectin, carrageenan, and vegetable oil, where the alginate beads are formed by slowly adding calcium chloride to the emulsion while stirring (Capela, Hay, & Shah, 2007). This method can easily enlarge the production scale, guarantee a high cellular survival rate, and make the microcapsule size well distributed (Kailasapathy, 2009). In addition, microencapsulation can form a protection shell of live lactobacillus against the detrimental gastric and bile-rich upper-intestinal environment.

Fructooligosaccharide (FOS) is a non-digestible dietary ingredient for monogastric animals that can be metabolized by colonic bacteria to produce short-chain fatty acids, such as butyrate (Gibson, Probert, Loo, Rastall, & Roberfroid, 2004). Howard, Gordon, Pace, Garleb, and Kerley (1995) confirmed that cell density and the number of stained cells in cecal mucosa of piglet could increase when FOS was supplemented to piglet feeds. The aim of the present study was therefore to investigate the effects of dietary supplementation of microencapsulated *L. plantarum* (MLP) and FOS on growth performance, blood immune parameters, intestinal morphology, and microbiota in weaned piglets.

Materials and methods

Animals and diets

Piglets were cared for and handed according to the Animal Ethics Committee Guidelines of Academy of State Administration of Grain (Beijing, China).

A total of 90 crossbred (Duroc × [Landrace × Large White]) piglets weaned at 28 ± 2 d and weighting 8.70 ± 0.47 kg were randomly allotted to 1 of 3 dietary groups based on body weight and sex. Each treatment was replicated with six replicate pens each with five piglets (two males and three females). Pigs were housed in 2.1 × 1.2 m pens in a temperature-controlled (28 ± 2°C) nursery room. *L. plantarum* strain ACCC 11016, originally isolated from feces of a healthy commercial pig, was obtained from the Agricultural Culture Collection of China. The MLP was produced by emulsion technology according to Dong et al. (2016). FOS was obtained from Baolingbao Biology Co. Ltd. (Shandong, China). Aureomycin was purchased from Jinxinnong Feed Co. Ltd. (Shenzhen, China).

The three dietary groups were: (1) antibiotic-free basal diet (CON); (2) basal diet supplemented with 0.03% aureomycin (ANT); (3) basal diet supplemented with 0.1 g kg^{-1} MLP (1×10^{10} cfu kg^{-1} of feed, calculated data; 1.6×10^{9} cfu kg^{-1} of feed, analyzed data) and FOS (1.5 g kg^{-1}) (SYN). The basal diet (Table 1) was formulated to meet or exceed NRC (2012) recommended nutrient requirements for 7- to 11-kg pigs. Feed and water were provided for *ad libitum* consumption.

Table 1. Ingredients and nutrient composition of the basal diet (as-fed basis).

Item	Basal diet
Ingredient, %	
Corn	61.47
Soybean meal	33.33
Soybean oil	1.90
Dicalcium phosphate	1.20
Salt	0.30
Limestone	0.90
L-Lysine hydrochloride (78%)	0.34
DL-Methionine	0.12
L-Threonine	0.09
Choline chloride	0.05
Vitamin premix[a]	0.05
Trace minerals premix[b]	0.25
Composition[c]	
Digestible energy(MJ/kg)	14.23
Crude protein	20.08
Calcium	0.81
Total phosphorus	0.67
Lysine	1.19

[a]Supplied the following (mg/kg): retinyl acetate, 7709 IU; cholecalciferol, 2200 IU; DL-α-tocopheryl acetate, 60 IU; menadione sodium bisulfite complex, 9 mg; riboflavin, 7.7 mg; vitamin B_{12}, 0.044 mg; D-calcium pantothenate, 33 mg; niacin, 33 mg; choline, 287 mg; and D-biotin 0.22 mg.

[b]Supplied the following (mg/kg): Mn (as $MnSO_4 \cdot H_2O$), 20 mg; Fe (as $FeSO_4 \cdot H_2O$), 100 mg; Zn (as $ZnSO_4 \cdot H_2O$), 100 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 15 mg; I (as KI), 0.60 mg; Se (as Na_2SeO_3), 0.10 mg.

[c]Calculated value for digestible energy and lysine, all other values are analyzed.

Growth performance

On days 0 and 28, body weight and feed intake were recorded after feed deprivation for 8 h and used to calculate average daily weight gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) for the 28-d growth trial. The incidences of diarrhea and mortality rates were monitored and recorded daily. Diarrhea incidence (%) = (total number of pigs with diarrhea) / (total number of pigs × experimental days) × 100 (Liu, Yu, Martínez, et al., 2017).

Plasma characteristics

On day 28, blood samples (5 mL) were collected from six piglets in each group (one piglet per pen) via jugular vein into EDTA-K_2 disodium vacuum tubes (Becton, Dickinson and Company, Franklin Lakes, USA). Total protein, albumin, blood urea nitrogen, total cholesterol, and glucose contents in plasma were measured using an automatic biochemical analyzer Cobas C501 (Roche, Mannheim, Germany). Superoxide dismutase level was analyzed using the xanthine oxidase technique, whereas the concentration of malondialdehyde (MDA) was determined using thiobarbituric method. Total antioxidant capacity was measured by colorimetry, according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Plasma IgA, IgG, and IgM were determined using double-antibody sandwich ELISA (Collier, Welsh, Carroll, & Laurenz, 2011).

Intestinal morphology

After sampling blood, pigs were killed by an intracardial administration of sodium pentobarbital (50 mg kg^{-1} body weight) and jugular exsanguination. Segments (2 cm each) of the duodenum (10 cm away from the pyloric junction), jejunum (proximal half of the remaining small intestine), and ileum (15 cm prior to the ileocaecal junction) were quickly isolated (Wang, Zeng, Mao, Wu, & Qiao, 2010) and flushed with saline. All intestinal segments were immediately fixed in 10% neutral-buffered formalin and then embedded in paraffin. Sections with 5-μm thickness of each sample were stained with hematoxylin–eosin for histomorphological examination. The sections were visualized using Coolpix 4500 (Nikon, Japanese). Villus height and crypt depth were measured using Motic Images 2000 software, version 1.3 (Xiamen, China).

Bacteriology

Digesta samples from colon were collected aseptically into sterile plastic containers and stored at 4°C until analysis (within 12 h). Samples were analyzed according to Torrallardona, Conde, Badiola, Polo, and Brufau (2003). Lactic acid bacterial counts were obtained by growth on de Man, Rogosa and Sharpe (MRS) agar (Beijing Land Bridge Technology Co. Ltd., Beijing, China) for 48 h at 37°C. *E. coli* was isolated and counted by growth on Eosin-Methylene Blue Agar (Hopebio, Qingdao, China) for 24 h at 37°C. Bacterial counts were expressed as $\log_{10}$ cfu/g.

Statistical analysis

Data were analyzed by one-way ANOVA using the general linear modeling (GLM) procedure of SAS Statistical Software (SAS Institute Inc., NC, USA). Differences among treatment means were tested using the Student–Newman–Keuls test and $P < .05$ were considered to indicate statistical significance.

Results

Growth performance

Compared with CON, dietary supplementation with SYN increased ($P < .05$) ADG and ADFI, and decreased ($P < .05$) the diarrhea ratio (Table 2). However, these response criteria did not differ ($P > .05$) between ANT and SYN groups.

Table 2. Effects of MLP and FOS on growth performance in weaned piglets[1].

Items	CON[2]	ANT[2]	SYN[2]	SEM	*P*-value
ADG[2] (g/d)	228[b]	265[ab]	335[a]	22.29	.016
ADFI[2] (g/d)	442[b]	460[ab]	553[a]	29.39	.045
F/G[2]	1.94	1.77	1.67	0.13	.331
Diarrhea ratio (%)	8.52[a]	7.13[b]	6.23[b]	0.44	.001

[1]Values are means and pooled SEM, $n = 6$. Within each row, values with different superscripts are different, $P < .05$.
[2]CON: the control group; ANT: basal diet supplemented with 0.03% aureomycin; SYN: basal diet supplemented with MLP and FOS.

Plasma biochemistry, antioxidant, and immune parameters

Pigs in the SYN and ANT groups had greater ($P < .05$) plasma albumin concentration than the CON group (Table 3). The blood urea nitrogen was lower ($P < .05$) in the SYN group than in the CON group. Pigs in the SYN group had a higher ($P < .05$) total antioxidant capacity in plasma than pigs in the CON and ANT groups (Table 4). However, the concentration of MDA and superoxide dismutase did not differ ($P > .05$) among the three groups. In addition, the plasma concentration of IgA and IgG was highest ($P < .05$) in the SYN group among these three treatments (Table 5), and also higher ($P < .05$) in the ANT group compared with the CON group.

Intestinal morphology and colon bacteria

Villus height and the ratio of villus height to crypt depth (VH:CD) were both higher in duodenum of SYN pigs, compared with the CON group (Table 6), whereas the VH:CD in jejunum of the SYN group tended to be higher compared with other groups ($P = .08$). The *E. coli* counts in the colon of the SYN group tended to be lower ($P = .069$) than that of the CON and ANT groups, whereas the lactic acid bacteria counts in the colon were greater ($P < .05$) for the SYN group than that in the other two groups (Table 7).

Discussion

Many studies have shown that synbiotics are good substitutes for antibiotics in feed (Estrada, Drew, & Van Kessel, 2001; Guerra-Ordaz et al., 2013). The reasons are that probiotics and prebiotics can synergistically promote growth of animals as well as improve their resistance to infections by equilibrating intestinal microbiota and stimulating the immune system (Fan, Chang, Yin, Wang, & Dang, 2015; Guerra-Ordaz et al., 2013; Liu et al., 2016). A previous study suggested that synbiotics containing *L. plantarum* and lactulose increased ADG in weaned pigs (Guerra-Ordaz et al., 2014). Supplementation with

Table 3. Effects of MLP and FOS on plasma biochemistry characteristic in weaned piglets[1].

Item	CON[2]	ANT[2]	SYN[2]	SEM	*P*-value
Total protein (g/L)	54.6	58	59.17	2.02	.295
Albumin (g/L)	24.80[b]	30.00[a]	29.17[a]	1.01	.008
Blood urea nitrogen (mmol/L)	4.55[a]	3.91[ab]	3.60[b]	0.23	.048
Total cholesterol (mmol/L)	2.18	2.04	2.08	0.11	.622
Glucose (mmol/L)	4.35	4.47	4.82	0.25	.420

[1]Values are means and pooled SEM, $n = 6$. Within each row, values with different superscripts are different, $P < .05$.
[2]CON: the control group; ANT: basal diet supplemented with 0.03% aureomycin; SYN: basal diet supplemented with MLP and FOS.

Table 4. Effects of MLP and FOS on plasma antioxide parameters in weaned piglets[1].

Item	CON[2]	ANT[2]	SYN[2]	SEM	*P*-value
MDA (nmol/mL)[2]	2.73	2.44	2.59	0.11	.182
SOD (U/mL)[2]	37.3	42.42	42.19	2.80	.362
T-AOC (U/mL)[2]	10.25[b]	10.45[b]	11.05[a]	0.19	.035

[1]Values are means and pooled SEM, $n = 6$. Within each row, values with different superscripts are different, $P < .05$.
[2]CON: the control group; ANT: basal diet supplemented with 0.03% aureomycin; SYN: basal diet supplemented with MLP and FOS; SOD: superoxide dismutase; T-AOC: total antioxidant capacity.

Table 5. Effects of MLP and FOS on plasma immunoglobulins in weaned piglets[1].

Item	CON[2]	ANT[2]	SYN[2]	SEM	*P*-value
IgA[2] (μg/mL)	99.99[c]	111.94[b]	181.33[a]	2.55	<.001
IgG[2] (μg/mL)	1641.53[c]	1782.26[b]	3381.07[a]	42.29	<.001
IgM[2] (μg/mL)	7.79	7.8	8.17	0.14	.147

[1]Values are means and pooled SEM, $n = 6$. Within each row, values with different superscripts are different, $P < .05$.
[2]CON: the control group; ANT: basal diet supplemented with 0.03% aureomycin; SYN: basal diet supplemented with MLP and FOS; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M.

Table 6. Effects of MLP and FOS on small intestinal histomorphology in weaned piglets[1].

Item	CON[2]	ANT[2]	SYN[2]	SEM	*P*-value
Duodenum					
VH (μm)[2]	421[b]	409[b]	467[a]	12.65	.022
CD (μm)[2]	172	174	183	5.83	.350
VH/CD	2.34[b]	2.31[b]	2.62[a]	2.34	.018
Jejunum					
VH (μm)	397	396	422	8.60	.170
CD (μm)	147	147	156	2.70	.092
VH/CD	2.87	2.72	2.92	0.06	.080
Ileum					
VH (μm)	318	337	327	18.36	.773
CD (μm)	116	116	110	4.30	.433
VH/CD	2.75	2.78	3.02	0.19	.508

[1]Values are means and pooled SEM, $n = 6$. Within each row, values with different superscripts are different, $P < .05$.
[2]CON: the control group; ANT: basal diet supplemented with 0.03% aureomycin; SYN: basal diet supplemented with MLP and FOS; VH: villus height; CD: crypt depth.

Table 7. Effects of MLP and FOS on colon bacterial counts (log_{10} CFU/g) in weaned piglets[1–3].

Item	CON[4]	ANT[4]	SYN[4]	SEM	*P*-value
E. coli	5.35	5.31	5.21	0.41	.069
Lactic acid bacteria	8.49[b]	8.48[b]	8.62[a]	0.53	.023

[1]Values are means and pooled SEM, $n = 6$.
[2]Bacterial counts are presented as log_{10} CFU/g wet weight.
[3]Within each row, values with different superscripts are different, $P < .05$.
[4]CON: the control group; ANT: basal diet supplemented with0.03% aureomycin; SYN: basal diet supplemented with MLP and FOS.

oligofructose and probiotics to an antibiotic-free creep feed during the pre-weaning period enhanced body weight gain of piglets (Shim, Verstegen, Kim, Kwon, & Verdonk, 2005). However, others have shown that synbiotics containing *L. plantarum* may not affect growth performance in pigs (Guerra-Ordaz et al., 2013). This may be attributed to the variability in the viable counts of probiotics in the feed. Probiotics can exert their beneficial effects only when their viable counts reach a certain quantity in the gastrointestinal tract (Guarner & Schaafsma, 1998). Guerra-Ordaz et al. (2013) administered *L. plantarum* daily by spraying the feed with a pure culture for each pig and supplemented diet with lactulose simultaneously. However, there was no significant effect on the growth performance of the pigs in their study, which might be attributed to the loss of viability for ingested probiotics. Our study used the emulsion technology to encapsulate *L. plantarum*, so as to enhance the ability of the probiotic products resist stress (Gerez, Font de Valdez, Gigante, & Grosso, 2012). Our results showed that ADG of pigs in the SYN group was increased by 46.9% and 26.4%, ADFI was increased by 25.1% and 20.2%, and diarrhea ratio was decreased by 16.3% and 12.6%, compared with pigs in the CON group and the ANT group,

respectively. This may be attributed to the high survival rate of *L. plantarum* colonizing in the intestine, which could produce organic acids and vitamins to decrease diarrhea ratio and improve growth performance (Fu & Mathews, 1999). On the other hand, FOS can promote probiotic production (Gibson et al., 2004; Herich et al., 2002), which may synergistically enhanced growth performance in weaned piglets. Work by Cunha et al. (1950) was the first to demonstrate that feeding pigs with aureomycin as an antibiotic improved weight gain and feed efficiency compared with the control group. Since that time, in-feed antibiotics have been widely researched and applied in the pig industry as growth-promoting additives. However, the growth response to aureomycin appeared to be dependent on increased feed intake (Brown, Becker, Terrill, & Card, 1952). In the present study, daily feed intake in the aureomycin group was not different from that of the CON group, which may also explain the lack of differences in weight gain between the two groups. Others have reported similar observations previously (Fan et al., 2015; Wang et al., 2012).

The content of albumin and globin reflect the digestibility and utilization of protein. Serum albumin is the most abundant blood protein in mammals, and is produced by the liver. It can regulate the oncotic pressure of blood and its deficiency can be related to the chronic hepatic or gastrointestinal diseases (Gbore & Egbunike, 2010). Kodner and Kudrimoti (2003) reported that content of serum urea nitrogen would increase when bodies suffer nephritis. Our results showed that plasma albumin level was increased by 17.62% in SYN group and increased by 20.97% in ANT group than CON group. And plasma blood urea concentration in SYN group was decreased by 20.88% compared with CON group. These results collectively demonstrated that our SYN enhanced protein digestibility and utilization in pigs.

Total antioxidant capacity level and superoxide dismutase values are important indices to evaluate antioxidant properties (Liu, Yu, Fang, et al., 2017). Concentration of MDA in plasma is generally used as a biomarker for radical-induced damage and peroxidation (Wang, Xu, An, Liu, & Feng, 2008). In the present study, plasma total antioxidant capacity value in SYN group was increased by 7.8% compared to the CON group. We postulated that FOS and *L. plantarum* produced organic acids which combined with metallic ions to synergistically improve the antioxidant capacity.

Concentrations of immunoglobulins are used as important response criteria to assess humoral immune response in humans and animals (Salim et al., 2013; Yuan et al., 2015). Naqid et al. (2015) reported that dietary *L. plantarum* B2984 and lactulose supplementation enhanced serum IgM and IgG concentrations against *S. typhimurium* infection in pigs. Similarly, in the present study, plasma IgA level in SYN pigs was increased by 81.3% and 62.0% than CON group and ANT group respectively. And plasma IgG level in SYN pigs was also increased by 106.0% and 89.7% than CON group and ANT group, respectively. These results confirmed that there was a more beneficial effect on humoral immune response with dietary supplementation of MLP and FOS than aureomycin.

Intestinal morphology changes significantly after weaning with respect to villus height, crypt depth, and villus-height-to-crypt-depth ratio (Pluske, Williams, & Aherne, 1996). Budiño et al. (2005) reported that synbiotic (*Bacillus licheniformis* + *Bacillus subtilis* and FOS) enhanced villus height and VH:CD at day 14 post-weaning. Similarly, the present study indicated that villus height and VH:CD in duodenum of SYN pigs increased by 11% and 12% than the CON group, respectively. These results could be due to the fact that MLP is able to successfully reach and adhere to the intestinal tract and produce

organic acids, and then support cell turn-over and promote activities of digestive enzymes. In addition, FOS may have been fermented by the probiotic to produce short-chain fatty acids, which could improve intestinal digestion and absorption.

Intestinal microbiota composition and their balance are closely related to health status (Konstantinov et al., 2006). Probiotics play an important role in maintaining intestinal ecosystem balance by promoting growth of beneficial bacteria and competing for nutrients and oxygen with harmful bacteria (Ng, Hart, Kamm, Stagg, & Knight, 2009). Estrada et al. (2001) reported that dietary *Bifidobacterium longum* and FOS supplementation increased the number of *Bifidobacteria* and decreased the number of *Clostridia* and anaerobes in feces of weaned piglets. Nemcová et al. (2007) indicated that the addition of *L. plantarum* and FOS had a significant decrease in the number of *E. coli* k88 adhering to the intestinal mucosa. Findings of the present study showed that the number of colonic *E. coli* in the ANT group decreased by 7.87%, whereas this decreased by 26.97% in SYN group than CON group. Furthermore, the number of colonic lactic acid bacteria in SYN group increased by 37.40% than in CON group. These observations may be due to the ability of *L. plantarum* to suppress the growth of harmful bacteria, especially *E. coli* k88 (*in vitro* data, not shown) and to enhance the growth of beneficial bacteria, consequently resulting in reduced diarrhea rate in pigs.

Conclusions

In conclusion, dietary supplementation of MLP and FOS increased growth performance and blood immunoglobulin concentrations, and improved intestinal morphology and microbiota, thus suggesting that this symbiotic may serve as an alternative to in-feed antibiotics in nursery pig diets.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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