Asian-Aust. J. Anim. Sci.
Vol. 20, No. 3 : 399 - 404
March 2007
www.ajas.info

Effect of Feeding Levels of Microbial Fermented Soy Protein on the Growth Performance, Nutrient Digestibility and Intestinal Morphology in Weaned Piglets

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ABSTRACT : To elucidate the efficacy of different levels of microbial-fermented soy proteins (FSP) on piglet performance, a total of 240 weaned piglets (L×Y×D, 22±3 d of age, 5.16±0.07 kg initial BW) were allotted to 4 treatment diets comprising control, FSP-3%, FSP-6% and FSP-9%. The fermented soybean product named Pepsoygen® was utilized for the study. There were 15 pigs per pen and 4 pens per treatment. The control diet contained 15% soybean meal (SBM), and SBM for the treatment diets was replaced at 3, 6 and 9% with FSP, respectively. Experimental diets were fed from 0 to 14 d (phase-I) after weaning and then a common commercial diet was fed from 15 to 35 d (phase-II). There was a linear ($p<0.05$) increase in ADG and ADFI at both phases of measurement. The feed to gain ratio was also improved, showing a linear ($p<0.01$) trend as the level of supplementation increased. Except for phosphorus, the digestibility of all other nutrients was improved linearly ($p<0.05$) in the FSP added diets. However, villous height and crypt depth were not affected by dietary treatments. No special effect on intestinal morphology was noticed between FSP-added and control diets. In conclusion, the growth, digestibility of nutrients and morphological changes in weaned pigs fed FSP showed improved performance at higher levels of supplementation. (**Key Words :** Piglets, Growth, Nutrient Digestibility, Intestine, Fermented Soy Protein)

INTRODUCTION

Soybean meal is an essential component in the feed formulation used for farm animals throughout the world. However, because of antigenic activity and antinutritional factors, young animals such as calves and piglets are sensitive to SBM and poor growth and digestive disorders are common when it is fed (Lalles, 1993).

In fact, milk products such as dried skim milk and dried whey as well as other animal protein sources such as spray-dried plasma proteins are typical feed ingredients in young pigs due to their high palatability and digestibility, but these are somewhat expensive. Therefore, specially processed soy products such as soy protein concentrate (SPC), soy protein isolate (SPI), and fermented soy protein (FSP) can alternatively be used in the starter diets. Fermented soybean meals were shown to contain proteins with high proportion of small peptides due to enzymatic degradation during fermentation process (Hong et al., 2004).

A fermented soybean meal (Pepsoygen®) was obtained from Genebiotech Co. Ltd., (Seoul, Korea) for this study to evaluate its effects at different supplemental levels on the performance, apparent total tract nutrient digestibility, and gut morphology in the weaned pigs fed for 14 d after weaning, and whether those effects last even after feeding a common diet for subsequent 21 d.

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Received October 20, 2005; Accepted July 6, 2006

MATERIALS AND METHODS

Animals and diets

Two hundred forty weaned pigs of 22±3 d of age (Landrace×Yorkshire×Duroc) were allotted to four treatments with four replicates in each, comprising 15 pigs with mixed sex (male: female ratio were the same among the treatments) in each replicate. The average body weight when weaned was 5.16±0.07 kg. The pigs were housed in partially slotted and concrete floor pens with a pen size of 1.90×2.54 m, with a self-feeder and nipple waterer to allow *ad libitum* access to feed and water. The dietary treatments include a diet containing SBM as a major protein source and three diets containing fermented soy protein (FSP) at 3, 6 and 9% by replacing equal amount of SBM. Iso-caloric (3,300 kcal/kg) and iso-proteinous (21.0% CP) diets were

Table 1. Formula and calculated composition of experimental diets for feeding trials (day 0 to 14)

	Control	FSP level (%)		
		3	6	9
Ingredient (%)				
Whey powder	38.00	38.00	38.00	38.00
Corn	30.38	30.90	31.15	31.35
SDPP	4.00	4.00	4.00	4.00
Biscuit by product	4.00	4.00	4.00	4.00
SBM (48%)	15.00	12.00	9.00	6.00
FSP-B	0.00	3.00	6.00	9.00
Fish meal (60%)	3.81	3.18	2.87	2.64
Animal fat	2.00	2.00	2.00	2.00
L-lysine HCl (78%)	0.32	0.34	0.34	0.34
DL-methionine (100%)	0.13	0.15	0.15	0.16
Lime stone	0.75	0.84	0.89	0.92
Salt	0.20	0.20	0.20	0.20
Vitamins premix1	0.30	0.30	0.30	0.30
Minerals premix2	0.20	0.20	0.20	0.20
Choline chloride (25%)	0.10	0.10	0.10	0.10
Apramycin	0.15	0.15	0.15	0.15
Mecadox	0.10	0.10	0.10	0.10
Sulfathiazol	0.10	0.10	0.10	0.10
Acidifier	0.20	0.20	0.20	0.20
Zinc oxide	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated composition				
ME (kcal/kg)	3,306	3,306	3,306	3,305
CP (%)	21.00	21.00	21.16	21.35
Lactose (%)	24.70	24.70	24.70	24.70
Ca (%)	0.82	0.82	0.82	0.82
Av. P (%)	0.41	0.40	0.40	0.40
Lys (%)	1.55	1.55	1.55	1.55
Met+cys	0.85	0.85	0.85	0.85
Thr (%)	0.91	0.91	0.92	0.93

[1] Supplied per kg diet: 9,600 IU vitamin A, 1,800 IU vitamin D_3, 24 mg vitamin E, 1.5 mg vitamin B_1, 12 mg vitamin B_2, 2.4 mg vitamin B_6, 0.045 mg vitamin B_{12}, 1.5 mg vitamin K_3, 24 mg pantothenic acid, 45 mg niacin, 0.09 mg biotin, 0.75 mg folic acid, 18 mg ethoxyquin.

[2] Supplied per kg diet: 162 mg Fe, 96 mg Cu, 72 mg Zn, 46.49 mg Mn, 0.9 mg I, 0.9 mg Co, 0.3 mg Se.

formulated, and lysine content (1.55%) was similar in all the diets. All the diets had the same protein contents by altering the contents of corn, and fishmeal as FSP replaces SBM. All the diets contained SBM with hulls whereas FSP was originally obtained from dehulled SBM.

All the diets met or slightly exceeded the nutrient requirements as suggested by NRC (1998). These diets were fed for only 14 d (phase I) in mash form, and then each group was fed a common commercial diet in crumbles during phase II (15-35 d). The composition of the starter diet (phase I, for 2 wk) is presented in Table 1. The experiment was conducted for 5 wk during which the body weights and feed intake were noted at the end of each phase of experimental feeding.

Apparent total tract nutrient digestibility

In order to study the effect of different levels of fermented soy protein source on apparent total tract nutrient digestibility, all pigs in all pens were fed their originally assigned diets mixed with chromic oxide (0.25%) from d 7 to 14 of phase I and fecal samples were collected from d 11 to 14 and pooled. The fecal samples were freshly collected from 5-6 pigs randomly from each pen each day to ensure no microbial modification of the nutrient contents. The fecal samples were dried in a forced-air drying oven at 60°C for 72 h and ground with a 1mm mesh Wiley mill for chemical analysis.

Ileal histometry and morphological studies

To study the effect of diets on gut morphology, villi height and crypt depth, representative pigs from each group (3 pigs per pen), were randomly selected and killed by electrocution at 14 d of age. Immediately after slaughter the small intestine was excised. The ileal contents (chyme) were removed. The small intestine was then immersed in 10% buffered formaline and then brought to laboratory for further studies.

The small intestinal segment was rinsed with 0.4 M KCL and then cut in 2 mm^2 small segments and submerged in a fixative solution (0.1 M collidine buffer, pH 7.3) containing 3% glutaraldehyde, 2% paraformaldehyde and 1.5% acrolein. Cross-sectional small intestine samples from the formalin preserved segments were fixed by standard paraffin embedding. Samples were sectioned at 6 μm and stained with azur A and eosine. Villous height and crypt depth were measured on the stained sections under microscope at 40× magnification equipped with an ocular micrometer. A minimum of 10 well-oriented intact villi was measured in duplicate specimens for each pig. Villous height was measured from the crypt base to the villous tip and all measurements (villous height and crypt depth) were made in 10-micrometer increments as mentioned by Cera et al. (1988).

Scanning electron microscopy (SEM) was performed on the 2 mm^2 specimens. Samples were dehydrated in an ethanol-freon series as described by Liepens and DeHaven (1978). Following platinum coating, specimens were viewed with an ISI scanning electron microscope (International Scientific Instruments, Inc. Milpitas, CA) and were photographed.

Analyses

Proximate analyses of the experimental diets were carried out following the AOAC (1990) methods. Gross energy and chromium were measured by a bomb calorimeter (Model 1261, Parr Instrument Co., Molin, IL) and an automated spectrophotometer (Shimadzu, Japan), respectively. Following acid hydrolysis in 6 N HCL at 105°C for 24 h, amino acid concentrations in SBM and FSP

Table 2. Crude protein and essential amino acid profiles of SBM and fermented protein source (as-fed basis)[1]

Protein sources[2]	SBM	FSP
Crude protein (%)	45.19	57.01
Arginine	3.27	3.83
Histidine	1.34	1.68
Isoleucine	1.85	2.42
Leucine	4.69	5.28
Lysine	2.87	3.02
Phenylalanine	2.00	2.79
Threonine	1.73	2.06
Valine	1.86	2.48
Methionine	0.48	0.80
Total	20.09	24.36
TEAA[3]/CP	0.44	0.43

[1] Tryptophan was not determined.
[2] SBM: Soybean meal; FSP: Fermented soybean protein.
[3] Total essential amino acid.

were analyzed by using a HPLC (Waters 486, USA). Sulfur containing amino acids was analyzed after cold performic acid oxidation (Moore, 1963) overnight with subsequent hydrolysis.

Statistical analysis

Data collected was subjected to statistical analysis using GLM procedure of SAS (1985) by using statistical software package using completely randomized design to study the linear and quadratic effects. The treatments were the main effects. The pens were the experimental units for all analysis. The level of significance was accepted at $p<0.05$, unless otherwise noted.

RESULTS AND DISCUSSION

Chemical composition of fermented soy protein

The crude protein and essential amino acid (EAA) profile of soy protein sources used is presented in Table 2. The TEAA/CP ratio almost remained similar for FSP and SBM. The tryptophan was not determined in any of the protein source. The CP content was higher in FSP (57.01%) than SBM (45.19%). The analyzed amino acid composition of fermented soy protein for EAA was nearly similar to SBM but the CP content was higher in FSP than SBM.

Growth performance

The average daily gain (ADG) was higher ($p<0.001$) in the pigs fed FSP diets than SBM at 0-14 d measurement showing a linear trend (Table 3). At 15-35 d, the ADG was higher ($p<0.01$) in the pigs fed FSP-B at higher levels (6 and 9%) than SBM; however, there was no difference at 3% level and SBM. Similar trend of higher ($p<0.001$) ADG in FSP than SBM followed at the overall (0-35 d) study and it was higher ($p<0.001$) at higher level of supplementation (6 and 9%) than lower level (3%). The higher ADG in FSP could be the effect of higher ($p<0.05$) average daily feed intake (ADFI) when compared with SBM. There was a linear increase in ADFI because of feeding processed soy proteins was also reported by Min et al. (2004). The growth rate (ADG) and ADFI is graphically presented in Figure 1. The feed to gain ratio was improved in FSP diets than to SBM at all levels showing a linear improvement in the present study. No quadratic trend was noticed for ADG, ADFI or F/G at any phase of measurement. The findings in the present study once again confirmed that FSP could significantly improve the ADG than SBM fed group that we reported earlier (Yun et al., 2005). The reason for lower ADG could be poor palatability; one of the distinct problems with SBM-based diets as was mentioned by Sohn et al. (1994). Although the palatability problem was not more prominently observed in the present research, but similar response of lower feed intake and ADG in SBM fed diet when compared with dried skim milk (DSM), ISP (isolated soy proteins), SDPP and wheat gluten fed diets

Table 3. Effect of dietary levels of fermented soy protein on growth performance in weaned pigs

Item	Control	Level (%)			SEM[1]	p value[2]	
		3	6	9		L	Q
Day 0-14							
ADG (g)	262[c]	274[b]	286[a]	286[a]	3.35	***[3]	NS[4]
ADFI (g)	394[b]	406[ab]	414[a]	412[a]	3.19	*	NS
F/G	1.51[a]	1.48[ab]	1.45[bc]	1.44[c]	0.01	**	NS
Day 15-35							
ADG (g)	407[b]	421[ab]	430[a]	434[a]	3.89	**	NS
ADFI (g)	631[b]	638[ab]	649[ab]	653[a]	3.40	*	NS
F/G	1.55[a]	1.52[ab]	1.51[ab]	1.50[b]	0.01	*	NS
Overall (day 0-35)							
ADG (g)	349[c]	362[b]	372[a]	375[a]	3.29	***	NS
ADFI (g)	537[c]	545[b]	555[a]	557[a]	2.58	***	NS
F/G	1.54[a]	1.50[b]	1.49[b]	1.48[b]	0.01	**	NS

[a, b, c] Values with different superscripts in the same row differ significantly ($p<0.05$).
[1] Pooled standard error of means. [2] L: Linear effects; Q: Quadratic effects.
[3] * $p<0.05$; ** $p<0.01$; *** $p<0.001$. [4] Not significant ($p>0.05$).

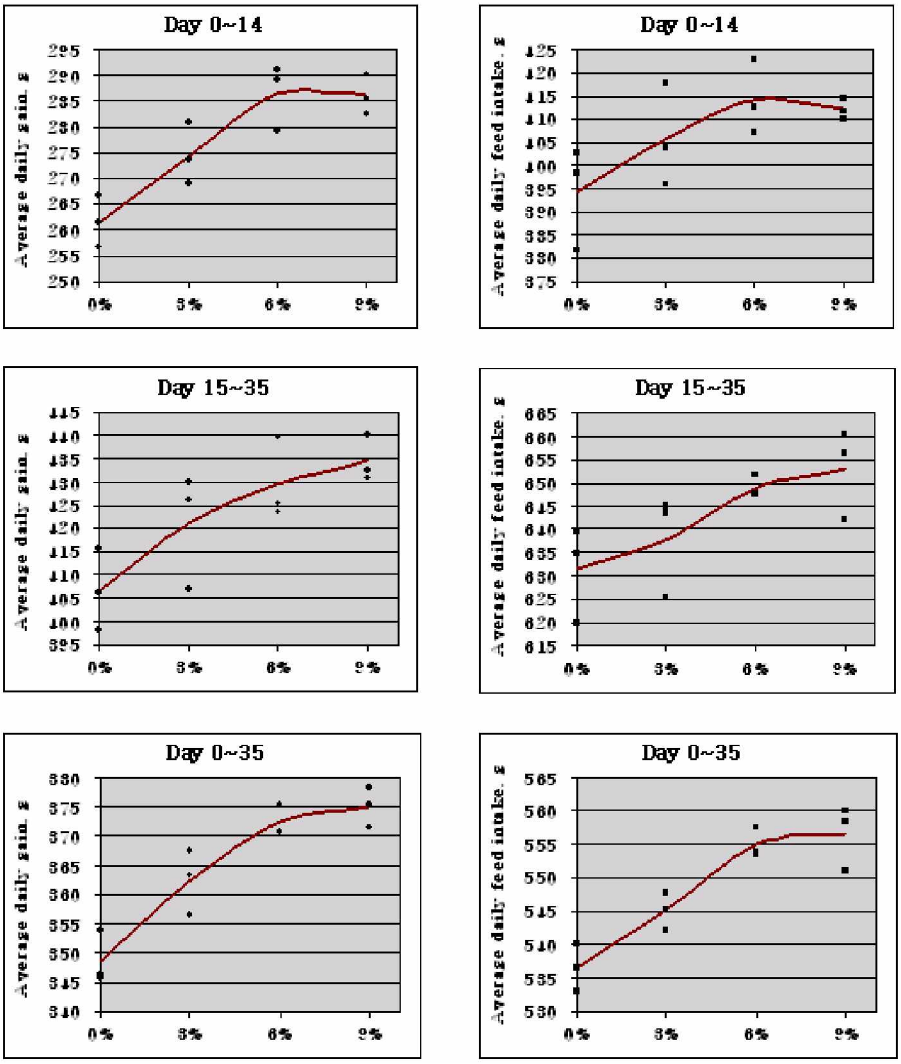

Figure 1. Growth and feed intake curves in weaned pigs fed with increasing levels of fermented soy protein.

Table 4. Effect of dietary levels of fermented soy protein on fecal nutrients digestibility in weaned pigs

Item	Control	Level (%)			SEM[1]	p value[2]	
		3	6	9		L	Q
DM	82.14[b]	83.84[a]	83.76[a]	84.33[a]	0.29	**[3]	NS[4]
GE	82.05[b]	83.49[a]	83.92[a]	84.30[a]	0.31	**	NS
CP	73.75[c]	77.31[b]	78.68[ab]	80.84[a]	0.86	***	NS
EE	63.09[b]	66.66[a]	68.40[a]	67.35[a]	0.70	**	*
Ash	54.18[b]	57.73[a]	58.22[a]	59.14[a]	0.67	**	NS
Ca	56.93[b]	56.45[b]	57.83[ab]	58.52[a]	0.31	*	NS
P	48.73[b]	53.43[a]	50.86[b]	49.77[b]	0.60	NS	**

[a, b, c] Values with different superscripts in the same row differ significantly ($p<0.05$).

[1] Pooled standard error of means. [2] L: Linear effects; Q: Quadratic effects.

[3] * $p<0.05$; ** $p<0.01$; *** $p<0.05$. [4] Not significant ($p>0.05$).

were also noted by Chae et al. (1999). It could be considered that the anti-nutritional factors in SBM that normally hinders performance was less effective in FSP fed group than SBM since fermentation removed such antigenic components. There are few studies, where fermented soybean proteins could serve as an alternative protein source in early-weaned pigs.

Apparent total tract nutrient digestibility

The data of apparent total tract nutrient digestibility conducted after 14 d of experimental feeding is presented in Table 4. The digestibility of dry matter, gross energy, crude protein, ether extract, ash and calcium was higher ($p<0.05$) in FSP fed diets than SBM showing linear trend. The higher nutrient's digestibility in FSP group had culminated into

Table 5. Effect of dietary levels of fermented soy protein on ileal villous height, crypt depth and villous height: crypt depth ratio in weaned pigs

Item	Control	Level (%)			SEM[1]	P-value[2]	
		3	6	9		L	Q
Villous height	326	335	329	336	4.96	NS[3]	NS
Crypt depth	223	218	215	213	3.96	NS	NS
Villous height:crypt depth	1.47	1.54	1.53	1.58	0.03	NS	NS

[1] Pooled standard error of means. [2] L: Linear effects; Q: Quadratic effects. [3] Not significant (p>0.05).

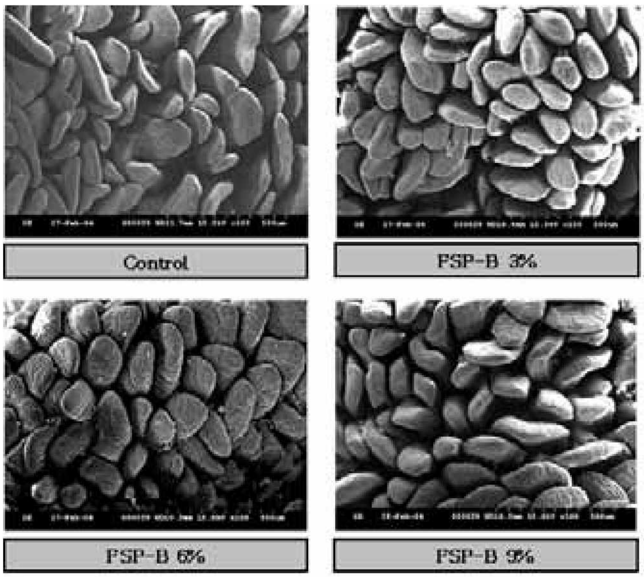

Figure 2. Scanning electron microscopic pictures of ileal villi in weaned pigs fed FSP at different levels.

increased weight gains in these animals. Higher DM and N digestibilities were also reported in pigs fed processed soy protein diet than to negative control (without processed soy protein) by Min et al. (2004). There was no linear difference in phosphorus digestibility. However, a quadratic trend was recorded when the diets were compared in the present study. The digestibility of nutrients was lowest in SBM than others. It is commonly noticed that when the feed intake is low, the nutrient digestibility is high. However, we could not find such effect in SBM fed animals. Even the digestibility of GE, CP, EE, calcium and phosphorus were lower in SBM fed animals as compared with other plant protein sources (FSP and rice protein concentrate) in our earlier studies (Yun et al., 2005). The lower nutrient digestibility in SBM than that of milk products based diets was also reported previously (Walker et al., 1986; Sohn et al., 1994). The poor digestibility of nutrients in pigs fed the SBM diet may be due to the presence of indigestible carbohydrate complexes (Walker et al., 1986), indigestible proteins such as glycinin and β-conglycinin (Li et al., 1991a), and (or) anti-nutritional factors, in addition to the incomplete development of digestive system in pigs (14 d old). The lower digestibility of nutrients lowered the ADG in this group.

Effect on villous height and crypt depth

The dietary protein sources did not show any impact on villous height at the ileum (Table 5). The crypt depth and the villous height to crypt depth ratio were not affected by dietary treatments. The villi of the small intestine change with age and with weaning from a long and fine-finger like shape to a short and thick tongue like shape (Cera et al., 1988). Hypersensitivity to antigens in diet may be responsible for the morphological changes in intestine was suggested by Li et al. (1990), but the present study failed to show any such changes. High levels of plant proteins that may contain strong antigens greatly affect villous height and gut morphology (Li et al., 1991b). Kelly et al. (1991) and Vente-Spreeuwenberg et al. (2004) reported that villous height and crypt depth were positively related to feed intake. Although the differences in feed intake in the present study were significant, it could not contribute to significant changes in the villous structures. Furthermore, Kelly et al. (1991) and Pluske et al. (1996) reported that pigs fed less feed, showed villous atrophy and increased crypt depth at all sites along the small intestine compared with pigs fed a higher quantity feed. The findings in our study were contradictory to the reports mentioned above and possibly this could be because of not much numerical differences in feed intake among treatments, although it was significant.

Effect on gut morphology

The photographs taken by scanning electron microscope (Figure 2) clearly depicted the effect of protein sources on the gut morphology. In our study, the villi structures by SEM were prominent, straight, finger- like, though shortened and densely located in FSP fed groups as compared with SBM. The luminal surfaces of the villi in each FSP group were smoother and these were in close association. The photographs showed shortened villi, but the villous tips were neither eroded in any of the groups nor showed any effects of atrophy. The villi in SBM group looked flattened with a broad diameter. However, Cera et al. (1988) found lengthened villi clearly evident at 14 d post-weaning in their studies; where they compared the villi with suckling animals of same age having long, narrow finger like morphological structures and longitudinally flattened tongue like villi in post-weaning pigs, that we also observed. The lower feed intake that commonly observed after weaning is a contributing factor to the villous morphology.

Gall and Chung (1982) also mentioned that food intake is more important rather than chronological age for the maturation of small intestine. In conclusion, the growth, digestibility of nutrients and morphological changes in weaned pigs fed FSP showed improved performance at higher levels of supplementation.

ACKNOWLEDGEMENTS

The authors appreciate for the partial financial support by the ARPC for carrying out this project.

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