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Original Research Article

Fermented soybean meal improves the growth performance, nutrient digestibility, and microbial flora in piglets

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ABSTRACT

In order to increase nutritive values of soybean meal (SBM), 3 species of microbes were used to ferment SBM. Through a 3 × 3 orthogonal design and parameter measurements of soybean peptide and anti-nutritional factor contents in the fermented soybean meal (FSBM), it was estimated that the best microbial proportion of *Bacillus subtilis*, *Hansenula anomala* and *Lactobacillus casei* was 2:1:2 for SBM fermentation ($P < 0.05$). The further piglet feeding experiment showed that 10% FSBM substitute for SBM had no significant effect on growth performance of suckling piglets (d 7–28) ($P > 0.05$). However, newly-weaned piglets (d 28–38) fed 10% FSBM and different levels of plasma protein obtained higher average daily gain (ADG) and feed conversion ratio (FCR), compared with those without FSBM but with 6% plasma protein ($P < 0.05$). Piglets (d 38–68) fed diets supplemented with FSBM and soybean protein concentrate (SBPC) at 3.75% and 7.5% respectively increased nutrient digestibility, fecal enzyme activity and lactic acid bacteria counts, and decreased fecal *Escherichia coli* counts ($P < 0.05$), compared with the control. These data indicated that FSBM had positive effects on nutrient digestibility and fecal microflora for piglets.

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1. Introduction

Soybean meal (SBM) is the major source of protein in swine diets. However, its application in piglet diets is limited due to some anti-nutritional factors and antigenic proteins, which interferes with digestion, absorption and utilization of nutrients (Holm et al., 1992; Hong et al., 2004).

Previous study showed that degradation of most antigenic proteins (glycinin and β-conglycinin) and protease inhibitors in SBM fermented by *Bacillus subtilis* improved intestinal morphology and digestive enzyme activities in weaned pigs (Feng et al., 2007). Fermentation of soybeans is also able to degrade proteins and carbohydrates to low molecular-weight and water-soluble compounds, which will facilitate nutrient digestibility and help to prevent diarrhea for piglets. In addition, it has been proposed that some microorganisms present in fermented products can inhibit intestinal colonization of pathogens that causes diarrhea in pigs (Kiers et al., 2003). The reduction of diarrhea is of great importance in pig production as it decreases the predisposition of these animals to *Escherichia coli* infection and improves feed efficiency, especially in weaning pigs (Pluske et al., 2002).

In piglet production, plasma protein (PP) and soybean protein concentrate (SBPC) are very common protein resources for improving animal immunity, health, feed availability and production performance. However, the high price of both protein resources restricts their usage in swine feed. Therefore, this study focused on high-quality FSBM production as a substitute for PP and SBPC for improving piglet performance with low cost.

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2. Materials and methods

2.1. Preparation of FSBM and its quality determination

Lactobacillus casei (CGMCC1.62), *B. subtilis* (CGMCC1.504) and *Hansenula anomala* (CGMCC2.881) were purchased from China General Microbiological Culture Collection Center (Beijing). The compositions (g/L) of De Man Rogosa Sharpe (MRS) medium for *L. casei* incubation were: tryptone 10, glucose 20, beef peptone 10, yeast extract 5, Tween-80 1 mL, K_2HPO_4 2, sodium acetate 5, sodium citrate 2, $MgSO_4$ 0.2, $MnSO_4$ 0.05. The medium was autoclaved at 0.15 MPa for 20 min, so did the following other media. *Lactobacillus* was incubated at a stationary state at 37 °C for 48 h. The compositions (g/L) of Luria–Bertani (LB) medium for *B. subtilis* and *E. coli* incubation were: tryptone 10, yeast extract 5, NaCl 10 at pH 7.0–7.2. *B. subtilis* and *E. coli* were incubated at 37 °C for 48 h with 200 rounds per minute (rpm). The compositions (g/L) of Yeast Extract Peptone Dextrose Medium (YPD) for *H. anomala* incubation were: peptone 20, glucose 20, and yeast extract 10 at pH 7.0–7.2. *H. anomala* was incubated at 30 °C for 48 h with 200 rpm.

Results from a former research in our laboratory showed that the optimal fermentation conditions of SBM for the combined microbes such as *B. subtilis*, *L. casei* and *H. anomala* were as follows. Soybean meal was mixed with water at a ratio of 3:1, autoclaved at 120 °C for 20 min, and cooled at room temperature. The different amount of microbes was added according to the design as shown in Table 1, in which groups 1–9 were in a 3 × 3 orthogonal design, groups 10–12 were the individual microbial fermentation, and group 13 was the control. There were 5 replicates for each treatment. Each sample was put into a 30-cm thick tank, fermented at 37 °C for 48 h, and dried at 50–55 °C, respectively. There were more than 1×10^6 colony-forming unit (cfu)/g visible microbes left in FSBM after drying. Trypsin inhibitor and soybean peptides were measured with the former protocols (Liener, 1996; Hong et al., 2004). Antigen protein was analyzed by using electrophoresis of SDS-PAGE that was carried out according to the previous method (Yin et al., 2007). The pH values of FSBM were measured using a pH meter. Amino acid concentrations in feedstuffs were measured using an automatic amino acid analyzer (Biochrom, UK).

2.2. The suckling and newly-weaned piglet trial

Twenty-five litters of 7-day-old suckling piglets were selected and divided into 5 groups with 5 litters for each group according to their body weight and sexuality (half male and half female). The piglets were fed 5 kinds of creep diets during suckling period, and weaned at 28 d of age. After weaning, the piglets were continually fed creep diets for 10 d. To reduce the influence from maternal effects, 25 milking sows with almost same breed, body weight, age, farrowing times, litter size and litter weight were selected. Five creep diets in Table 2 were designed as follows:

1) The basal diet containing 10% SBM and 6% plasma protein;
2) The basal diet containing 10% FSBM and 6% plasma protein;
3) The basal diet containing 10% FSBM and 4% plasma protein;
4) The basal diet containing 10% FSBM and 2% plasma protein;
5) The basal diet containing 10% FSBM and 0 plasma protein.

2.3. The elder piglet trial

Two hundred and fifty 35-day-old pigs were assigned to 5 groups with 5 replications for each group and 10 piglets for each replication. The experimental period was 30 d. Five diets in Table 3 were designed as follows:

1) The basal diet as control group;
2) The basal diet with 3.75% FSBM substitute for SBM and wheat bran;
3) The basal diet with 7.50% FSBM substitute for SBM and wheat bran;
4) The basal diet with 3.75% SBPC substitute for SBM and wheat bran;
5) The basal diet with 7.50% SBPC substitute for SBM and wheat bran.

In both experiments, the feed and water were given to piglets *ad libitum*. The piglets were weighed at initial and terminal experiment after they were fasted for 12 h. The diarrhea rates and mortality were recorded daily. Diarrhea rates were calculated as follows:

$$\text{Diarrhea rates}(\%) = 100 \times [(\text{Diarrhea frequency}/\text{Total number of pigs in each group})/\text{Experimental days}].$$

Feed intake in each group was calculated and recorded once a week. The temperature and relative humidity in shed were maintained at 25 ± 2 °C and 65%–70%, respectively. The temperature in the nursery box was set at 33 ± 2 °C for the first week, 30 ± 2 °C for the second week, and then kept at 25 ± 2 °C. The suckling piglets were given creep feed from the age of 7 d. The feeding management and immunization program were conducted according to the general requirements of the pig farm.

2.4. Determination of nutrient digestibility

Before the end of feeding experiment for the elder piglets, 5 piglets in each group were selected, and each piglet was separately put in a metabolic cage. The fresh feces were collected 3 times daily without contamination for each piglet for 3 d (30% of the feces were

Table 1
The experimental design for fermented soybean meal (FSBM) (vol/wt, %).[1]

Groups	*Bacillus subtilis*	*Hansenula anomala*	*Lactobacillus casei*
FSBM-1	0.5	0.5	0.5
FSBM-2	0.5	1.0	1.0
FSBM-3	0.5	1.5	1.5
FSBM-4	1.0	0.5	1.0
FSBM-5	1.0	1.0	1.5
FSBM-6	1.0	1.5	0.5
FSBM-7	1.5	0.5	1.5
FSBM-8	1.5	1.0	0.5
FSBM-9	1.5	1.5	1.0
FSBM-10	1.0	0.0	0.0
FSBM-11	0.0	1.0	0.0
FSBM-12	0.0	0.0	1.0
Control	0.0	0.0	0.0

[1] The addition of microbes during soybean meal fermentation was adjusted to 4% (vol/wt) with distilled water from group 1 to 12, and 4% distilled water was added in the control group. The visible counts for each microbe were 1×10^9 colony-forming unit (cfu)/mL.

Table 2
Diet compositions and nutrient levels for suckling and newly-weaned piglets.

Item	Groups				
	SBM + 6% PP	FSBM + 6% PP	FSBM + 4% PP	FSBM + 2% PP	FSBM + 0 PP
Ingredients, %					
Baked corn meal[1]	10.00	10.00	10.00	10.00	10.00
Corn meal	43.27	44.69	45.28	47.65	49.17
Soybean meal	10.00	0.00	0.00	0.00	0.00
Fermented soybean meal	0.00	10.00	10.00	10.00	10.00
Baked soybean meal[1]	10.00	8.71	10.00	10.00	10.00
Whey powder	10.00	10.00	10.00	10.00	10.00
Plasma protein powder	6.00	6.00	4.00	2.00	0.00
Fish meal	5.00	5.00	5.00	5.00	5.00
Soybean oil	2.00	2.00	2.00	2.00	2.00
Limestone	1.57	1.62	1.52	1.07	1.49
Dicalcium phosphate	0.81	0.63	0.85	0.93	0.99
NaCl	0.35	0.35	0.35	0.35	0.35
Premix[2]	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Nutrient composition, %					
Digestive energy, MJ/kg	15.06	15.06	15.06	15.06	15.06
Crude protein	21.11	21.05	21.09	21.10	21.08
Ash	5.70	5.72	5.71	5.75	5.77
Crude fat	6.51	6.62	6.60	6.55	6.57
Crude fiber	2.21	2.19	2.16	2.20	2.23
Calcium	1.05	1.06	1.07	1.05	1.08
Phosphorus	0.60	0.62	0.61	0.63	0.60
Lysine	1.44	1.44	1.43	1.42	1.42
Methionine	0.35	0.35	0.35	0.34	0.34

SBM = soybean meal; FSBM = fermented soybean meal; PP = plasma protein.
[1] They were treated using microwave at 60 kW and 120 °C for 4 min and then crashed.
[2] Premix provided the following amounts per kilogram complete diet: vitamin A 13,500 IU, vitamin D 2,150 IU, vitamin E 15 IU, vitamin K 3 mg, vitamin B_{12} 0.024 mg, vitamin B_1 1.8 mg, vitamin B_2 6 mg, niacin 24 mg, D-pantothenic acid 15 mg, folic acid 0.3 mg, biotin 4.5 mg, choline 5,000 mg, Cu 100 mg, Zn 100 mg, Fe 100 mg, Mn 100 mg, I 0.3 mg, Se 0.3 mg.

Table 3
Diet compositions and nutrient levels for weaned piglets.

Item	Groups				
	Control	3.75% FSBM	7.5% FSBM	3.75% SBPC	7.5% SBPC
Ingredients, %					
Corn meal	64.65	64.65	64.65	64.65	64.65
Wheat bran	10.00	10.00	10.00	10.00	10.00
SBM	18.63	14.50	10.18	12.56	6.53
FSBM	0.00	3.75	7.50	0.00	0.00
Soybean protein concentrate	0.00	0.00	0.00	3.75	7.50
Baked soybean meal[1]	2.50	2.50	2.50	2.50	2.50
Wheat meal	0.23	0.88	1.47	2.82	5.10
Soybean oil	0.90	0.63	0.63	0.63	0.63
Limestone	0.90	0.91	0.92	0.91	0.94
Dicalcium phosphate	0.84	0.83	0.80	0.83	0.80
NaCl	0.35	0.35	0.35	0.35	0.35
Premix[2]	1.00	1.00	1.00	1.00	1.00
Nutrient composition, %					
Digestive energy, MJ/kg	13.80	13.81	13.82	13.81	13.83
Crude protein	17.65	17.67	17.63	17.65	17.65
Ash	2.90	2.85	2.90	2.85	2.85
Crude fat	1.45	1.46	1.45	1.47	1.46
Fiber	0.85	0.86	0.86	0.85	0.87
Calcium	0.81	0.82	0.80	0.83	0.81
Phosphorus	0.60	0.59	0.59	0.60	0.59
Lysine	0.83	0.82	0.82	0.82	0.82
Methionine	0.25	0.25	0.25	0.25	0.25

FSBM = fermented soybean meal; SBPC = soybean protein concentrate.
[1] It was treated using microwave at 60 kW and 120 °C for 4 min and then crashed.
[2] Premix provided the following amounts per kilogram complete diet: vitamin A 10,000 IU, vitamin D_3 1,500 IU, vitamin E 60 IU, vitamin K 4.5 mg, vitamin B_{12} 0.028 mg, vitamin B_1 5 mg, vitamin B_6 1.7 mg, niacin 35 mg, D-pantothenic acid 20 mg, folic acid 0.85 mg, biotin 0.47 mg, choline 500 mg, Cu 150 mg, Zn 140 mg, Fe 130 mg, Mn 30 mg, I 0.35 mg, Se 0.25 mg.

collected each time). The fecal samples of each piglet were dried, ground and mixed to determine the concentrations of nutrients and 4 mol/L hydrochloric acid (HCl) insoluble ashes. Crude protein (CP), crude fat (CF), calcium (Ca), and phosphorus (P) in diets and feces were determined with Kjeldahl, ether extract, potassium permanganate ($KMnO_4$) and ammonium molybdate ($(NH_4)_6Mo_7O_{24}$) protocols, respectively (Jurgens, 1997). The nutrient digestibility was determined by using the endogenous indicator (4 mol/L HCl insoluble

ashes) protocol (Jurgens, 1997). The calculating form was made as follows: Nutrient apparent digestibility = 100 − [100 × (Indicator content in diets/Indicator content in feces × Nutrient content in feces/Nutrient content in diets)].

2.5. Determining the counts of E. coli and lactic acid bacteria in pig feces

Five grams of fresh feces from each of 5 elder piglets in each group were collected sterilely, diluted 10^5 to 10^9 folds with 0.9% physiological saline for *E. coli* and with anaerobic solution for lactic acid bacteria (Shapton and Board, 1972), and then vortexed completely. The mixtures (0.1–0.2 mL) were dispensed onto the plates with eosin methylene blue agar for determining *E. coli* counts or into anaerobic roll tubes with MRS agar for determining lactic acid bacteria counts. The bacteria were incubated for 2 d at 37 °C, and then the cfu/g was used to express bacterial counts calculated as logarithm (lg) value.

2.6. Determination of enzyme activity and plasma immunoglobulin

Five grams of feces were mixed with 45 mL 0.9% physiological saline (NaCl) in a 250−mL conical flask, shaken at 250 × *g* for 30 min and then filtrated with 4 − fold gauze. The filtrate was centrifuged at 12,000 × *g* for 15 min. The supernatant was used for determining fecal enzyme activity. One enzyme unit is defined as the activity that hydrolyses 1 μmol substrate per min. Protease activity was measured by the modified method with azocasein as the substrate (Lynn and Clevette-Radford, 1984). Lipase activity was measured according to the former method (Erlanson-Albertsson et al., 1987). Amylase activity was determined by using a kit (No. 700) from Sigma Chemical Company.

For immunoglobulin (Ig) analyses, blood samples were collected in heparinized Vacutainer tubes by a puncture of the jugular vein of each piglet at the end of experiment. Plasma was obtained after centrifugation of blood samples at 2,000 × *g* for 10 min and stored at −20 °C until analysis. The concentrations of total IgG, IgA and IgM in the plasma were measured using the pig Ig ELISA quantification kit (Bethyl Laboratories).

2.7. Statistical analysis

Experimental data were expressed as the means and standard errors. The data were analyzed using the ANOVA procedures of Statistical Analysis Systems Institute, 1992. Duncan's test was used to compare treatment means. Differences were considered to be statistically significant at $P < 0.05$.

3. Results

3.1. Determination of optimal microbial proportion for SBM fermentation

Table 4 shows that the best microbial proportion of *B. subtilis*, *H. anomala* and *L. casei* was 2:1:2 in FSBM-4 group, in which there was the highest content of soybean peptide (14.36% vs. 2.16%) and the lowest content of trypsin inhibitor (9.75 vs. 35.44 mg/g, $P < 0.05$). The combined microbial fermentation was better than the individual microbial fermentation ($P < 0.05$). Fig. 1 indicates that the antigen proteins decreased after microbial fermentation.

3.2. Effect of FSBM on suckling and newly-weaned piglet growth

The 10% FSBM substitute for SBM under different levels of plasma protein addition had no significant effect on suckling piglet production ($P > 0.05$); however, the FSBM diet with 2% plasma protein could decrease diarrhea rate and mortality (Table 5). For the newly-weaned piglets, average daily gain (ADG) and the feed conversion rate (FCR) in the diets with 10% FSBM was better than that in the control group ($P < 0.05$), indicating that FSBM was better than plasma protein for improving piglet production.

Table 4
The pH values, soybean peptide and trypsin inhibitor contents of fermented soybean meal (FSBM).[1]

Groups	pH	Soybean peptides, %	Trypsin inhibitor, mg/g
FSBM-1	7.57 ± 0.42^{a}	11.42 ± 0.45^{d}	10.53 ± 0.36^{e,f}
FSBM-2	6.92 ± 0.11^{b}	12.32 ± 0.35^{c,d}	11.37 ± 0.56^{c,d,e}
FSBM-3	5.59 ± 0.27^{c}	7.06 ± 0.32^{e}	11.58 ± 0.42^{c,d}
FSBM-4	6.90 ± 0.07^{b}	14.36 ± 0.25^{a}	9.75 ± 0.64^{f}
FSBM-5	7.02 ± 0.19^{b}	11.66 ± 0.51^{d}	12.14 ± 0.12^{c}
FSBM-6	7.00 ± 0.24^{b}	12.22 ± 0.25^{c,d}	10.81 ± 0.32^{d,e}
FSBM-7	7.65 ± 0.29^{a}	12.82 ± 0.41^{b,c}	11.16 ± 0.36^{c,d,e}
FSBM-8	6.92 ± 0.14^{b}	13.92 ± 0.42^{a}	11.44 ± 0.64^{c,d,e}
FSBM-9	7.09 ± 0.14^{b}	14.26 ± 0.47^{a}	10.74 ± 0.36^{d,e,f}
FSBM-10	7.12 ± 0.17^{b}	7.01 ± 0.72^{e}	12.55 ± 1.34^{c}
FSBM-11	6.89 ± 0.67^{b}	4.83 ± 0.42^{f}	15.34 ± 1.42^{b}
FSBM-12	4.51 ± 0.47^{d}	4.26 ± 0.43^{f}	16.18 ± 1.62^{b}
SBM	6.90 ± 0.06^{b}	2.16 ± 0.21^{g}	35.44 ± 0.53^{a}

SBM = soybean meal.
$^{a–f}$ Within a column, means without a common superscript differ ($P < 0.05$).
[1] FSBM-1 to FSBM-12 are designed in Table 1. Data represent means ± SE of 5 replicates.

3.3. Effect of FSBM on elder piglet production performance

Table 6 shows that the elder piglets fed the diet containing FSBM and SBPC had lower FCR ($P < 0.05$) and diarrhea rates than the piglets fed SBM; however, ADG among the groups was insignificant. Table 7 indicates that the addition of FSBM and SBPC in piglet diet could significantly increase nutrient digestibility, fecal enzyme activity and *Lactobacillus* counts, decrease fecal *E. coli* counts compared with the other groups ($P < 0.05$), but there was no significant effect on plasma immunoglobulin ($P > 0.05$).

4. Discussion

Soybean meal is the main protein resource for animal production; however, some anti-nutritional factors such as trypsin inhibitor and antigen protein reduce its nutritional value and inhibit animal production. Plasma protein and SBPC are better protein resources, but they are expensive. How to increase SBM nutritional value by microbial fermentation for replacing the expensive protein resources is very important for animal production.

To increase FSBM nutritive value, many species of microbes such as *Aspergillus oryzae* (Kim et al., 2007), lactic acid bacteria (Cho et al., 2007) and *B. subtilis* (Kiers et al., 2003) have been used independently. It was reported that FSBM with *B. subtilis* can be used as a highly digestible protein source in piglet diets due to the extensive hydrolysis of protein to amino acids and peptides (Yoonyi et al., 2012); *Lactobacillus* can improve intestinal function, promote nutrient digestion and absorption, and regulate immune function (Vanbelle et al., 1990). Yeast can transfer the soybean protein to high-quality microbial protein (Gao et al., 2007). Therefore, the above 3 species of microbes are selected in this study. This result showed that SBM fermented with the optimal microbial proportion could significantly decrease trypsin inhibitor and antigen protein contents, and increase soybean peptide contents by 6 folds, which was better than the previous reports with the individual microbial fermentation (Mital and Garg, 1990; Hachmeister and Fung, 1993). The reason may be due to the

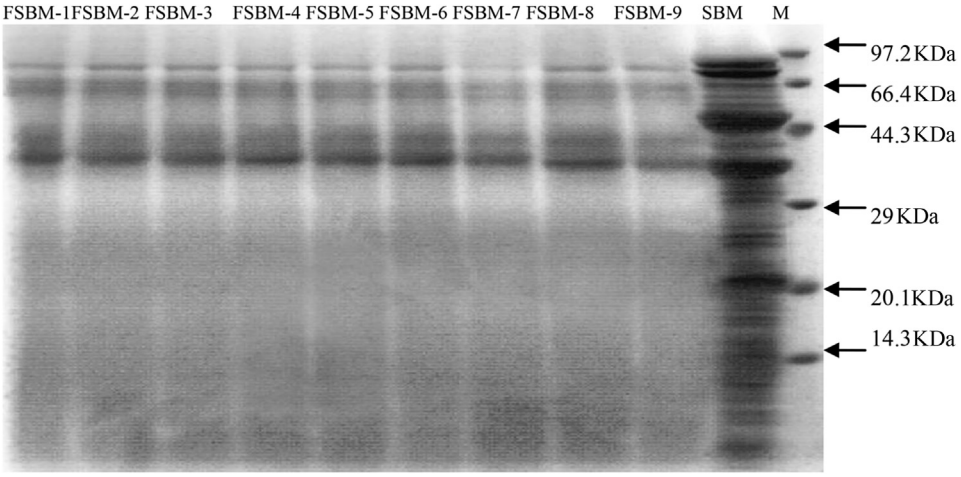

Fig. 1. Sodium dodecyl sulfate (SDS) – polyacrylamide gel electrophoresis (PAGE) map of fermented soybean meal (FSBM) and soybean meal (SBM). M = protein marker; FSBM-1 to FSBM-9 are designed in Table 1.

Table 5
Effects of fermented soybean meal (FSBM) on production performance of suckling and newly-weaned piglets.[1]

Item	Groups				
	SBM + 6% PP	FSBM + 6% PP	FSBM + 4% PP	FSBM + 2% PP	FSBM + 0 PP
Suckling piglets (d 7–28)					
Initial weight, kg	2.93 ± 0.68	2.80 ± 0.79	2.83 ± 0.34	2.95 ± 0.49	3.05 ± 0.26
Final weight, kg	8.30 ± 1.31	7.88 ± 1.01	8.13 ± 1.30	8.18 ± 1.03	7.90 ± 0.96
Average daily gain, g	383.57 ± 80.52	362.86 ± 77.09	378.57 ± 68.32	373.57 ± 9.53	346.43 ± 15.52
Daily feed intake, g	5.87	4.55	4.80	4.39	4.22
Diarrhea rate, %	4.64	3.21	2.86	1.61	2.32
Mortality, %	15.00	15.00	12.50	2.50	7.50
Newly-weaned piglets (d 28–38)					
Initial weight, kg	8.30 ± 1.31	7.88 ± 1.01	8.13 ± 1.30	8.18 ± 1.03	7.90 ± 0.96
Final weight, kg	10.14 ± 1.44	10.31 ± 1.60	10.74 ± 1.05	10.71 ± 1.23	10.31 ± 1.46
Average daily gain, g	184.33 ± 30.16^{b}	242.69 ± 35.72^{a}	261.47 ± 41.59^{a}	252.75 ± 40.64^{a}	240.93 ± 24.44^{a}
Daily feed intake, g	291.24 ± 30.52^{b}	337.34 ± 35.32^{a,b}	376.52 ± 38.16^{a}	369.02 ± 37.75^{a}	342.12 ± 33.19^{a}
Feed conversion rates	1.58 ± 0.11^{a}	1.39 ± 0.12^{b}	1.44 ± 0.06^{a,b}	1.46 ± 0.08^{a,b}	1.42 ± 0.06^{a,b}
Diarrhea rate, %	7.86	5.24	4.12	4.04	4.89
Mortality, %	0.00	0.00	0.00	0.00	0.00

SBPC = soybean protein concentrate.
$^{a-b}$ Within a row, means without a common superscript differ ($P < 0.05$).
[1] Data represent means ± SE of 5 replicates.

Table 6
Effects of fermented soybean meal (FSBM) on growth performance of elder piglets.[1]

Item	Groups				
	Control	3.75% FSBM	7.5% FSBM	3.75% SBPC	7.5% SBPC
Initial weight, kg	11.16 ± 1.88	10.35 ± 1.87	10.37 ± 1.49	10.61 ± 1.67	10.94 ± 1.83
Final weight, kg	23.50 ± 2.31	22.40 ± 1.97	23.06 ± 2.58	22.63 ± 2.57	23.66 ± 2.50
Average daily gain, g	404.51 ± 18.29	401.76 ± 22.97	423.18 ± 20.36	400.75 ± 22.41	424.38 ± 22.77
Daily feed intake, g	902.06	863.78	863.29	833.56	886.95
Feed conversion rates	2.23 ± 0.04^{a}	2.15 ± 0.05^{a,b}	2.04 ± 0.04^{c}	2.08 ± 0.06^{b,c}	2.09 ± 0.03^{b,c}
Diarrhea rates, %	0.53	0.33	0.13	0.40	0.20

SBPC = soybean protein concentrate.
$^{a-c}$ Within a row, means without a common superscript differ ($P < 0.05$).
[1] Data represent means ± SE of 5 replicates.

combined microbial cooperation during fermentation. It also showed that all the essential amino acids and protein contents in FSBM were higher than those in SBM. The main reasons are the lower moisture, reciprocal translocation of nutrients and organic matter loss by microbial fermentation.

The present experiment showed that FSBM can replace plasma protein in piglet diets for improving ADG and FCR, and decease diarrhea rate and mortality. Previous research has shown that pigs fed a diet containing FSBM had a greater ADG and FCR than the control group (Zamora and Veum, 1979; Kim et al., 2005; Jones et al., 2010). These growth-promoting effects may be due to the improvement of nutrition value of FSBM (Feng et al., 2007) and the elimination of anti-nutritive factors after fermentation (Hong et al., 2004). Why is FSBM able to decrease diarrhea rate and mortality for suckling piglets? The reasons may be the beneficial microbes existing in FSBM to inhibit the pathogenic microbial proliferation

Table 7
Nutrient digestibility, fecal enzyme activity, microbial flora and plasma immunoglobulin of elder piglets.[1]

Item	Groups				
	Control	3.75% FSBM	7.5% FSBM	3.75% SBPC	7.5% SBPC
Crude protein, %	77.43 ± 0.37^{d}	85.39 ± 1.37^{a,b}	87.44 ± 2.05^{a}	80.27 ± 2.10^{c,d}	82.77 ± 1.31^{b,c}
Crude fat, %	48.45 ± 0.64^{d}	60.06 ± 2.86^{bc}	71.08 ± 4.48^{a}	53.99 ± 7.40^{c,d}	62.14 ± 1.97^{b}
Calcium, %	69.89 ± 3.68^{c}	77.01 ± 1.71^{a,b}	82.2 ± 1.17^{a}	74.27 ± 5.01^{b,c}	70.75 ± 2.88^{c}
Phosphorus, %	57.76 ± 5.16^{b}	55.95 ± 2.63^{b}	65.75 ± 5.09^{a}	66.78 ± 3.53^{a}	70.87 ± 3.48^{a}
Protease, U/g	77.43 ± 0.37^{d}	85.39 ± 1.37^{a,b}	87.44 ± 2.05^{a}	80.27 ± 2.1^{c,d}	82.77 ± 1.31^{b,c}
Amylase, U/g	48.45 ± 0.64^{d}	60.06 ± 2.86^{b,c}	71.08 ± 4.48^{a}	53.99 ± 7.40^{c,d}	62.14 ± 1.97^{b}
Lipase, U/g	69.89 ± 3.68^{c}	77.01 ± 1.71^{a,b}	82.20 ± 1.17^{a}	74.27 ± 5.01^{b,c}	70.75 ± 2.88^{c}
Lactic acid bacteria, cfu/g	8.51 ± 0.23^{b}	9.38 ± 0.15^{a}	9.26 ± 0.13^{a}	9.28 ± 0.07^{a}	9.27 ± 0.10^{a}
E. coli, cfu/g	7.42 ± 0.18^{a}	6.47 ± 0.27^{b}	6.45 ± 0.11^{b}	7.41 ± 0.28^{a}	7.19 ± 0.43^{a,b}
IgG, g/L	3.22 ± 1.05	2.08 ± 0.22	2.09 ± 0.15	2.86 ± 1.08	2.74 ± 0.82
IgA, g/L × 10^{-1}	0.17 ± 0.06	0.17 ± 0.06	0.23 ± 0.06	0.17 ± 0.06	0.20 ± 0.00
IgM, g/L	1.01 ± 0.12	0.94 ± 0.38	0.88 ± 0.24	1.038 ± 0.35	0.86 ± 0.16

FSBM = fermented soybean meal; SBPC = soybean protein concentrate.
[a–d] Within a row, means without a common superscript differ ($P < 0.05$).
[1] Data represent means ± SE of 5 replicates.

(Yin et al., 2012) as well as the reduction or removal of antigenic proteins by microbial fermentation (Zhao et al., 2008).

Even though FSBM and SBPC have no significant effect on elder piglet's production and plasma immunoglobulin, they are able to improve nutrient availability. The significant promoting effect of FSBM on nutrient digestibility in the piglets corroborated the previous observation with growing pigs (Zamora and Veum, 1979; Sarkar and Tamang, 1995). The improvement of nutrient utilization may be caused by the productions of enzymes, soybean peptides, beneficial microbes and other unknown products during microbial fermentation of SBM. The high fecal enzyme activities and lactic acid bacteria counts and low fecal *E. coli* counts by feeding FSBM proved the above deduction.

In conclusion, the optimal microbial proportion of *Bacillus subtilis*, *H. anomala* and *L. casei* at 2:1:2 can increase soybean peptide content and nutritive value of SBM, and decrease anti-nutritional factor content in SBM. The feeding experiments of suckling and weaned piglets showed that FSBM could replace plasma protein and SBPC in diets for piglet production and economical profit.

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