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Characteristics of Solid-state Fermented Feed and its Effects on Performance and Nutrient Digestibility in Growing-finishing Pigs

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ABSTRACT : This study investigated the effects of solid-state fermentation of a compound pig feed on its microbial and nutritional characteristics as well as on pig performance and nutrient digestibility. A mixed culture containing *Lactobacillus fermentum*, *Saccharomyces cerevisae* and *Bacillus subtilis* was used for solid-state fermentation and solid-state fermented feed samples were collected on days 0, 1, 2, 3, 5, 7, 10, 15, 20 and 30 for microbial counts and chemical analysis. Lactic acid bacteria increased rapidly during the first three days of fermentation and then slowly declined until day 10 and, thereafter, the counts were maintained at about 6.7 log cfu/g for the duration of the fermentation period. Enterobacteria also increased during the first two days, and then fell below the detectable level of the analysis (3.0 log cfu/g). The pH of the fermentation substrate declined from 6.1 at the start of fermentation to 5.7 by day 30. The water-soluble protein content increased from 8.2 to 9.2% while the concentration of acetic acid increased from 16.6 to 51.3 mmol/kg over the 30-day fermentation. At the end of the 30-day fermentation, the solid-state fermented feed was used in a pig feeding trial to determine its effects on performance and nutrient digestibility in growing-finishing pigs. Twenty crossbred barrows (14.11±0.77 kg BW) were allotted into two dietary treatments, which comprised a regular dry diet containing antibiotics and a solid-state fermented feed based diet, free of antibiotics. There was no difference due to diet on pig performance or nutrient digestibility. In conclusion, solid-state fermentation resulted in high counts of lactic acid bacteria and low counts of enterobacteria in the substrate. Moreover, feeding a diet containing solid-state fermented feed, free of antibiotics, can result in similar performance and nutrient digestibility in growing-finishing pigs to a regular diet with antibiotics. (**Key Words :** Solid-state Fermented Feed, Microflora, Nutrient Content, Growing-finishing Pigs)

INTRODUCTION

Fermentation is widely used to produce healthy foods for people and animals and interest in its application in animal feeding is increasing due to the total ban of antibiotic growth promoters in the European Union (Canibe et al., 2006). The application of fermentation technology in animal feeds can be categorized into that involving fermented raw materials and that involving fermented liquid feed.

Raw materials such as soybean and soybean meal (Hong et al., 2004; Cho et al., 2007; Kim et al., 2007), cottonseed meal (Zhang et al., 2006), barley (Canibe and Jensen, 2007), wheat (Canibe and Jensen, 2007) and farm by-products (Oboh and Akindahunsi, 2005; Ramli et al., 2005; Oduguwa et al., 2007) can be fermented, with the aim of eliminating anti-nutritional factors such as gossypol in cottonseed meal (Zhang et al., 2006) and trypsin inhibitor in soybean meal (Hong et al., 2004), improving nutrient digestibility (Cho et al., 2007; Kim et al., 2007) and enriching the quality of protein (Oduguwa et al., 2007). Then the fermentation end products can be incorporated into diets as feed ingredients.

Feeding fermented liquid feed to lactating sows has been reported to reduce the numbers of coliforms while increasing the counts of lactic acid bacteria in the feces of newborn piglets (Demecková et al., 2002). In addition, fermented liquid feed has been reported to reduce the counts of enterobacteriaceae along the entire gastrointestinal tract (van Winsen et al., 2001; Canibe and Jensen, 2003) and to increase lactic acid bacteria numbers in the stomach of growing pigs (van Winsen et al., 2001). The high concentrations of organic acids in the fermented liquid feed (Canibe and Jensen, 2003; Canibe et al., 2006) are believed to be responsible for the reduction in pathogens

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Table 1. Composition of substrate for fermentation

Item	%
Soybean meal	51.81
Corn gluten meal	22.02
Rapeseed meal	3.89
Peanut meal	3.89
Calcium hydrogen phosphate	5.18
Limestone	5.18
Salt	2.98
Choline chloride	0.13
Lysine-HCl	1.68
Mineral and vitamin premix[1]	3.24

[1] Supplied per kilogram of premix: Cu, 30,000 mg; Fe, 19,000 mg; Zn, 18,000 mg; Mn, 9,600 mg; I, 80 mg; Se, 70 mg; retinyl acetate, 800,000 IU; cholecalciferol, 240,000 IU; DL-α-tocopheryl acetate, 2,400 IU; menadione sodium bisulfite complex, 480 mg; thiamine, 150 mg; riboflavin, 480 mg; pyridoxine, 240 mg; vitamin B_{12}, 1.6 mg; nicotinic acid, 3,100 mg; D-Ca-pantothenate, 1,900 mg; folic acid, 100 mg; biotin, 6 mg; choline chloride, 40,000 mg.

(van Winsen et al., 2001).

There is little literature on the use of fermented compound feed manufactured according to solid-state fermentation. However, it may also have potential to be efficient in promoting animal health and growth. Therefore, the present study was conducted to investigate the nutritional and microbial characteristics of solid-state fermented feed during the fermentation process and to determine the effects of solid-state fermented feed on performance and nutrient digestibility in growing-finishing pigs when fed in the absence of antibiotics.

MATERIALS AND METHODS

Manufacture of solid-state fermented feed

A mixed liquid bacterial culture, containing *Lactobacillus fermentum* (CGMCC No. 0843), *Saccharomyces cerevisae* (CGMCC No. 2.1793) and *Bacillus subtilis* (CGMCC No. 7.5), was prepared with 5.03 log cfu/ml of lactic acid bacteria, 5.74 log cfu/ml of yeast and 5.45 log cfu/ml of bacillus. The raw material used as the substrate for the fermentation was a mixed compound feed formulated as shown in Table 1. The substrate was mixed with the liquid culture in a ratio of about 10:3 (w/v). The mixture was then packaged in multi-layer, polythene bags with a capacity of 25 kg equipped with a one-way valve to allow release of carbon dioxide produced during fermentation (Rou Duoduo Biotechnology Co., Beijing, China). Fermentation was performed in these bags at 25±5°C. Samples of the solid-state fermented feed were collected on day 0, 1, 2, 3, 5, 7, 10, 15, 20 and 30 for microbial counts and chemical analysis.

Microbiological counts of solid-state fermented feed

Ten-fold dilutions of the solid-state fermented feed samples were prepared for microbial enumeration. Lactic acid bacteria were enumerated on de Man, Rogosa and Sharp agar following anaerobic incubation at 37°C for 36 h. Enterobacteria were enumerated on McConkey agar following aerobic incubation at 37°C for 24 h. Bacillus were enumerated on Mixed Nutrient agar plates at 37°C for 24 h. Yeasts were enumerated on agar plates (10 g yeast extract, 20 g bacteriological peptone, 20 g D-glucose and 15 g agar per liter) following aerobic incubation at 30°C for 36 h.

Chemical analysis of solid-state fermented feed samples

The dry matter and crude protein content of the fermented feed were determined according to AOAC (1990). Homogenates (5 g of sample+50 ml of normal saline) of fermented feed samples were prepared to determine water-soluble protein. The mixture was centrifuged at 6,000 rpm for 10 min. The water-soluble protein in the supernatant was determined according to the method established by Bradford (1976) using bovine serum albumin as the standard. A 1 g sample was dissolved in 10 ml water and then centrifuged at 6,000 rpm for 10 min and pH of the supernatant was measured with a pocket-sized pH meter (Hanna instruments, Woonsocket, Rhode Island, US).

Short chain fatty acids were analyzed by Gas Chromatography (Hewlett Packard HP 6890 GC System, Santa Clara, CA). Homogenates (10 g sample+30 ml sterile distilled water) of fermented feed were centrifuged at 6,000 rpm for 10 min. A sample of supernatant (1 ml) was transferred into a centrifuge tube with a total capacity of 2 ml and 0.5 ml of acetonitrile was added to deproteinize the sample. After 5 min of centrifugation at 10,00 rpm, 1 ml supernatant was then mixed with 0.2 ml 2-ethylbutyric acid as the internal standard. Short chain fatty acids were analyzed using a gas chromatograph equipped with a flame ionization detector and a polyethylene glycol column (30.0 m×250 μm×0.25 μm). Helium was used as the carrier gas at 2 ml/min. Lactic acid was determined using the enzymatic kit produced by Nanjin Jiancheng Bioengineering Research Studio (Nanjin, China) according to the manufacturer's instructions.

Animal feeding trial

At the end of the 30-day fermentation, the solid-state fermented feed was used for a feeding trial to determine its effects on pig performance and nutrient digestibility. This trial was performed at the Monogastric Animal Metabolism Laboratory of China Agricultural University (Beijing, China). All animals used in this experiment were maintained according to the principles of the China Agricultural University Animal Care and Use Committee.

Twenty crossbred barrows (Duroc×Yorshire×Landrace) with an initial body weight of 14.11±0.77 kg were divided by weight into two groups (10 per treatment). The pigs were

Table 2. Composition of experimental diets and nutrient levels

Item	14-25 kg		25-65 kg		65-95 kg	
	Control	SFF[1]	Control	SFF	Control	SFF
Ingredient						
Maize (%)	70.0	60.5	67.5	65.0	70.0	70.0
Soybean meal (%)	20.0	12.0	20.0	11.0	20.0	9.0
Fish meal (%)	6.0	-	3.0	-	-	-
Wheat bran (%)	-	2.5	4.5	4.0	5.0	6.0
Soybean oil (%)	-	-	1.0	-	1.0	-
Solid-state fermented feed (%)	-	25.0	-	20.0	-	15.0
Mineral and vitamin premix (%)[2]	4.0	-	4.0	-	4.0	-
Nutrient composition[3]						
Metabolizable energy (Mcal/kg)	3.21	3.21	3.22	3.22	3.23	3.23
Crude protein (%)	18.04	18.02	16.63	16.66	14.99	14.99
Lysine (%)	1.20	1.24	0.97	0.96	0.73	0.71
Calcium (%)	0.96	0.96	0.65	0.65	0.48	0.48
Total phosphorus (%)	0.80	0.81	0.51	0.50	0.51	0.50

[1] SFF = Solid-state fermented feed.

[2] 14-25 kg (per kilogram premix): Cu, 5,000 mg; Fe, 2,800 mg; Zn, 2,500 mg; Mn, 1,300 mg; I, 15 mg; Se, 10 mg; Ca, 200 g; P, 20 g; Salt, 80 g; retinyl acetate, 125,000 IU; cholecalciferol, 38,000 IU; DL-α-tocopheryl acetate, 380 IU; menadione sodium bisulfite complex, 75 mg; thiamine, 25 mg; riboflavin, 75 mg; pyridoxine, 40 mg; vitamin B_{12}, 0.25 mg; nicotinic acid, 500 mg; D-Ca-pantothenate, 300 mg; folic acid, 16 mg; biotin, 1 mg; choline chloride, 6,000 mg; colistin sulfate, 375 mg.

25-65kg (per kilogram premix): Cu, 3,500 mg; Fe, 2,200 mg; Zn, 2,200 mg; Mn, 1,100 mg; I, 10 mg; Se, 8 mg; Ca, 180 g; P, 20 g; Salt, 80 g; retinyl acetate, 100,000 IU; cholecalciferol, 30,000 IU; DL-α-tocopheryl acetate, 300 IU; menadione sodium bisulfite complex, 60 mg; thiamine, 20 mg; riboflavin, 60 mg; pyridoxine, 30 mg; vitamin B_{12}, 0.2 mg; nicotinic acid, 400 mg; D-Ca-pantothenate, 250 mg; folic acid, 13 mg; biotin, 0.8 mg; choline chloride, 5,000 mg; lysine, 3.2%; flavomycin, 125 mg.

65-95 kg (per kilogram premix): Cu, 2,500 mg; Fe, 1,600 mg; Zn, 1,600 mg; Mn, 800 mg; I, 8 mg; Se, 6 mg; Ca, 150 g; P, 10 g; Salt, 80 g; retinyl acetate, 100,000 IU; cholecalciferol, 25,000 IU; DL-α-tocopheryl acetate, 250 IU; menadione sodium bisulfite complex, 45 mg; thiamine, 15 mg; riboflavin, 50 mg; pyridoxine, 25 mg; vitamin B_{12}, 0.15 mg; nicotinic acid, 300 mg; D-Ca-pantothenate, 200 mg; folic acid, 10 mg; biotin, 0.6 mg; choline chloride, 4,000 mg; lysine, 3.2%; flavomycin, 125 mg.

[3] Determined values except for ME.

fed either a corn-soybean meal-based control diet formulated with antibiotics or an antibiotic-free diet containing the solid-state fermented feed. The trial was conducted in three phases with the initial phase conducted from 14-25 kg BW with the test diet containing 25% solid-state fermented feed, a second phase conducted from 25-65 kg BW with the test diet containing 20% solid-state fermented feed and the final phase conducted from 65-95 kg BW with the test diet containing 15% solid-state fermented feed. The diets were formulated to contain similar levels of all nutrients (Table 2). It should be noted that the vitamin-mineral premix for the diets containing solid-state fermentation product were placed into the initial fermentation substrate and therefore a vitamin-mineral premix was not included in the solid-state fermented feed-based diets. To avoid the feed going moldy caused by its high water content, the solid-state fermented feed-based diet was never formulated more than two days before feeding.

The pigs were individually housed in stainless steel metabolism crates equipped with a plastic-coated, totally slatted floor during the whole experiment. The pens were 0.5×1.1 m during the initial phase and 0.6×1.4 m during the final two phases. Pigs were fed twice daily until full while fresh water was available all the times. One weak pig in both groups was removed from the experiment during the initial phase.

Feed intake and body weight were recorded at the end of each period. Fecal collection trays were inserted below each crate during the last three days of each experimental period and a total fecal collection was conducted to allow the determination of nutrient digestibility.

Chemical analysis of feed and feces

The dry matter, crude protein, calcium and phosphorus in diets or feces were determined according to AOAC (1990) and gross energy in feces was measured by an adiabatic bomb calorimeter (Model 1281, Parr, Moline, IL). Feed samples were hydrolyzed with 6 N HCl for 24 h at 110°C prior to determination of lysine content using High Performance Liquid Chromatography (Hitachi L-8800 Amino Acid Analyzer, Tokyo, Japan).

Statistical analysis

Data relating to microbial counts and chemical analysis of solid-state fermented feed were calculated using Microsoft Office Excel (2003). Data for nutrient digestibility and performance were analyzed using ANOVA of SAS (SAS Institute, 1996).

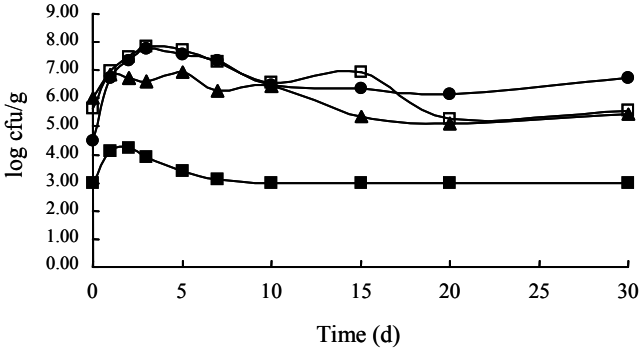

Figure 1. LAB (●), yeasts (□), bacillus (▲) and enterobacteria (■) counts of solid-state fermented feed during fermentation. Detectable level for enterobacteria (3.0 log cfu/g) was applied at day 10 and later days when no colony was detected on the plates.

RESULTS

Microbial characteristics of solid-state fermented feed during fermentation

The microbial counts of solid-state fermented feed during fermentation are shown in Figure 1. The counts of lactic acid bacteria increased rapidly during the first three days of fermentation and then slowly declined until day 10 and, thereafter, the counts were maintained at about 6.7 log cfu/g for the duration of the fermentation period. Enterobacteria increased during the first two days, and then fell below the detectable level of the analysis (3.0 log cfu/g). Counts of yeasts also showed a rapid increase during the first three days of fermentation, maintaining an elevated level until day 15 whereupon the levels dropped back to the initial level of 5.6 log cfu/g. Bacillus showed a small increase from 6.0 to 6.9 log cfu/g during the first 5 days of incubation but dropped to 5.4 log cfu/g by day 30.

Chemical analysis of solid-state fermented feed

Dry matter content dropped from 66.0% to about 64.5% after 5 days of fermentation and remained at this level until the 30th day (Table 3). Crude protein was relatively constant at around 30.5%. However, water-soluble protein increased from 8.2 to 9.2% and the ratio of water-soluble protein to crude protein increased by more than two percentage units over the 30-day incubation; pH dropped from 6.16 down to 5.73 after 30 days fermentation.

Table 3. Nutritional characteristics of solid-state fermented feed during fermentation[1]

	Dry matter (%)	pH	Crude protein (% as fed)	Water soluble protein (% as fed)	WSP/CP (%)
Time (d)					
0	66.0±0.13	6.16±0.06	30.2±0.20	8.2±0.26	27.1±1.03
1	65.6±0.52	6.06±0.01	30.8±0.14	8.4±0.16	27.2±0.65
2	65.2±0.11	6.02±0.02	30.3±0.81	8.4±0.29	27.7±0.22
3	65.0±0.42	5.89±0.01	30.3±0.15	8.1±0.00	26.8±0.13
5	64.4±1.44	5.85±0.04	30.5±0.69	8.2±0.32	26.9±0.45
7	64.4±0.69	5.85±0.08	30.7±0.29	8.0±0.29	25.9±0.70
10	64.2±0.43	5.86±0.00	30.8±0.57	8.7±0.03	28.4±0.42
15	64.7±1.36	5.76±0.05	30.6±0.08	9.1±0.55	29.6±1.87
20	63.9±0.07	5.79±0.01	30.3±0.46	9.2±0.10	30.4±0.78
30	64.6±1.16	5.73±0.09	31.0±0.02	9.2±0.32	29.8±1.02

[1] Values are means±standard deviation (n = 2).

Table 4. Short chain fatty acid and lactic acid concentration during fermentation[1]

Item	Lactic acid	Acetic acid	Propionic acid	Isobutyric acid	Butyric acid	Isovaleric acid	Valeric acid
	mmol/kg						
Time (d)							
0	15.0±0.47	16.7±2.23	0.1±0.07	ND[2]	0.9±0.14	0.4±0.15	0.4±0.18
1	10.1±0.59	32.6±1.79	0.1±0.08	ND	0.7±0.01	0.5±0.06	0.2±0.01
2	11.2±0.00	33.4±0.78	0.3±0.15	ND	0.7±0.22	0.5±0.02	0.2±0.06
3	8.2±2 .00	46.4±3.38	0.3±0.11	0.4±0.02	0.7±0.02	0.5±0.29	0.2±0.01
5	7.6±0.71	34.5±1.71	0.3±0.06	0.3±0.20	0.7±0.07	0.4±0.01	0.1±0.01
7	9.7±1.82	42.2±3.06	0.6±0.47	0.8±0.65	0.6±0 .07	0.3±0.00	0.1±0.02
10	11.7±0.24	32.9±1.51	1.1±0.11	1.3±0.06	0.7±0.04	0.4±0.13	0.1±0.00
15	11.5±0.24	46.4±1.06	1.6±1.72	1.3±1.44	0.6±0.00	0.2±0.01	ND
20	10.3±0.47	42.6±4.51	1.2±0.32	1.2±0.20	0.6±0.00	0.2±0.01	ND
30	14.4±0.59	51.3±2.76	2.0±0.11	2.4±2.49	0.4±0.09	0.3±0.08	ND

[1] Values are means±standard deviation (n = 2). Data are on original sample basis. [2] Not detected.

Data regarding the short chain fatty acid and lactic acid concentrations during fermentation are shown in Table 4. The concentration of lactic acid decreased during the first seven days of incubation and then rose back to the initial level of about 15 mmol/kg by the end of the 30-day incubation. Acetic acid concentration increased dramatically from 16.7 to 51.3 mmol/kg after 30 days of fermentation. The concentrations of propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid were low and varied little throughout the incubation.

Pig performance and nutrient digestibility

There was no significant difference between the two diets in terms of average daily gain, daily intake or feed conversion for the three experimental periods with the exception of a significantly ($p<0.05$) higher feed conversion for pigs fed the solid-state fermented feed- based diet during the second phase (Table 5).

The digestibility of energy, crude protein, calcium and phosphorus did not differ between the two diets during all three experimental periods (Table 6). The solid-state fermented feed-based diet showed a trend to improved digestibility of crude protein in phase three ($p = 0.10$).

DISCUSSION

Spontaneous (natural) fermentation without starter inoculum is sometimes used to process fermented feed (Canibe and Jensen, 2003; Canibe et al., 2006; Canibe and Jensen, 2007) while controlled fermentation with inoculum has wider application (van Winsen et al., 2001; Demecková et al., 2002; Kiers et al., 2003; Canibe et al., 2007; Niven et al., 2007). Controlled fermentation typically involves lactic acid bacteria, molds, yeasts and bacillus as inoculum because they can achieve more predictable results under optimal conditions (Giraffa, 2004).

A combined liquid culture of *Lactobacillus fermentum*, *Saccharomyces cerevisae* and *Bacillus subtilis* was used as the inoculum for the current study. *Saccharomyces cerevisae* was added to consume the oxygen inside the fermenting bag to promote the growth of anaerobes including lactic acid bacteria and bacillus. The *Bacillus subtilis* used was screened by Guo et al. (2006) and has been shown to be a potential alternative to antibiotics for use in animal feeds because it decreased the counts of *E. coli* shed in the feces of piglets. The *Lactobacillus fermentum,* which was isolated from the gut of a healthy

Table 5. Effects of solid-state fermented feed (SFF) on performance of growing-finishing pigs

Item		Diet		SEM	p
		Control	SFF		
14-25 kg	Daily gain (g)	392	433	24	0.26
	Daily intake (g)	682	740	36	0.30
	Feed conversion	1.9	1.71	0.08	0.12
25-65 kg	Daily gain (g)	784	759	19	0.35
	Daily intake (g)	1,850	1,935	33	0.09
	Feed conversion	2.37	2.55	0.05	0.01
65-95 kg	Daily gain (g)	1,097	1,150	48	0.45
	Daily intake (g)	3,449	3,604	124	0.39
	Feed conversion	3.16	3.15	0.06	0.90
14-95 kg	Daily gain (g)	766	787	23	0.56
	Daily intake (g)	2,000	2,099	49	0.17
	Feed conversion	2.62	2.68	0.04	0.33

Table 6. Effects of solid-state fermented feed (SFF) on nutrient digestibility in growing-finishing pigs

Item		Diet		SEM	p
		Control (%)	SFF (%)		
14-25 kg	Energy	89.12	88.83	0.86	0.82
	Crude protein	86.85	87.66	1.70	0.74
	Calcium	71.07	68.36	1.47	0.22
	Phosphorus	59.30	57.38	1.96	0.50
25-65 kg	Energy	87.35	88.15	0.63	0.39
	Crude protein	88.23	88.82	0.78	0.60
	Calcium	52.82	51.53	2.86	0.76
	Phosphorus	39.93	39.83	1.57	0.97
65-95 kg	Energy	88.88	89.86	0.46	0.15
	Crude protein	89.24	90.52	0.52	0.10
	Calcium	42.48	45.00	2.75	0.52
	Phosphorus	31.06	30.60	2.08	0.88

piglet, has been shown to be effective in preventing diarrhea in *E. coli* challenged piglets (Huang et al., 2003) and acted as the main bacterial contributor in the fermentation.

Feed fermentation has been divided into two phases by Canibe and Jensen (2003). The first phase is characterized by low levels of lactic acid bacteria and yeasts, high pH and a blooming of enterobacteria while the second phase is characterized by high levels of lactic acid bacteria and yeasts, low pH and low counts of enterobacteria. This same pattern was also achieved in the present trial, with the counts of lactic acid bacteria, yeasts and enterobacteria during fermentation indicating that a successful fermentation occurred. It appeared that a steady state can be reached after 5 days at 25±5°C.

The final counts of lactic acid bacteria (6.7 log cfu/g) in this trial are much lower than that achieved with fermented liquid feed, which can reach as high as 9.0-9.4 log cfu/g (van Winsen et al., 2001; Demecková et al., 2002; Canibe and Jensen, 2003; Canibe et al., 2006; Canibe et al., 2007). The lower counts of lactic acid bacteria may be caused by the lower moisture content of solid-state fermented feed as the feed to water ratio used was just 10:3 (w/v) while feed to water ratios of 1:2 (w/v) or even 1:2.75 (w/v) are reported with fermented liquid feed (van Winsen et al., 2001; Demecková et al., 2002; Canibe and Jensen, 2003; Canibe et al., 2006; Canibe et al., 2007). The transportation of nutrients and ions is greater in media with a higher water content, thus leading to higher lactic acid bacteria in fermented liquid feeds.

The counts of yeast showed a large increase to 7.8 log cfu/g but dropped back to 5.6 because of the absence of oxygen during the latter stages of fermentation. The ratio of yeast to lactic acid bacteria was about 1:100 and the same ratio was summarized by Corsetti and Settanni (2007) in their review on sourdough fermentation. The final counts of bacillus were 5.4 log cfu/g in this trial while a level of 5.3 log cfu/g feed was reported to produce a significant increase in lactobacilli counts and a numerical decrease in *E. coli* counts in the faecal samples of piglets as described by Guo et al. (2006). So the level of bacillus in the solid-state fermented feed used in the present trial should have been sufficient to produce positive effects on pig health.

The decrease in the dry matter content of the solid-state fermentation product (1.5%) was likely caused by the consumption of carbohydrate by yeast and aerobic bacteria. The absence of oxygen during later stages of fermentation would impair the growth and metabolism of yeast, so further decrease of dry matter would not occur. The crude protein content remained unchanged indicating that there was no loss of nitrogen during fermentation. The exact reason for the two percentage unit increase in water-soluble protein is unclear, but one potential explanation might be that microorganisms hydrolyze water-insoluble proteins into soluble forms, such as free amino acids and small-size peptides, with better absorbability (Hong et al., 2004; Tonheim et al., 2007).

Lactic acid bacteria are classified according to the end products of fermentation into homofermentative or heterofermentative lactic acid bacteria, and the *Lactobacillus fermentum* used in this trial belongs to the latter classification. In addition to lactic acid, the principle end products of this class of lactic acid bacteria are CO_2, ethanol and acetic acid (Corsetti and Settanni, 2007). This would partially explain the dramatic increase in acetic acid observed during fermentation in the present study, which increased to 51.3 mmol/kg, or about three times the initial concentration. An increase of a similar magnitude has been previously reported (van Winsen et al., 2001; Canibe and Jensen, 2003; Canibe et al., 2006; Canibe and Jensen, 2007; Hong and Lindberg, 2007). Filya and Sucu (2007) proved in their study that heterolactic fermentation was helpful in aerobic stability of fermented crops due to the high levels of acetic and propionic acid in fermentation end products.

The decrease in pH from 6.16 to 5.73 which occurred during fermentation was most likely the result of increased acetic acid as the concentrations of propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid remained below 2.5 mmol/kg after 30-day fermentation. Fermented liquid feed usually has a pH of 3.8-4.5 (van Winsen et al., 2001; Beal et al., 2002; Demecková et al., 2002; Canibe and Jensen, 2003; Canibe and Jensen, 2007; Canibe et al., 2007), which is obviously lower than that obtained in the current study according to solid-state fermentation. One possible reason for the lower pH with liquid fermented feed might be that the high water content stimulates lactic acid fermentation thus producing more organic acids.

Nutrient digestibility was unaffected during all the three experimental periods except for a trend for solid-state fermentation feed to improve the digestibility of crude protein in phase three. Tonheim et al. (2007) indicated that water-soluble protein is easier to digest than water-insoluble proteins, so the increase in water-soluble protein in the solid-state fermented feed may increase the crude protein digestibility.

Both positive and negative effects of fermented liquid feed on pig performance have been reported (Canibe and Jensen, 2003; Canibe and Jensen, 2007; Canibe et al., 2007). However, literature on solid-state fermented feed is scarce. In the present trial, there was no significant difference between the two diets on pig performance indicating that solid-state fermented feed is a potential alternative to antibiotic inclusion in diets fed to growing-finishing pigs. The possible mechanism of solid-state fermented feed in

replacing antibiotics in this study may be due to the following characteristics gained from solid-state fermentation: i) High counts of lactic acid bacteria and bacillus and low counts of enterobacteria; ii) High concentration of organic acids and low pH; iii) Increase of water soluble protein to crude protein ratio (WSP/CP).

In conclusion, solid-state fermentation resulted in high counts of lactic acid bacteria and low counts of enterobacteria of substrate. Moreover, feeding a diet containing solid-state fermented feed, free of antibiotics, can result in similar performance and nutrient digestibility in growing-finishing pigs as a regular diet with antibiotics.

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