TÜBİTAK

Research Article

Turk. J. Vet. Anim. Sci.
2012; 36(2): 143-151
© TÜBİTAK
doi:10.3906/vet-1012-652

Effect of fermented protein feedstuffs on pig production performance, nutrient digestibility, and fecal microbes

Juan CHANG[1], Qingqiang YIN[1,2,*], Pengpeng WANG[1], Weimin WANG[1], Ruiyu ZUO[1], Qiuhong ZHENG[1], Junxi LIU[2]

[1]College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, CHINA

[2]Henan Engineering and Technology Research Center of Feed Microbes, Zhoukou, 466000, CHINA

Received: 01.01.2011

Abstract: In order to determine the availability of fermented protein feedstuffs (FPFS)—such as cottonseed, blood, and feather meals—with Aspergillus oryzae, 2 phases of feeding experiments were adopted for 7 weeks. Sixty 58-day-old crossbred pigs were assigned to 6 groups, 10 pigs per group. Group 1 was the control; groups 2, 3, and 4 were supplemented with 6%-7%, 12%-14%, and 18%-21% FPFS, respectively, by replacing the same percentage of soybean meal (SBM); group 5 was supplemented with 12%-14% unfermented protein feedstuffs (UFPFS) by replacing the same percentage of SBM; group 6 was on the same base as group 3 with digestive energy (DE) balanced as in group 1. The results showed: (1) The soluble amino acids in FPFS were increased by 211% (58.06 vs. 18.68 g/kg), compared with UFPFS; (2) the average daily gain (ADG) increased ($P < 0.05$) in group 2, compared with the other groups; ADG in group 3 was higher than that in group 5 ($P < 0.05$); and (3) nutrient digestibility in the groups supplemented with FPFS was higher than in the group supplemented with UFPFS ($P < 0.05$). The results suggest that a 6%-7% FPFS supplementation to replace SBM in pig diets would be ideal and economic.

Key words: Pig, Aspergillus oryzae, protein feedstuffs, fermentation, production performance

Introduction

Soybean meal (SBM) is the most widely used protein source in the formulation of pig diets. Because its price is higher than the lower quality protein resources such as cottonseed meal (CSM), blood meal, and feather meal, understanding how to use low-quality protein resources becomes very important in reducing feed costs.

Cottonseed meal has long been considered a potential source of protein with high protein content for animals; however, its application is limited due to the presence of the toxic gossypol, low lysine levels, and high fiber content (1). A number of methods have been developed for removing gossypol from cottonseed, and microbial fermentation should be one promising detoxification method (2-4). The other lower quality protein resources such as feather and blood meals also have high protein content; however, they have limited application because of their poor nutrient digestibility and variability (5,6). It is feasible that both nutrient digestibility and amino acid balance may be improved by microbial action (7).

* E-mail: QQZ22@yahoo.com.cn

Fermentation is a unique process with great potential for recycling certain low-quality feed stuffs into useful animal feeds. Fermentation with *Aspergillus oryzae* (*A. oryzae*) could decrease gossypol in CSM (3) and enhance the small-size peptide content in SBM (8). In addition, *A. oryzae* has the capacity to produce enzymes such as amylase, cellulose, and protease, which can hydrolyze soybean constituents and contribute to the development of a desirable texture, flavor, and aroma in the product (9). Research has shown that SBM fermented by *A. oryzae* could significantly improve growth performance, feed utilization, and intestinal enzyme activities in broilers (10). CSM fermented by *Aspergillus niger* increased the levels and digestibility of methionine (Met), lysine (Lys), and threonine (Thr) (3). The fermented soybean has also been shown to inhibit *Escherichia coli* (*E. coli*) infection and shorten the duration of diarrhea in piglets (11). There were also some reports regarding fermented blood and feather meals (7,12), but there are fewer studies on fermentation of plant and animal protein feedstuffs.

In the present study the fermentation of CSM, blood, and feather meals by *A. oryzae* was studied. The effect of the partial replacement of SBM with the fermented protein feedstuffs on pig production performance, nutrient digestibility, and fecal microbes was determined in order to verify whether it can be used as a new kind of protein feed resource for animals.

Material and methods

Incubation of *A. oryzae*

The *A. oryzae* used in this study was kept in our laboratory, isolated from a cow rumen, and identified by 26 S rDNA. The *A. oryzae* was incubated at 30 °C for 3 days in potato dextrose agar (PDA) medium (0.6% soluble starch, 0.2% yeast extract, 0.5% peptone, 2% dextrose, 2% agar, 0.2% KH_2PO_4, 0.03% $MgSO_4 \cdot 7H_2O$). Spore suspension was obtained by scraping off the spores from pure cultures and suspending them in sterile 0.9% physiological saline. Spore counts were determined to be approximately 1×10^9 cfu/mL.

Preparation of fermented protein feedstuffs (FPFS)

The protein feedstuffs consisted of 60.0% CSM, 12.5% blood meal, 12.5% feather meal, 10.0% wheat bran, and 5.0% corn. It was divided into 2 parts; 1 part was not fermented as the control, and 1 part was fermented by *A. oryzae*. The fermentation was carried out under the following conditions: 4% (v/w) spore solution, 40% moisture, pH 6, and incubation for 48 h at 30 ° . The fermented sample was dried at 50-60 °C up to 90% dry matter, and then ground.

Chemical analysis

The samples for chemical determination were prepared by putting 5 g of unfermented protein feedstuffs (UFPFS) or FPFS in 20 mL of 0.9% physiological saline (w/v) and stirring for 30 min. The samples were then centrifuged for 5 min at 13,000 × *g*, and the supernatants were kept for the following determinations. The soluble amino acids from the supernatants and the total amino acids from the original UFPFS or FPFS without suspension were determined by 835-50 High-Speed Amino Acid Analyzer (Hitachi, Japan), using the protocol described by Sarkar et al. (13). The soluble amino acid concentrations in the supernatants were adjusted to the concentrations in dry FPFS. The amylase activity was assayed by the method of Yoo et al. (14): 1 amylase unit (U) was defined as the amount of enzyme catalyzing the conversation of 1 mg of starch in 5 min under assay conditions. The protease activity was determined by the method of Sandhya et al. (15): 1 unit of protease activity was defined as the amount of enzyme that liberated 1 μg of tyrosine per minute. Cellulase activity was measured by using carboxymethycellulose (CMC) as the substrate, and 1 unit of enzyme activity was defined as 1 μmol of glucose production per hour (16).

The experimental design, animals, diets, and feeding programs

Sixty 58-day-old crossbred pigs [(Landrace × Yorkshire) × Duroc] with an average initial body weight (BW) of 17.53 ± 0.86 kg were used in this trial. Pigs were assigned to 6 groups according to their BW and sex, 10 pigs per group, in 1 pen (5 males and 5 females). Every pig had its own identification code for statistical analysis of average daily gain (ADG).

A 2-period feeding program was adopted in this experiment. After nursing, the pigs were fed with the phase 1 diet for 3 weeks, followed by the phase 2 diet for 4 weeks. The diets were mash feed, and the experiment was designed as follows:

Group 1: Basal diet.

Group 2: Basal diet [7% (w/w) SBM removed] + 7% (w/w) FPFS in phase 1.

Basal diet [6% (w/w) SBM removed] + 6% (w/w) FPFS in phase 2.

Group 3: Basal diet [14% (w/w) SBM removed] + 14% (w/w) FPFS in phase 1.

Basal diet [12% (w/w) SBM removed] + 12% (w/w) FPFS in phase 2.

Group 4: Basal diet [21% (w/w) SBM removed] + 21% (w/w) FPFS in phase 1.

Basal diet [18% (w/w) SBM removed] + 18% (w/w) FPFS in phase 2.

Group 5: Basal diet [14% (w/w) SBM removed] + 14% (w/w) UFPFS in phase 1.

Basal diet [12% (w/w) SBM removed] + 12% (w/w) UFPFS in phase 2.

Group 6: Basal diet [14% (w/w) SBM removed] + 14% (w/w) FPFS in phase 1; digestive energy (DE) was balanced with soybean oil, as in group 1.

Basal diet [12% (w/w) SBM removed] + 12% (w/w) FPFS in phase 2; DE was balanced with soybean oil, as in group 1.

The composition and nutrient levels of the diets used in the 2 consecutive experiments (Tables 1 and 2) were prepared according to the recommended

Table 1. Feed compositions (%) and nutrient levels (%) of the experimental diets in phase 1.

Groups	1	2	3	4	5	6
Feed compositions						
Corn	64.40	64.35	64.30	64.26	64.30	61.31
Soybean meal	26.00	19.00	12.00	5.00	12.00	12.50
FPFS	0.00	7.00	14.00	21.00	0.00	14.00
NFPFS	0.00	0.00	0.00	0.00	14.00	0.00
Wheat bran	6.00	6.00	6.00	6.00	6.00	6.00
Soybean oil	0.00	0.00	0.00	0.00	0.00	2.50
Calcium carbonate	1.25	1.25	1.25	1.25	1.25	1.25
Dicalcium phosphate	0.90	0.90	0.90	0.90	0.90	0.90
Salt	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine·HCl	0.15	0.20	0.25	0.29	0.25	0.24
Premix compound	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient levels and energy						
Crude protein	17.91	17.91	17.92	17.92	17.92	17.86
Ca	0.76	0.76	0.77	0.77	0.77	0.76
Total P	0.53	0.53	0.55	0.55	0.55	0.53
Available P	0.29	0.29	0.30	0.30	0.30	0.29
Lysine	0.93	0.93	0.93	0.93	0.93	0.93
DE (MJ/kg)	13.40	13.13	12.86	12.58	12.86	13.41
Price (Chinese Yuan/kg)	2.24	2.22	2.16	2.10	2.10	2.36

Note: vitamin and mineral premix provided (per kilogram of diet): 150 mg, Fe (ferrous sulfate); 130 mg, Zn (zinc oxide); 50 mg, Mn (manganese oxide); 15 mg, Cu (copper sulfate); 0.9 mg, I (potassium iodate); 0.3 mg, Se (sodium selenite); 11,000 IU, vitamin A; 1,100 IU, vitamin D_3; 100 IU, vitamin E; 3.5 mg, vitamin K; 15 mg, niacin; 10 mg of pantothenic acid; 3.50 mg, riboflavin; 0.025 mg, vitamin B_{12}; 0.35 mg, biotin; 0.3 mg, folacin; 20 mg, pyridoxine; 6 mg, thiamine; and 300 mg, choline. DE was calculated, and other nutrients were measured.

Table 2. Feed compositions (%) and nutrient levels (%) of the experimental diets in phase 2.

Groups	1	2	3	4	5	6
Feed compositions						
Corn	64.83	64.79	64.75	64.71	64.75	62.06
Soybean meal	22.00	16.00	10.00	4.00	10.00	10.50
FPFS	0.00	6.00	12.00	18.00	0.00	12.00
NFPFS	0.00	0.00	0.00	0.00	12.00	0.00
Wheat bran	10.00	10.00	10.00	10.00	10.00	10.00
Soybean oil	0.00	0.00	0.00	0.00	0.00	2.20
Calcium carbonate	1.10	1.10	1.10	1.10	1.10	1.10
Dicalcium phosphate	0.70	0.70	0.70	0.70	0.70	0.70
Salt	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine·HCl	0.12	0.16	0.20	0.24	0.20	0.19
Premix compound	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient levels and energy						
Crude protein	16.77	16.77	16.78	16.78	16.78	16.74
Ca	0.65	0.65	0.66	0.66	0.66	0.65
Total P	0.51	0.51	0.53	0.53	0.53	0.53
Available P	0.25	0.25	0.26	0.26	0.26	0.26
Lysine	0.85	0.85	0.85	0.85	0.85	0.85
DE (MJ/kg)	13.21	12.97	12.74	12.50	12.74	13.23
Price (Chinese Yuan/kg)	2.15	2.10	2.06	2.01	2.01	2.21

Note: vitamin and mineral premix provided (per kilogram of diet): 150 mg, Fe (ferrous sulfate); 130 mg, Zn (zinc oxide); 50 mg, Mn (manganese oxide); 15 mg, Cu (copper sulfate); 0.9 mg, I (potassium iodate); 0.3 mg, Se (sodium selenite); 11,000 IU, vitamin A; 1,100 IU, vitamin D_3; 100 IU, vitamin E; 3.5 mg, vitamin K; 15 mg, niacin; 10 mg of pantothenic acid; 3.50 mg, riboflavin; 0.025 mg, vitamin B_{12}; 0.35 mg, biotin; 0.3 mg, folacin; 20 mg, pyridoxine; 6 mg, thiamine; and 300 mg, choline. DE was calculated, and other nutrients were measured.

standards (17). The pigs were weighed at the beginning, middle, and end of the experiment and fasted for 12 h before weighing. The experimental period was 49 days, and the pre-trial period was 7 days. Feed and water were given to the pigs ad libitum. The feed intake in each group was recorded once a week. The temperature in the shed was 25-35 °C during the trial.

Determination of nutrient digestibility

At the end of the feeding experiment fresh feces were collected, without contamination, from 5 pigs in each group for 3 days, 3 times daily (35% of the feces were collected each time). The feces samples from each pig during the 3 day collections were dried, ground, and mixed to determine the concentrations of nutrients and 4 N hydrochloric acid (HCl) insoluble ashes. Crude protein (CP), crude fat (CF), calcium (Ca), and phosphorus (P) in the diets and feces were determined by Kjeldahl, ether extract, potassium permanganate ($KMnO_4$), and ammonium molybdate [$(NH_4)_6Mo_7O_{24}$] protocols, respectively (18). The nutrient digestibilities were determined by using the endogenous indicator [4 N hydrochloric acid (HCl) insoluble ashes] protocol (18). The calculation was made as follows: nutrient apparent digestibility = 100 − (100 × indicator content in feed/indicator content in feces × nutrient content in feces/nutrient content in feed).

Determination of the number of *E. coli* and lactic acid bacteria in pig feces

Fresh feces (5 g) from each of the 5 pigs were collected sterilely, diluted 10^5-10^9 folds with 0.9% physiological saline for *E. coli*, with anaerobic solution for lactic acid bacteria (19), and then vortexed completely (300 rounds/min). The mixtures (0.2-0.3 mL) were dispensed onto the plates with eosin methylene blue agar for determining *E. coli* or into anaerobic roll tubes with MRS agar for determining lactic acid bacteria. The compositions of eosin methylene blue agar were (g/L): peptone, 10; lactose, 10; eosin, 0.4; methylene blue, 0.065; agar, 14; K_2HPO_4, 2; and pH, 7.2 ± 0.4. The compositions of MRS agar were (g/L): tryptone, 10; glucose, 20; beef peptone, 10; yeast extract, 5; agar, 20; Tween 80, 1 mL; K_2HPO_4, 2; sodium acetate, 5; sodium citrate, 2; $MgSO_4$, 0.2; $MnSO_4$, 0.05; and pH, 6.2-6.6. The bacteria were incubated for 2 days at 37 °C, and then the colonies were counted.

Statistical analysis

Experimental data were expressed as means and standard errors. The data were analyzed using the ANOVA procedures of the Statistical Analysis Systems Institute (SAS 6.0). Duncan's multiple range test was used to compare treatment means. Differences were considered statistically significant at $P < 0.05$.

Results

Chemical composition of FPFS

The soluble amino acid and total amino acid profiles of the UFPFS and FPFS were listed in Table 3. The soluble amino acids and total amino acids of FPFS were increased by 211% and 2.06%, compared with those of UFPFS. The activities of protease, amylase, and cellulase of the fermented protein feedstuffs were 1821.27, 2778.35, and 5562.43 U/g, respectively.

Table 3. Amino acid compositions of unfermented and fermented protein stuffs (g/kg, 90% dry matter).

Components	Soluble amino acids		Total amino acids	
	unfermented protein feedstuffs	fermented protein feedstuffs	unfermented protein feedstuffs	fermented protein feedstuffs
Aspartic acid	1.66	0.22	49.20	51.90
Glutamic acid	5.60	14.67	88.90	92.10
Serine	1.23	0.47	30.00	29.20
Arginine	0.10	0.07	47.70	48.60
Glycine	0.69	4.81	46.70	43.90
Threonine	0.80	0.10	21.10	22.00
Proline	0.44	4.93	39.50	38.40
Alanine	1.68	5.24	29.80	29.80
Valine	1.20	5.58	33.80	34.20
Methionine	0.23	1.36	4.90	5.70
Cystine	0.03	0.13	11.90	13.00
Isoleucine	0.80	3.49	21.20	22.60
Leucine	1.68	6.16	40.60	41.20
Phenylalanine	0.87	4.61	25.00	27.30
Histidine	0.47	3.08	15.80	15.50
Lysine	1.10	2.84	20.40	22.00
Tyrosine	0.11	0.31	19.40	20.00
Total amino acids	18.68	58.06	546.00	557.50

Effect of the FPFS on pig production

The effects of FPFS on pig production are presented in Table 4. In phase 1, ADG in the basal diet and the diet with 7% FPFS was higher than that in the other groups ($P < 0.05$). In phase 2, ADG in the diet with 6% FPFS was higher than in the other groups ($P < 0.05$). Overall, the ADG and economic benefits in group 2 were higher than in the basal diet and other groups ($P < 0.05$), while ADG in groups 3, 4, 5, and 6 was lower than in the control group ($P < 0.05$). ADG decreased with increasing levels of FPFS additions to pig diets. ADG in the diet with 12%-14% UFPFS was lower than in the other groups ($P < 0.05$). The diet of group 6 with DE balanced as in group 1 had no positive effect on ADG and nutrient digestibility. In addition, daily intake (DI) in group 2 was a little higher than that in group 1 ($P > 0.05$), and feed conversion (FC) in groups 1 and 2 was a little higher than in the other groups ($P > 0.05$).

Effect of the FPFS on nutrient digestibility

Table 5 showed that the digestibility of CP, Ca, and P in the groups supplemented with FPFS was higher than in the group supplemented with UFPFS ($P < 0.05$). It could be concluded that nutrient digestibility could be enhanced by the fermentation process.

The changes of microbes in feces affected by the FPFS

Table 6 indicated that the *E. coli* counts in group 2 decreased only insignificantly ($P > 0.05$), while the counts of lactic acid bacteria increased significantly ($P < 0.05$), compared with the control group. There were no significant differences between groups 2 and 3 in the number of *E. coli* and lactic acid bacteria.

Table 4. Production performances.

Groups	1	2	3	4	5	6
Phase 1 (3 weeks)						
ADG (g)	587.27 ± 40.76^{A}	567.73 ± 45.41^{A}	516.82 ± 53.37^{B}	490.45 ± 75.39^{BC}	473.64 ± 58.37^{C}	522.27 ± 39.00^{B}
DI (Kg)	1.28	1.29	1.27	1.25	1.25	1.25
FC	0.46	0.44	0.41	0.39	0.38	0.42
Cost*	4.69	4.77	5.06	5.12	5.30	5.29
Phase 2 (4 weeks)						
ADG (g)	682.26 ± 78.98^{B}	787.74 ± 49.68^{A}	660.97 ± 67.12^{BC}	647.74 ± 48.82^{BC}	606.45 ± 31.92^{D}	636.77 ± 61.05^{BC}
DI (Kg)	1.94	2.15	2.03	1.92	1.93	1.94
FC	0.35	0.37	0.33	0.34	0.31	0.33
Cost*	6.11	5.73	6.33	5.96	6.40	6.73
Overall (7 weeks)						
ADG (g)	642.76 ± 38.49^{B}	696.42 ± 20.25^{A}	601.13 ± 37.29^{C}	582.57 ± 68.02^{CD}	551.89 ± 26.95^{D}	589.25 ± 44.43^{CD}
DI (Kg)	1.66	1.79	1.71	1.64	1.65	1.66
FC	0.39	0.39	0.35	0.37	0.34	0.35
Cost*	5.55	5.39	5.86	5.65	5.99	6.18

Note: each value represents mean ± SE of 10 replicates per treatment. In the same row, significant differences at $P \leq 0.05$ levels are indicated by the different letters (A, B, C, D). Data followed by the same letter in the same row are not significantly different from each other ($P > 0.05$). *Cost: cost per unit of gain estimated in Chinese Yuan.

Table 5. The digestibilities of crude protein, crude fat, calcium, and phosphorus (%).

Groups	Crude protein	Crude fat	Calcium	Phosphorus
1	77.94 ± 1.23^{A}	80.57 ± 1.44^{A}	52.07 ± 1.49^{A}	44.45 ± 1.49^{B}
2	78.98 ± 1.59^{A}	80.65 ± 1.62^{A}	52.72 ± 1.52^{A}	46.71 ± 2.01^{B}
3	74.75 ± 1.32^{B}	78.75 ± 2.60^{A}	54.28 ± 2.68^{A}	49.46 ± 1.36^{A}
4	74.40 ± 2.56^{B}	79.89 ± 1.50^{A}	53.48 ± 1.75^{A}	51.46 ± 3.71^{A}
5	69.62 ± 1.46^{C}	71.72 ± 3.61^{B}	46.44 ± 1.89^{B}	40.97 ± 2.93^{C}
6	72.33 ± 2.35^{B}	76.33 ± 2.33^{A}	53.49 ± 1.01^{A}	47.71 ± 0.32^{B}

Note: each value represents mean ± SE of 5 replicates per treatment. In the same column, significant differences at $P \leq 0.05$ levels are indicated by the different letters (A, B, C). Data followed by the same letter in the same column are not significantly different from each other ($P > 0.05$).

Table 6. The counts of *E. coli* and lactic acid bacteria in feces.

Groups	*E. coli*	Lactic acid bacteria
	($\times 10^{6}$ cfu/g)	($\times 10^{9}$ cfu/g)
1	7.09 ± 0.12^{B}	7.68 ± 0.07^{BC}
2	6.87 ± 0.24^{B}	8.16 ± 0.38^{A}
3	7.23 ± 0.13^{B}	8.07 ± 0.44^{AB}
4	7.67 ± 0.27^{A}	7.29 ± 0.11^{D}
5	7.93 ± 0.06^{A}	7.56 ± 0.24^{CD}
6	7.70 ± 0.26^{A}	7.97 ± 0.22^{AB}

Note: each value represents mean ± SE of 5 replicates per treatment. In the same column, significant differences at $P \leq 0.05$ levels are indicated by the different letters (A, B, C, D). Data followed by the same letter in the same column are not significantly different from each other ($P > 0.05$).

Discussion

Chemical compositions of FPFS

This research showed that the fermentation process could increase soluble amino acid concentrations and slightly affect total amino acid contents. Similar results have been reported in fermented soybean meal by other investigators (13,20). The high concentrations of soluble amino acids may be due to the growth of microflora, which can secrete protease to aid in the digestion of protein feedstuffs for amino acid production. In addition, the microorganisms can convert substrates into microbial protein and other biological compounds.

Effect of the FPFS on pig production and nutrient digestibility

The results indicated that group 2, with 7% or 6% FPFS replacing SBM, had the best effect on improving pig production and economic benefits. It was suggested that the optimum addition of FPFS in pig diets was 6%-7%. Many researchers showed that fermented soybean meal improves pig production and feed conversion (21,22). There were also reports

that fermentation could increase the nutritional value of poultry feather and blood meals (2,7) and decrease the gossypol content of cottonseed meal (3). It was reported that fermented soybean meal with *A. oryzae* improved the apparent digestibility of dry matter and CP more effectively than unfermented soybean meal (23). This experiment also showed that protein feedstuffs fermented with *A. oryzae* were better able to increase nutrient digestibility than unfermented protein feedstuffs. This may be mostly due to the elimination of anti-nutritional factor and the degradation of large-size protein in FPFS (8).

The nutrient digestibilities in group 2 were even higher than in the basal diet, but this tendency was not found in groups 3 and 4. The reason may be that the protein feedstuffs contain cottonseed, feather, and blood meals, which have poor digestibility before fermentation. Fermentation could improve nutrient digestibility of cottonseed, feather, and blood meals to some extent; a small addition of FPFS to pig diets could have a positive effect on pig production and nutrient digestibility, while a large FPFS addition may have a negative effect. In conclusion, the reason FPFS has the ability to improve pig production may be the fermenting functions, which decrease anti-nutritional factors, produce enzymes, improve nutrient availability, and maintain gut microbial balance.

The changes of microbes in feces affected by FPFS

Lactobacillus and *Bifidobacterium* are considered the main beneficial microorganisms because of their potential to inhibit the growth of putrefactive and pathogenic bacteria (24). Pluske et al. reported that a healthy gut environment (e.g., low counts of enteropathogenic *E. coli*) could affect voluntary feed intake (25). In the current study, the diet of group 2 successfully increased the counts of *Lactobacillus* and decreased the counts of *E. coli* in pig fecal samples, which may have contributed to the positive effect on production performance of a 6%-7% FPFS replacement of soybean meal in pig diets.

In conclusion, FPFS with *A. oryzae* could increase soluble amino acid concentrations and multi-enzyme production. FPFS supplemented in pig diets could significantly improve the ADG and nutrient digestibilities, increase *Lactobacillus* counts, and decrease the *E. coli* counts in pig feces, compared with UFPFS. The optimum addition of FPFS in pig diets was 6%-7%. Results suggest that FPFS is a new kind of beneficial protein feed resource for pig production.

References

1. Sterling, K.G., Costa, E.F., Henry, M.H., Pesti, G.M., Bakalli, R.I.: Responses of broiler chickens to cottonseed- and soybean meal-based diets at several protein levels. J. Poult. Sci., 2001; 81: 217-226.
2. Rahma, E.H., Narasingo, R.M.S.: Gossypol removal and functional properties of protein produced by extraction of glanded cottonseed with different solvents. J. Food Sci., 1984; 49: 1057-1060.
3. Zhang, W.J., Xu, Z.R., Zhao, S.H., Sun, J.Y., Yang, X.: Development of microbial fermentation process for detoxification of gossypol in cottonseed meal. Anim. Feed Sci. Technol., 2007; 135: 176-186.
4. Nagalakshmi, D., Sastry, V.R.B., Agrawal, D.K.: Detoxification of undecorticated cottonseed meal by various physical and chemical methods. Anim. Nutr. Feed Technol., 2002; 2: 117-126.
5. Moughan, P.J., Dong, G.Z., Pearson, G., Wilkinson, B.H.P.: Protein quality in blood meal. II. The effect of processing on in vivo nitrogen digestibility in rats, protein solubility and FDNB-available lysine. J. Anim. Feed Sci. Technol., 1999; 79: 309-320.
6. Apple, J.K., Boger, C.B., Brown, D.C., Maxwell, C.V., Friesen, K.G., Roberts, W.J., Johnson, Z.B.: Effect of feather meal on live animal performance on carcass quality and composition of growing-finishing swine. J. Anim. Sci., 2003; 81: 172-181.
7. Bertsch, A., Coello, N.: A biotechnological process for treatment and recycling poultry feathers as a feed ingredient. J. Bioresource Technol., 2005; 96: 1703-1708.
8. Hong, K.J., Lee, C.H., Kim, S.W.: *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and soybean meal. J. Food Med., 2004; 7: 430-436.
9. Hachmeister, K.A., Fung, D.Y.: Tempeh: a mold-modified indigenous fermented food made from soybeans and/or cereal grains. Crit. Rev. Microbiol., 1993; 19: 137-188.

10. Feng, J., Liu, X., Xu, Z.R., Wang, Y.Z., Liu, J.X.: Effects of fermented soybean meal on digestive enzyme activities and intestinal morphology in broilers. J. Poult. Sci., 2007; 86: 1149-1154.

11. Kiers, J.L., Meijer, J.C., Nout, M.J.R., Rombouts, F.M., Nabuurs, M.J.A., van der Meulen, J.: Effect of fermented soya beans on diarrhoea and feed efficiency in weaned piglets. J. Appl. Microbiol., 2003; 95: 545-552.

12. Mncene, W.B., Tuitoek, J.K., Muiruri, H.K.: Nitrogen utilization and performance of pigs given diets containing a dried or undried fermented blood/molasses mixture. Feed Sci. Technol., 1999; 78: 239-247.

13. Sarkar, P.K., Jones, L.J., Craven, G.S., Somerset, S.M., Palmer, C.: Amino acid profiles of kinema, a soybean-fermented food. Food Chem., 1997; 59: 69-57.

14. Yoo, Y.G., Hong, G., Hatch, R.T.: Comparison of α-amylase activities from different assay methods. J. Biotechnol. Bioengineer, 1987; 1: 147-151.

15. Sandhya, C., Sumantha, A., Szakacs G., Pandey, A.: Comparative evaluation of neutral protease production by *Aspergillus oryzae* in submerged and solid-state fermentation. J. Process Biochemistry, 2005; 40: 2689-2694.

16. Shauna, M., McKelvey, R., Murphy, A.: Analysis of wide-domain transcriptional regulation in solid-state cultures of *Aspergillus oryzae.* J. Ind. Microbiol. Biotechnol., 2010; 37: 455-469.

17. National Research Council: Nutrient Requirement of Swine. 10th Ed. National Academy Press, Washington, DC. 1998; 1-30.

18. Jurgens, M.H.: Animal Feeding and Nutrition. Eighth Ed. Kendall/Hunt Publishing Company, Iowa. 1997; 63-66.

19. Shapton, D.A., Board, R.G.: Isolation of anaerobes. Academic Press Inc. Ltd., London. 1972; 25-76.

20. Regalado, G., Zamora, T., Veum, L.: Nutritive value of whole soybeans fermented with *Aspergillus oryzae* or *Rhizopus oligosporus* as evaluated by neonatal pigs. J. Nutr., 1988; 118: 438-444.

21. Kim, Y.G., Lohakare, J.D., Yun, J.H., Heo, S., Chae, B.J.: Effect of feeding levels of microbial fermented soy protein on the growth performance, nutrient digestibility and intestinal morphology in weaned piglets. Asian-Aust. J. Anim. Sci., 2007; 20: 399-404.

22. Sarkar, P.K., Tamang, J.P.: Changes in the microbial profile and proximate composition during natural and controlled fermentations of soybeans to produce kinema. Food Chem., 1995; 12: 317-325.

23. Feng, J., Liu, X., Xu, Z.R., Lu, Y.P., Liu, Y.Y.: The effects of *Aspergillus oryzae* fermented soybean meal on growth performance, digestibility of dietary components and activities of intestinal enzymes in weaned piglets. J. Anim. Feed Sci., 2006; 134: 295-303.

24. Paton, A.W., Morona, R., Paton, J.C.: Designer probiotics for prevention of enteric infections. Nat. Rev. Microbiol., 2006; 4: 193-200.

25. Pluske, J.R., Hampson, D.J., Williams, I.H.: Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest. Prod. Sci., 1997; 51: 215-236.