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EFFECTS OF SOYBEAN MEAL FERMENTED BY *LACTOBACILLUS* SPECIES AND *CLOSTRIDIUM BUTYRICUM* ON GROWTH PERFORMANCE, DIARRHEA INCIDENCE, AND FECAL BACTERIA IN WEANING PIGLETS*

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Abstract

Fermented soybean meal (FSBM) has been widely investigated as a nutritional strategy for reducing the use of fish meal (FM) and antibiotic growth promoters. Microbial fermentation by using bacteria can increase the bioavailability of nutrients and reduce the levels of antinutritional factors in soybean meal (SBM). In this study, we evaluated whether FSBM produced from *Lactobacillus* species and *Clostridium butyricum* improves growth performance, diarrhea incidence, and fecal bacteria in weaning piglets. Eighty-four crossbred male piglets with an average initial body weight of 8.36±0.63 kg were randomly allotted to 3 dietary treatments consisting of 7 replicate stalls with 4 piglets each. The dietary treatments were: (1) 3% FM in the diet; (2) 5% FSBM in the diet; and (3) 3% FM in the diet plus 4 mg/kg antibiotic growth promoters (AGP). We determined that growth performance was unaffected in FSBM-fed weaning piglets compared with a FM group. Similar to the AGP group, FSBM supplementation significantly reduced diarrhea incidence in weaning piglets. The number of fecal *Lactobacillus* species significantly increased in 28-day-old FSBM-fed weaning piglets compared with the other groups. Compared with AGP, FSBM has the highest inhibitory effect on the number of fecal *Enterobacteriaceae* at 28 d old. Furthermore, serum immunoglobulin G and immunoglobulin A levels in FSBM-fed weaning piglets significantly increased at the same age. These results together indicate that FSBM can replace FM in the diets of weaning piglets without affecting growth performance. Furthermore, similar to AGP, FSBM could improve diarrhea incidence, fecal bacteria, and immunoglobulin levels in weaning piglets. Therefore, SBM fermented by *Lactobacillus* species and *C. butyricum* demonstrated high potential for development as swine feed ingredients.

Key words: *Clostridium butyricum,* fermented soybean meal, growth, *Lactobacillus,* piglet

Soybean meal (SBM) is a commonly used source of vegetable protein in animal feed because of its low cost and high nutritional value (Cromwell, 2012). Several an-

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tinutritional factors such as trypsin inhibitor, lectin, α-amylase inhibiting factor, and soybean antigens have been identified in crude SBM (Grant, 1989). These factors directly limit soybean protein utilization and have a detrimental effect on the small intestine in nursing pigs (Dunsford et al., 1989; Li et al., 1990). The use of SBM as only protein source negatively affects growth performance in piglets (Genova et al., 2018). Our previous research and other studies have demonstrated that antinutritional factors in SBM can be efficiently degraded through microbial fermentation (Hong et al., 2004; Chi and Cho, 2016; Zhang et al., 2016; Su et al., 2018).

The partial replacement of fish meal (FM) with fermented soybean meal (FSBM) produced from a single bacterial strain in diets has no negative effect on growth performance in weaning piglets (Rojas and Stein, 2015; Jeong and Kim, 2015). Our recent research demonstrated that multistrain microbial FSBM could efficiently improve the nutritional value of FSBM (Su et al., 2018). Whether SBM fermented by *Lactobacillus* species and *Clostridium butyricum* could provide an alternative to FM as an ingredient in swine feed has yet to be verified.

Antibiotic growth promoters (AGP) can positively affect porcine gut microbiota, thereby improving pig health and growth performance. The European Union has banned the use of AGP in animal production. Therefore, finding alternative solutions for preventing microbial infection in pigs is urgent. FSBM can improve growth performance, digestive enzyme activities, intestinal morphology and gut microbiota in piglets (Feng et al., 2007; Yuan et al., 2017; Wang et al., 2018; Zhang et al., 2018). Furthermore, FSBM can alleviate diarrhea incidence in weaned pigs under an enterotoxigenic *Escherichia coli* challenge (Kiers et al., 2003). Our recent study demonstrated that FSBM produced from *Lactobacillus* species and *C. butyricum* exhibits antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Su et al., 2018). However, studies have mainly used *Aspergillus* or *Lactobacillus* species for SBM fermentation. No reports have determined whether FSBM produced from *Lactobacillus* species and *C. butyricum* affects diarrhea incidence and the number of fecal bacteria in weaning piglets. Whether SBM fermented by *Lactobacillus* species and *C. butyricum* could provide an antibiotic alternative for pigs therefore remains unclear.

Based on previous studies, we hypothesize that FSBM can replace FM in the diets without affecting growth performance and FSBM in the diets could improve diarrhea incidence in weaning piglets. This study could be applied to evaluate whether FSBM produced from multibacterial strains as an alternative to FM and antibiotics has an effect on growth performance and diarrhea incidence in weaning piglets.

Material and methods

All experiments were performed in accordance with the approved guidelines. The animal protocol was approved by the National Ilan University Institutional Animal Care and Use Committee.

Microorganisms

Lactobacillus acidophilus (BCRC10695), *L. delbrueckii* (BCRC10696), and *L. salivarius* (BCRC12574) were purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan). *C. butyricum* MIYAIRI 588 was purchased from Miyarisan Pharmaceutical (Tokyo, Japan).

Solid-state fermentation of SBM

Solid-state fermentation of SBM by using multiple strains was performed in accordance with the procedure described by Su et al. (2018). SBM was first mixed with water at an initial moisture content of 50% in an anaerobic space bag and autoclaved at 121°C for 30 min. The cooled SBM was inoculated with 3% (v/w) inoculums containing *L. acidophilus*, *L. delbrueckii*, *L. salivarius*, and *C. butyricum*, mixed carefully under sterile conditions and incubated in a chamber at 37°C. After 2 d of fermentation, samples of FSBM were dried at 50°C for 12 h and homogenized through mechanical agitation. The fermented powder was then stored at 4°C prior to analysis. To determine colony forming units (CFUs), the FSBM was diluted 10-fold serially with 0.85% NaCl and then plated on selective media for microbial enumeration. A De Man, Rogosa, Sharpe (MRS; Sigma-Aldrich, St. Louis, MO, USA) agar plate and a brain heart infusion (Sigma-Aldrich, St. Louis, MO, USA) agar plate were used to determine the number of *Lactobacillus* species and *C. butyricum*, respectively. The agar plates were incubated in an anaerobic chamber at 37°C for 24 h. Bacterial growth was counted, analyzed, and expressed as CFUs per gram (CFU/g). The average number of colonies of *Lactobacillus* species and *C. butyricum* was 1×10^8 CFU/g FSBM, respectively.

Animal study

Eighty-four crossbred ((Landrace × Yorkshire) × Duroc, 28-day-old) male piglets with an average initial body weight of 8.36 ± 0.63 kg were involved in the study. Piglets were randomly allotted to 3 dietary treatments: (1) FM: 3% FM in the diet; (2) FSBM: 5% FSBM in the diet; and (3) FM + AGP: 3% FM in the diet plus 4 mg/kg flavomycin (AGP). Diets (Table 1) were formulated to meet or exceed the nutrient requirements recommended by the National Research Council (Nutrient Requirements for Swine, 2012). There were 4 pigs per stall and 7 replicate stalls per treatment. The experimental period was 28 d. All piglets were housed in stalls (1.5 × 2.1 m) with slatted plastic floors. The piglets were adapted to the basal diets and the housing for 3 days before starting the 28-day experiment. Room temperature was set at 30°C at the beginning of the study and then gradually reduced to 24°C by the end. The lighting schedule was 10L:14D throughout the experiment. Food and water were offered *ad libitum* throughout the experimental period. The individual body weight, average daily feed intake, average daily weight gain, and feed conversion ratio were recorded at 1, 14, and 28 d old. The average final body weight of piglets was 18.27 ± 1.42 kg.

Table 1. Composition of experimental diets (as-fed basis)

Item	FM[1]	FSBM[2]	FM + AGP[3]
Ingredient (g kg^{-1}):			
corn, yellow	570.0	560.0	570.0
soybean meal, 44% CP	260.0	275.0	260.0
fish meal, 60% CP	30.0	0	30
fermented soybean meal	0	50.0	0
dried whey	85.0	85.0	85.0
soybean oil	35.0	55.0	35.0
$CaCO_3$, 38% Ca	5.0	5.0	5.0
$CaHPO_4$	12.0	20.0	12.0
salt	4.0	4.0	4.0
choline, 50%	0.8	0.8	0.8
L-Lysine, 98%	3.5	3.0	3.5
vitamin, premix[4]	1.0	1.0	1.0
mineral, premix[5]	1.0	1.0	1.0
Chemical composition (g kg^{-1}):			
crude protein	191.2	191.8	191.2
analyzed calcium	8.0	8.1	8.0
analyzed total phosphorus	7.0	7.6	7.0
lysine	14.5	14.5	14.5
methionine + cystine	6.5	6.5	6.5
phenylalanine + tyrosine	15.5	14.3	15.5
ME (kcal/kg)	3567.6	3537.1	3567.6

[1]FM = 3% fish meal in the diet.

[2]FSBM = 5% fermented soybean meal in the diet.

[3]FM + AGP = 3% fish meal in the diet plus 4 mg/kg flavomycin.

[4]Supplied per kg diet: vitamin A, 6000 IU as vitamin A acetate; vitamin D, 900 IU as D-activated animal sterol; vitamin E, 30 IU as alpha tocopherol acetate; vitamin K_3, 3 mg as menadione dimethylpyrimidinol bisulfite; riboflavin, 6 mg; pantothenic acid, 18 mg as calcium pantothenate; niacin, 60 mg as nicotinamide and nicotinic acid; and vitamin B_{12}, 30 µg.

[5]Supplied per kg diet: Cu, 20 mg as copper sulfate; Zn, 100 mg as zinc oxide; Fe, 140 mg as iron sulfate; Mn, 40 mg as manganese sulfate; Se, 0.1 mg as sodium selenite; and I, 0.2 mg as potassium iodate.

Diarrhea incidence analysis

Diarrhea incidence was recorded daily in each stall throughout the entire experiment through direct observation conducted by 2 evaluators. The piglets were considered to have diarrhea when the fecal consistency was level 2 or 3, as described previously by Liu et al. (2010) and calculated as follows:

Diarrhea incidence (%) = [the number of pigs with diarrhea in each stall × diarrhea d/(4 pigs × 28 d)] × 100.

Fecal bacteria population analysis

Fresh feces were collected by massaging the rectums of 2 pigs in each stall (14 pigs per treatment) at 1, 14, and 28 d of age. The pooled fecal sample (1 g) from each stall was diluted using 9 mL of 0.85% NaCl and then homogenized. Viable counts of *Lactobacillus* species in the fecal samples were performed by plating serial 10-fold dilutions (in 0.85% NaCl) onto MRS agar plates and incubating them under anaerobic conditions at 37°C for 24 h. Viable counts of *Enterobacteriaceae* in the fecal samples were conducted by plating serial 10-fold dilutions (in 0.85% NaCl) onto lysogeny broth (Sigma-Aldrich, St. Louis, MO, USA) agar plates which were then cultured in an aerobic incubator at 37°C for 24 h. The *Lactobacillus* species and *Enterobacteriaceae* colonies were counted immediately after removal from the incubator.

Serum immunoglobulin G and immunoglobulin A analysis

Blood samples were collected in nonheparinized tubes from the anterior vena cava using a 10 mL syringe and 20 gauge × 3.8 cm sterile needle from 2 random pigs in each stall (14 pigs per treatment) at 14 and 28 d old. Blood samples were centrifuged for 15 min at 3000 × g at 4°C; the serum was then stored at −4°C until determination of immunoglobulin G (IgG) and immunoglobulin A (IgA) concentration. Serum immunoglobulin levels were measured using an enzyme-linked immunosorbent assay kit (Bethyl Laboratories, Inc., Montgomery, TX, USA).

Statistical analysis

All experimental data were subjected to analysis of variance (ANOVA) appropriate for a completely randomized design using the general linear model (GLM) procedure employed by SAS (SAS Institute, Cary, NC, USA). The stall was used as the experimental unit to analyze growth performance and diarrhea incidence, whereas an individual piglet was used as the experimental unit for analysis of fecal microbiota and serum immunoglobulins. Statistically significant effects were further analyzed, and means were compared using Duncan's multiple range tests. $P<0.05$ was considered statistically significant.

Results

Effects of multistrain microbial FSBM on growth performance and diarrhea incidence in weaning piglets

The results revealed that the replacement of FM with FSBM did not affect body weight, daily weight gain, daily feed intake, and feed conversion ratio in weaning piglets compared with the FM group (Table 2; $P = 0.66$ for body weight; $P = 0.46$ for average daily weight gain; $P = 0.40$ for average daily feed intake; $P = 0.20$ for feed conversion ratio). Growth performance was not improved by antibiotic supplementation in weaning piglets compared with the FM group during the experimental period. However, the incidence of diarrhea in antibiotic-fed weaning piglets significantly

decreased compared with the FM group during the experimental period (Table 3; P≤0.05). Similarly, FSBM-fed weaning piglets exhibited a lower incidence of diarrhea compared with the FM group during the experimental period (Table 3; P≤0.05). No significant difference was observed in the incidence of diarrhea between FSBM-fed and antibiotic-fed weaning piglets. These results indicate that FSBM could be used as an alternative protein source without altering growth performance in weaning piglets. Furthermore, similar to antibiotics, FSBM supplementation significantly improved the incidence of diarrhea in weaning piglets.

Table 2. Effect of multistrain FSBM on the growth performance of weaning piglets

Group	FM[1]		FSBM[2]		FM + AGP[3]		P-value
	Mean	SD	Mean	SD	Mean	SD	
Weight (kg/head)							
1 d	8.11[4]	0.28	8.06	0.34	8.07	0.33	0.9528
14 d	11.92	1.09	11.60	0.57	11.71	0.78	0.7726
28 d	18.75	2.21	18.01	0.72	18.27	1.26	0.6612
Average daily weight gain (kg/d)							
1–14 d	0.229	0.35	0.233	0.01	0.241	0.04	0.9937
15–28 d	0.451	0.04	0.450	0.05	0.498	0.10	0.3932
1–28 d	0.381	0.06	0.350	0.03	0.380	0.06	0.4627
Average daily feed intake (kg/d)							
1–14 d	0.295	0.02	0.294	0.01	0.296	0.03	0.9851
15–28 d	0.667	0.02	0.657	0.07	0.635	0.05	0.4995
1–28 d	0.552	0.09	0.501	0.04	0.520	0.07	0.4033
Feed conversion ratio							
1–14 d	1.307	0.11	1.263	0.03	1.295	0.02	0.4601
15–28 d	1.486	0.10	1.462	0.04	1.437	0.06	0.4523
1–28 d	1.390	0.07	1.433	0.07	1.370	0.05	0.1988

[1]FM = 3% fish meal in the diet
[2]FSBM = 5% fermented soybean meal in the diet.
[3]FM + AGP = 3% fish meal in the diet plus 4 mg/kg flavomycin.
[4]Values are expressed as mean ± SD (n = 7).

Table 3. Effect of multistrain FSBM on diarrhea incidence among weaning piglets

Group	FM[1]		FSBM[2]		FM + AGP[3]		P-value
	Mean	SD	Mean	SD	Mean	SD	
Diarrhea (%)							
1–28 d	16.67 a[4]	1.29	7.14 b	1.26	9.52 b	1.38	0.0001

[1]FM = 3% fish meal in the diet.
[2]FSBM = 5% fermented soybean meal in the diet.
[3]FM + AGP = 3% fish meal in the diet plus 4 mg/kg flavomycin.
[4]Values are expressed as mean ± SD (n = 7).
a, b – means within a row that have no common letter are significantly different (P≤0.05) according to Duncan's test.

Table 4. Effect of multistrain FSBM on fecal *Lactobacillus* species and *Enterobacteriaceae* of weaning piglets

Group	FM[1]		FSBM[2]		FM + AGP[3]		P-value
	Mean	SD	Mean	SD	Mean	SD	
Lactobacillus species (Log CFU/g)							
1 d	8.35[4]	0.17	8.24	0.11	8.11	0.13	0.1672
14 d	8.02	0.08	8.23	0.12	8.14	0.10	0.1030
28 d	7.56 a	0.22	8.78 b	0.01	7.49 a	0.30	0.0005
Enterobacteriaceae (Log CFU/g)							
1 d	4.65	0.05	4.65	0.01	4.67	0.04	0.7950
14 d	4.73 a	0.04	4.62 ab	0.07	4.54 b	0.03	0.0069
28 d	5.05 a	0.04	4.66 c	0.09	4.83 b	0.04	0.0006

[1]FM = 3% fish meal in the diet.
[2]FSBM = 5% fermented soybean meal in the diet.
[3]FM + AGP = 3% fish meal in the diet plus 4 mg/kg flavomycin.
[4]Values are expressed as mean ± SD (n = 14).
a, b, c – means within a row that have no common letter are significantly different (P≤0.05) according to Duncan's test.

Table 5. Effect of multistrain FSBM on total IgG and IgA concentrations of weaning piglets

Group	FM[1]		FSBM[2]		FM + AGP[3]		P-value
	Mean	SD	Mean	SD	Mean	SD	
IgG (mg/mL)							
14 d	3.70[4]	0.72	3.97	1.77	3.64	1.28	0.8700
28 d	3.99 b	1.30	5.52 a	1.61	3.76 b	1.32	0.0437
IgA (mg/mL)							
14 d	0.21 b	0.04	0.44 a	0.25	0.22 b	0.06	0.0087
28 d	0.22 b	0.04	0.48 a	0.27	0.23 b	0.05	0.0054

[1]FM = 3% fish meal in the diet.
[2]FSBM = 5% fermented soybean meal in the diet.
[3]FM + AGP = 3% fish meal in the diet plus 4 mg/kg flavomycin.
[4]Values are expressed as mean ± SD (n = 14).
a, b – means within a row that have no common letter are significantly different (P≤0.05) according to Duncan's test.

Effects of multistrain microbial FSBM on fecal bacteria population and serum immunoglobulin levels in weaning piglets

FSBM supplementation in weaning piglets did not change the number of *Lactobacillus* in fecal samples collected at 1 and 14 d old. By contrast, the number of *Lactobacillus* in fecal samples increased remarkably in FSBM-fed weaning piglets compared with the FM and FM + AGP groups (Table 4; P≤0.05). Antibiotic supplementation in weaning piglets significantly reduced the number of *Enterobacteriaceae* in fecal samples collected at 14 and 28 d old (Table 4; P≤0.05). The number of *Enterobacteriaceae* in fecal samples also decreased in FSBM-fed weaning piglets at 28 d old (Table 4; P≤0.05). Furthermore, the inhibitory effect of FSBM on fecal *Enterobacteriaceae* number was higher than was that of antibiotics (Table 4; P≤0.05). Immunity analysis revealed no significant difference in the serum IgG levels of weaning piglets of all groups at 14 d old (Table 5; P = 0.87). However, FSBM supplementation in weaning piglets significantly promoted serum IgG levels

compared with the FM and FM + AGP groups (Table 5; $P \leq 0.05$). Similarly, serum IgA levels significantly increased in FSBM-fed weaning piglets at 14 and 28 d old compared with the FM and FM + AGP groups (Table 5; $P \leq 0.05$). These results indicate that FSBM containing a multistrain of probiotics could alter the fecal bacteria population and improve serum immunoglobulin levels in weaning piglets.

Discussion

In this study, we demonstrated that replacing dietary FM with FSBM produced from *Lactobacillus* species and *C. butyricum* did not affect growth performance in weaning piglets. Dietary supplementation with FSBM significantly reduced diarrhea incidence in weaning piglets.

FM is widely used as an animal feed in the diets of young pigs because of its ideal amino acid composition and the high digestibility of nutrients (Fowler, 1997; Kim and Easter, 2001). It has been demonstrated that microbial fermentation of SBM could eliminate antinutritional factors and increase nutrient value by producing proteolytic enzymes, thereby liberating free amino acids (Hong et al., 2004; Chi and Cho, 2016; Zhang et al., 2016; Su et al., 2018). Dietary supplementation with FSBM in weaned pigs can improve the feed conversion rate, amino acid digestibility, intestinal morphology, immunity, and digestive enzyme activities (Kiers et al., 2003; Cho et al., 2007; Feng et al., 2007; Cho and Kim, 2011). However, the cost and risks associated with diseases from FM has become a major concern in pig production. In addition, it has been reported that high levels of fish meal in the diet have a higher incidence of diarrhea in weaned piglets (Wu et al., 2015). Diets with fish meal tend to be more fermentable in the intestine, thereby increasing the toxic bacterial metabolites, such as polyamines and NH_3 (Wen et al., 2018). The polyamines and NH_3 could increase the incidence of diarrhea in piglets (Porter and Kenworthy, 1969; Dong et al., 1996). Thus, a cheaper and safer protein source must be developed for piglet diets. Yun et al. (2005) reported that FM supplementation in weaning piglets exhibited more favorable effects on growth performance, nutrient digestibility, and gut morphology than SBM. By contrast, several studies have demonstrated that FM could be completely replaced with FSBM during the 4-week post-weaning period in pigs without affecting growth performance, nutrient digestibility, and gut morphology (Yun et al., 2005; Jones et al., 2010; Rojas and Stein, 2015). Jeong and Kim (2015) determined that partial replacement of FM with FSBM has no negative effect on growth performance in weaning piglets. We also determined that growth performance was unaffected in FSBM-fed weaning piglets compared with FM-fed weaning piglets. Overall, the results indicate that FSBM could serve as a partial replacement protein source for SBM in diets fed to weaned pigs. Topics that must be investigated in future research include i) whether FSBM produced from multibacterial strains generates more free amino acids and ii) the amount of essential amino acids compared with FM. The effect of SBM fermented by *Lactobacillus* species and *C. butyricum* on nutrient digestibility, intestinal morphology and biochemistry

parameters (urinary urea nitrogen, blood urea nitrogen, creatinine, glucose and diamine oxidase) in weaning piglets must also be assessed.

Post-weaning diarrhea in pigs strongly affects feed efficiency and is commonly associated with bacterial infection in the pig intestine (Pluske et al., 2002). Probiotics are used in livestock to improve gut health and nutrient utilization (Abudabos et al., 2015; Liao and Nyachoti, 2017). Lactic acid produced from *Lactobacillus* species and butyric acid produced from *Clostridium butyricum* can reduce pH value to inhibit the growth of pathogens (Zhang et al., 2009; Wang et al., 2014). SBM fermented by *Lactobacillus* species and *C. butyricum* showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Su et al., 2018). Research has reported that FSBM containing probiotics could improve gastrointestinal flora and inhibit the intestinal colonization of pathogens that cause diarrhea in pigs (Kiers et al., 2003; Wang et al., 2018). The antinutritional factors could be removed during microbial fermentation of SBM, thereby increasing nutrient value (Hong et al., 2004; Chi and Cho, 2016; Zhang et al., 2016; Su et al., 2018). Dietary supplementation with FSBM improves growth performance, nutrient digestibility, and microbial flora in piglets (Canibe and Jensen, 2003; Mukherjee et al., 2016; Yuan et al., 2017; Wang et al., 2018). Jeong and Kim (2015) also reported that FSBM containing *Enterococcus faecium* or *Bacillus subtilis* supplementation could increase the number of *Lactobacillus* in fecal samples compared with FM treatment, whereas no significant difference was observed in the number of fecal *Escherichia coli* after FSBM treatments. Our previous study showed that FSBM containing *Lactobacillus* species and *C. butyricum* exhibited antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Su et al., 2018). In the present study, we also demonstrated that FSBM supplementation increased the number of *Lactobacillus* in fecal samples of weaning piglets compared with FM supplementation. Furthermore, the number of fecal *Enterobacteriaceae* in FSBM-fed piglets was significantly reduced compared with FM treatment. These results are consistent with studies conducted by Yuan et al. (2017) that revealed that the addition of FSBM produced from *L. casei*, *B. subtillis*, and *Hansenula anomala* in piglet diets could increase the number of *Lactobacillus* and reduce the number of *Escherichia coli* in the feces. However, research has demonstrated that diarrhea in piglets does not significantly improve after FSBM supplementation (Jeong and Kim, 2015; Yuan et al., 2017). This difference may contribute to the bacteria strains used in the fermentation of SBM. Specifically, *Lactobacillus* species and *C. butyricum* can reduce pH value by producing lactic acid and butyric acid, respectively, thereby efficiently inhibiting the growth of intestinal pathogens (Su et al., 2018). This may create a more acidic environment in the gastrointestinal tract that prevents diarrhea in piglets. Whether SBM fermented by *Lactobacillus* species and *C. butyricum* could decrease fecal *Escherichia coli* counts has not been determined.

Serum immunoglobulin concentrations have been considered indicators of systemic immune status in piglets (Blecha and Kelley, 1981; Machado-Neto et al., 1987). Deng et al. (2012) reported that the coadministration of *B. subtilis* and *L. salivarius* in piglets increased the number of intestinal-IgA-producing cells. Furthermore, dietary supplementation with *B. subtilis* and *B. licheniformis* in weaned piglets promotes serum IgG production (Ahmed et al., 2014). Supplementation of

FSBM produced from *L. casei*, *B. subtillis*, and *H. anomala* in piglet diets did not promote serum immunoglobulin levels (Yuan et al., 2017). However, SBM fermented by *L. plantarum*, *B. subtilis,* and *Saccharomyces cerevisiae* significantly increased serum IgG, IgM, and IgA levels in weaned piglets (Zhu et al., 2017). In the present study, we also demonstrated that FSBM containing *Lactobacillus* species and *C. butyricum* potentiates the immunity of weaning piglets by increasing serum IgG and IgA levels compared with the FM group. Furthermore, Li et al. (2018) reported that *C. butyricum* exerts beneficial effects on intestinal health by improving intestinal barrier function and alleviating inflammatory reactions. These findings indicate that FSBM associated with probiotics improve immunity in piglets

Conclusions

This paper provides evidence that FSBM can replace FM in the diets of weaning pigs without affecting growth performance. Furthermore, similar to antibiotics, FSBM has a positive effect on fecal bacteria, diarrhea prevention, and immunoglobulin levels in weaning piglets. Thus, SBM fermented by *Lactobacillus* species and *C. butyricum* may serve as alternative protein sources and growth promoters for swine diets following the ban on antibiotics.

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