Asian-Aust. J. Anim. Sci.
Vol. 23, No. 11 : 1496 - 1502
November 2010

www.ajas.info

Effects of Diet Complexity and Fermented Soy Protein on Growth Performance and Apparent Ileal Amino Acid Digestibility in Weanling Pigs

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ABSTRACT : Two experiments were conducted to evaluate the effects of diet complexity and fermented soy protein on growth performance and amino acid digestibility. In Exp. 1, a total of 120 crossbred weanling pigs (5.68±0.80 kg BW) were randomly allocated into 4 treatments. Each treatment had 6 replicate pens comprising 5 pigs in each replicate. Experimental diets consisted of simple (soybean meal as protein source) and complex (soybean meal, rice protein concentrate, potato protein concentrate and fish meal as protein sources) diets; each diet contained 0 or 5% fermented soy protein (FSP), respectively. Dietary treatments included: i) simple diet; ii) simple diet with 5% FSP; iii) complex diet; iv) complex diet with 5% FSP. Pigs were provided each experimental diet for 20 d (phase 1) and then fed the same common diet for 10 d (phase 2). During days 0-10, pigs fed FSP diets had greater ADG than those fed non-FSP diets ($p<0.05$). G/F in FSP treatments was significantly higher than that in non-FSP treatments ($p<0.05$) from days 0 to 10. Throughout the overall period, G/F was greater in FSP treatments compared with non-FSP treatments ($p<0.05$). On d 10, N digestibility was higher in pigs fed FSP diets than in those fed non-FSP diets ($p<0.05$). Diet complexity did not affect growth performance and nutrient digestibility ($p>0.05$) in this experiment. In Exp 2, 12 ileal-cannulated, weanling barrows were housed in individual metabolism crates and randomly assigned to 1 of 4 treatments (same as Exp. 1) by using a 4×4 Latin square design. Among the essential amino acids, apparent ileal digestibility (AID) of Met and Val were increased in pigs fed FSP diets compared with those fed non-FSP diets ($p<0.05$). AID of Met, Phe and total essential amino acids were higher in pigs fed complex diets than in those fed simple diets ($p<0.05$). Among the non-essential amino acids, AID of Ala in FSP treatments was greater than that in non-FSP treatments ($p<0.05$). In addition, Asp, Cys, Glu, Pro, Ser and total non-essential amino acid digestibilities in pigs fed complex diets were higher compared with those fed simple diets ($p<0.05$). Interaction was observed in AID of Met, Asp and Pro. In conclusion, these results indicated that feeding of 5% FSP to nursery pigs improved feed efficiency and AID of amino acids, and diet complexity did not maximize the growth performance of pigs in the subsequent phase. (**Key Words :** Apparent Ileal Amino Acid Digestibility, Diet Complexity, Fermented Soy Protein, Growth Performance, Pigs)

INTRODUCTION

Soybean meal contains (SBM) several anti-nutritional factors, in particular, trypsin inhibitors and some of oligosaccharides which depress growth rate and efficiency of nutrient utilization when fed to swine (Anderson et al., 1979). This may be due to the presence of indigestible carbohydrate complexes (Li et al., 1990), antigenic soy proteins (Li et al., 1990), and residual trypsin inhibitors (Lallès, 2000) which lead to the digestive disturbances. With the advent of new feed processing technology, novel sources have been recognized to replace animal protein sources. Some studies have indicated that feed efficiency and AA digestibility were improved in pigs fed fermented soybean meal (FSP) (Min et al., 2004; Kim et al., 2007; Cho et al., 2008). Kim et al. (2005) reported FSP can be used up to 10% in a weanling pig diet and successfully replaced the use of dried skim milk when the lactose contents were matched. Feng et al. (2007) indicated that FSP was beneficial to growth performance, digestibility of dietary components and activities of intestinal enzymes in piglets. The fermentation process is thought to eliminate residual trypsin inhibitors and some oligosaccharides in soybean meal that can decrease pig performance (Baker, 2000) as well as a greater concentration of CP and AA than conventional SBM (Kim et al., 2010).

Tokach et al. (1994) reported that when nursery diets are

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Received March 21, 2010; Accepted May 31, 2010

formulated, a primary objective is to select ingredients that will stimulate feed intake and maximize performance and this can be accomplished by increasing the complexity of diet. But this strategy frequently makes the diet more expensive. Previous research has demonstrated that complex diets improved nursery pig performance during the early postweaning period (Whang et al., 2000; Wolter et al., 2003).

Although there were many studies that investigated the effects of FSP or diet complexity on nursery pigs, to the best of our knowledge, no researches were conducted to evaluate the interactive effects of diet complexity and dietary FSP on pigs. Therefore, the objective of the current study was to examine the effects of dietary fermented soy protein and diet complexity, which contains soybean meal, rice protein concentrate, potato protein concentrate, fish meal and dietary FSP, on growth performance and apparent ileal amino acid digestibility in weanling pigs.

MATERIALS AND METHODS

Experimental design, animals and diets

Exp. 1. The experiment received prior approval from the Animal Protocol Review Committee of Dankook University. One hundred and twenty ((Yorkshire×Landrace)×Duroc) pigs (weaned on d 21±3) with average initial BW of 5.68±0.80 kg were randomly allotted to one of four treatments with six replications per treatment and five pigs per pen according to initial BW in accordance with a randomized complete block design. All of the pigs were housed in an environmentally controlled nursery facility with slatted plastic flooring and a mechanical ventilation system. Each pen was equipped with a one-sided, stainless-steel self-feeder and a nipple drinker that allowed pigs unlimited access to feed and water throughout the experiment.

Dietary treatments were as follows: i) simple diet; ii) simple diet with 5% FSP; iii) complex diet; iv) complex diet with 5% FSP. A two-phase nursery feeding program was employed in the experiment, with diets formulated to provide all of the nutrients to meet or exceed NRC (1998) requirements (Tables 1 and 2). The experiment lasted for 30 d and pigs were fed phase 1 diet for 20 d, and then switched to the same commercial phase 2 diet for 10 d to determine the effects of previous diets on subsequent performance.

Exp. 2 Twelve weaned pigs (6.13±0.92 kg) had 7 d to adapt to their surroundings before the surgery. Pigs had *ad libitum* access to feed during this period. Feed was withdrawn from each animal 12 h before surgery, but the pigs had continuous access to water. Pigs were surgically fitted with a simple T-cannula at approximately 10 cm cranial to the ileo-cecal junction, according to procedures adapted from Sauer et al. (1983). The cannulation site was cleaned daily with soft detergent and warm water during the entire experiment. In addition, anesthesia was then induced by injecting the pigs with Strenial (Janssen Pharmaceutica, Belgium) and Zoletil 50 (Virbac Lab, Korea). The pigs were fed 2 meals daily until full feeding was achieved 4 d after surgery. A period of 10 d was allowed for surgical recovery before the beginning of the experiment.

The twelve ileal-cannulated, weanling barrows were housed in individual metabolism crates and randomly assigned to 1 of 4 treatments (same as Exp. 1) by using a 4×4 Latin square design. The 4 feeding periods consisted of 4 d diet acclimation followed by d 5 of freshly feces samples collection and d 6 and 7 of ileal-digesta collection. The samples were then stored at -20°C until analysis. Ileal digesta were collected for 12 h periods between the morning and evening feeding (8:00 am and 8:00 pm) on d 6 and 7. After feeding, every 20 min the digesta were emptied into plastic containers and placed on ice. The daily feed allowance was $0.095\times BW^{0.75}$, as proposed by NRC (1998). Chromic oxide (0.20%) was added as an inert indicator to allow digestibility determination. The samples of digesta were then freeze-dried and finely ground for further analysis.

Producing fermented soy protein

Common soybean meal was fermented by inoculating *Aspergillus oryzae* GB-107 under anaerobic conditions for 48 h. FSP was produced by solid-state fermentation techniques (Genebiotech, Korea).

Sampling and measurements

Individual pig BW and pen feed consumption were measured on d 10, 20 and 30 of the experiment and utilized in the determination of average daily gain (ADG), average daily feed intake (ADFI) and gain: feed ratio (G/F).

Chromium oxide (Cr_2O_3) was added to the diet at 0.20% as an indigestible marker to calculate the digestibility coefficient on d 10, 20 and 30 for 5 d prior to the collection. On d 10, 20 and 30, fecal grab samples were then collected at random from at least two pigs in each pen. After collection, all feed and feces samples were stored immediately at -20°C until analysis. Before chemical analysis, fecal samples were dried at 70°C for 72 h and finely ground to pass through a 1 mm screen. The procedures utilized for the determination of DM and N was conducted in accordance with the methods established by the AOAC (1990). Chromium levels were determined via UV absorption spectrophotometry (Shimadzu, UV-1201, Japan). Nitrogen was determined by a Kjectec 2300 Nitrogen Analyzer (Itecator, Tecator AB, Hoganas, Sweden). Amino acid digestibility of the experimental feed was

Table 1. Experimental diets composition for d 0-20 (as-fed basis)

Diet complexity	Simple		Complex	
FSP level	0%	5%	0%	5%
Ingredient (%)				
Expanded corn	31.74	32.97	40.64	38.90
Whey powder	15.58	15.58	15.58	15.58
Soybean meal (dehulled)	33.09	27.09	14.96	14.99
Fermented soy protein	-	5.00	-	5.00
Rice protein concentrate	-	-	3.75	2.50
Potato protein concentrate	-	-	3.75	2.50
Fish meal	-	-	3.75	2.50
Bakery by-product	10.00	10.00	10.00	10.00
Lard	5.12	4.92	3.48	3.82
Tricalcium phosphate	1.32	1.31	1.09	1.16
Yeast culture	1.00	1.00	1.00	1.00
Organic acid	0.63	0.63	0.63	0.63
Zinc oxide	0.30	0.30	0.30	0.30
Salt	0.20	0.20	0.20	0.20
L-lys·HCl (98%)	0.27	0.27	0.28	0.27
DL-met (97%)	0.20	0.19	0.13	0.15
Thr (96%)	0.19	0.18	0.11	0.13
Vitamin premix[1]	0.18	0.18	0.18	0.18
Mineral premix[2]	0.18	0.18	0.18	0.18
Chemical composition[3]				
ME (kcal/kg)	3,557	3,559	3,533	3,543
NE (kcal/kg)	2,640	2,640	2,640	2,640
CP	21.00	21.00	21.00	21.00
Lys	1.40	1.41	1.40	1.41
Met	0.50	0.50	0.52	0.52
Met+Cys	0.85	0.85	0.86	0.86
Thr	0.83	0.83	0.87	0.86
Ca	0.78	0.78	0.78	0.78
Total P	0.66	0.67	0.63	0.64
Lactose	10.50	10.50	10.50	10.50

[1] Provided per kg diet: 20,000 IU of vitamin A; 4,000 IU of vitamin D_3; 80 IU of vitamin E; 16 mg of vitamin K_3; 4 mg of thiamine; 20 mg of riboflavin; 6 mg of pyridoxine; 0.08 mg of vitamin B_{12}; 120 mg of niacin; 50 mg of Ca-pantothenate; 2 mg of folic acid and 0.08 mg of biotin.
[2] Provided per kg diet: 80 mg of Fe; 140 mg of Cu; 179 mg of Zn; 12.5 mg of Mn; 0.5 mg of I; 0.25 mg of Co and 0.4 mg of Se.
[3] Calculated value.

determined, following acid hydrolysis with 6N HCl at 110°C for 24 h, using an amino acid analyzer (Biochrom 20, Pharmacia Biotech, England). Sulfur-containing amino acids were analyzed after cold performic acid oxidation overnight and subsequent hydrolysis.

Statistical analysis

All data in this experiment were analyzed by ANOVA using a 2×2 factorial arrangement of treatments, with the pen being considered as the experimental unit. The ANOVA was performed according to the GLM procedures of SAS (1996). The main effects of the diet complexity and FSP treatments, as well as the interaction were included in the model. When a significant interaction was observed, the means of each treatment were compared using Fisher's protected least significant difference test. Variability in the data is expressed as the standard error of the means (SE) and probability values less than 0.05 were considered significant.

RESULTS

Growth performance

Table 3 presents the effects of diet complexity and

Table 2. Compositions of basal diets (as-fed basis)

Ingredient (%)	Phase 2 (days 20 to 30)
Extruded maize	48.42
Extruded soyabean meal	35.07
Extruded oat	5.00
Soyabean oil	4.03
Fish meal	3.00
Monocalcium phosphate	0.90
Organic acid	0.10
Tricalcium phosphate	0.98
Yeast culture	0.60
Zinc oxide	0.30
Salt	0.20
Vitamin premix[1]	0.18
Mineral premix[2]	0.13
L-lysine·HCl	0.12
Antioxidant	0.05
DL-methionine	0.02
Chemical composition (%)	
ME (kcal/kg)	3,336
CP	21.97
Lys	1.40
Met	0.40
Ca	0.90
P	0.80

[1] Provided per kg diet: 20,000 IU of vitamin A; 4,000 IU of vitamin D_3; 80 IU of vitamin E; 16 mg of vitamin K_3; 4 mg of thiamine; 20 mg of riboflavin; 6 mg of pyridoxine; 0.08 mg of vitamin B_{12}; 120 mg of niacin; 50 mg of Ca-pantothenate; 2 mg of folic acid and 0.08 mg of biotin.

[2] Provided per kg diet: 80 mg of Fe; 140 mg of Cu; 179 mg of Zn; 12.5 mg of Mn; 0.5 mg of I; 0.25 mg of Co and 0.4 mg of Se.

[3] Calculated value.

dietary FSP on growth performance in weanling pigs. During d 0-10, pigs fed FSP diets had greater ADG than those fed non-FSP diets (p<0.05). ADG was not affected by dietary treatments during d 10-20, d 20-30, d 0-20 or the overall period (p>0.05). No significant differences in ADFI were observed throughout the whole period (p>0.05). During d 0-10 and the overall period, G/F in FSP treatments was significantly higher compared with non-FSP treatments (p<0.05). No interaction was observed in growth performance throughout the experiment.

DM and N digestibility

DM and N digestibility are presented in Table 4. During d 0-10, N digestibility was higher in pigs fed FSP diets than those fed non-FSP diets (p<0.05) and the highest DM and N digestibility were observed in pigs fed the complex diet with FSP and the lowest in those fed the simple diet without FSP (p<0.05). Diet complexity did not influence DM and N digestibility (p>0.05). No interactive effect was detected in this trial.

Apparent ileal amino acid digestibility

Table 5 shows AID of amino acid digestibility. AID of Met and Val among the essential amino acid were increased in pigs fed FSP diets compared with those fed non FSP diets (p<0.05). AID of Met, Phe and total essential amino acid were higher in pigs fed complex diets than those fed simple diets (p<0.05). Among the non essential amino acid, AID of Ala in FSP treatments was greater than that in non-FSP treatments (p<0.05). Besides, AID of Asp, Cys, Glu, Pro, Ser and total non essential amino acid in pigs fed complex diets were higher than those fed simple diets (p<0.05). Interactive effect was observed in AID of Met, Asp and Pro (p<0.05).

DISCUSSION

In the current study, ADG and G/F during d 0-10 as well as overall G/F was increased by FSP treatments. In agreement with our study, Jones et al. (2010) observed pigs fed increasing concentrations of FSP had improved G/F. Yun et al. (2005) reported that pigs fed fermented soy product grew faster during d 0-28 than those fed soybean meal diet. Cho et al. (2008) demonstrated that although feeding fermented soybean meal increased G/F and His and Lys digestibility, ADG was not improved. The improvement in G/F may be due to the extensive hydrolysis of protein resulting in readily available free amino acids and peptides which is a major characteristic of these fermentations (Sarkar et al., 1997; Kiers et al., 2000). However, FSP improved ADFI as dietary FSP level increased (Min et al., 2004), which was not confirmed in our study. We suggest that further research about the effect of FSP on ADFI is needed. Diet complexity had no effect on ADG, ADFI and G/F during every experimental period. When nursery diets are formulated, a primary objective is to select ingredients that will stimulate feed intake and maximize performance, which can be accomplished by increasing the complexity of the diet (Tokach et al., 1994). Besides, simple diets which contain high dietary soybean meal levels result in villus hypersensitivity to the soybean protein that decrease pig gains (Li et al., 1991). However, our data did not indicate adequate pig performance with the diet complexity. These results partially agree with Dritz et al. (1996) who indicated that diet complexity did not affect ADG and ADFI during the 7.0 to 18.9 kg period. In addition, the composition of protein sources used in our experiment was quite different from others. Most weanling diets contained high levels of animal proteins such as blood meal, plasma protein and fish meal, while our protein sources were composed of soybean meal, rice protein concentrate, potato protein concentrate and fish meal. Therefore, our data indicated that diet

Table 3. Effect of diet complexity and dietary FSP on growth performance in weaned pigs[1]

Item	Simple		Complex		SE[2]	p-value		
	-FSP	+FSP	-FSP	+FSP		FSP	Diet	Diet×FSP
d 0-10								
ADG (g)	224	242	239	249	6	0.03	0.19	0.64
ADFI (g)	336	321	354	337	13	0.33	0.76	0.43
G/F	0.667	0.754	0.675	0.739	0.025	0.02	0.60	0.89
d 11-20								
ADG (g)	486	491	480	488	17	0.74	0.51	0.86
ADFI (g)	844	866	854	831	26	0.62	0.94	0.51
G/F	0.576	0.567	0.562	0.587	0.018	0.30	0.77	0.40
d 21-30								
ADG (g)	560	583	574	585	16	0.22	0.67	0.73
ADFI (g)	950	947	1,017	945	29	0.66	0.43	0.58
G/F	0.589	0.616	0.596	0.632	0.019	0.57	0.86	0.61
d 0-20								
ADG (g)	355	366	360	369	9	0.65	0.86	0.47
ADFI (g)	590	594	604	584	15	0.48	0.77	0.51
G/F	0.602	0.616	0.596	0.632	0.014	0.55	0.63	0.42
d 0-30								
ADG (g)	423	439	431	441	10	0.42	0.81	0.63
ADFI (g)	710	711	742	704	17	0.28	0.72	0.92
G/F	0.596	0.617	0.581	0.626	0.013	0.03	0.37	0.52

[1] Fermented soy protein was contained 0 or 5% in simple and complex diets, respectively. [2] Pooled standard error.

complexity, which included a large amount of vegetable protein sources, had no effect on growth performance in the early-weaned period. This research failed to show any interaction in growth performance between FSP and diet complexity. Further studies are needed to evaluate the effects of FSP and diet complexity.

The improvement in N digestibility in FSP treatments could mirror the differences on ADG during d 0-10, which agreed with Min (2006) who reported that FSP did not affect DM digestibility while N digestibility was improved as FSP levels increased. Due to a high proportion of small peptide and low anti-nutritional (trypsin inhibitor, oilgosaccharides level) factors in FSP by microbial fermentation (*Aspergillus oryzae* and *Bacillus* spp.), beneficial results were expected but our study failed to identify FSP effect on DM and N digestibility for early-weaned pigs except digestibility of N during d 0-10. Besides, there were no significant differences on DM and N digestibility between simple diet with FSP and complex diet without FSP. The digestibility of DM and N was increased numerically in FSP diets and complex diets without statistical significance. This may reflect the lack of complex diets effect on growth performance in this experiment. No interactive effects were observed in nutrient digestibility which may be attributed to the high complexity of complex diets even without FSP. As we know, complex diets could

Table 4. Effect of diet complexity and dietary FSP on nutrient digestibility in weaned pigs[1]

Item	Simple		Complex		SE[2]	p-value		
	-FSP	+FSP	-FSP	+FSP		FSP	Diet	Diet×FSP
d 10								
DM	0.768	0.802	0.804	0.814	0.012	0.18	0.35	0.78
N	0.688	0.734	0.723	0.764	0.017	0.03	0.19	0.61
d 20								
DM	0.758	0.788	0.789	0.787	0.012	0.31	0.75	0.63
N	0.699	0.715	0.705	0.708	0.018	0.25	0.45	0.73
d 30								
DM	0.750	0.778	0.782	0.787	0.138	0.11	0.84	0.79
N	0.689	0.703	0.696	0.708	0.195	0.33	0.67	0.54

[1] Fermented soy protein was contained 0 or 5% in simple and complex diets, respectively. [2] Pooled standard error.

Table 5. Effect of diet complexity and dietary FSP on AID of amino acid in weaned pigs[1]

Item	Simple		Complex		SE[2]	p-value		
	-FSP	+FSP	-FSP	+FSP		FSP	Diet	Diet×FSP
Essential amino acid (EAA)								
Arginine	0.87	0.86	0.88	0.84	0.01	0.34	0.46	0.78
Histidine	0.69	0.72	0.70	0.67	0.02	0.23	0.62	0.52
Isoleucine	0.66	0.67	0.70	0.69	0.02	0.45	0.76	0.67
Leucine	0.68	0.72	0.73	0.68	0.02	0.18	0.42	0.35
Lysine	0.79	0.83	0.80	0.82	0.01	0.76	0.26	0.42
Methionine	0.72	0.73	0.77	0.81	0.01	0.03	<0.01	0.04
Phenylalanine	0.68	0.72	0.75	0.74	0.01	0.25	0.02	0.37
Threonine	0.73	0.76	0.76	0.75	0.01	0.44	0.56	0.73
Valine	0.66	0.71	0.68	0.69	0.01	0.04	0.71	0.28
Total EAA	0.72	0.74	0.75	0.74	0.01	0.35	0.03	0.91
Non essential amino acid (NEAA)								
Alainine	0.67	0.71	0.68	0.70	0.01	0.04	0.56	0.87
Asparatic acid	0.73	0.76	0.79	0.76	0.01	0.44	0.02	0.04
Cystine	0.69	0.63	0.70	0.70	0.01	0.29	0.03	0.41
Glutamic acid	0.77	0.78	0.81	0.81	0.01	0.57	0.03	0.34
Glycine	0.65	0.70	0.69	0.67	0.01	0.74	0.51	0.86
Proline	0.68	0.70	0.74	0.78	0.02	0.27	0.01	0.04
Serine	0.73	0.77	0.79	0.78	0.08	0.66	0.01	0.57
Tyrosine	0.67	0.72	0.74	0.67	0.02	0.70	0.48	0.85
Total NEAA	0.70	0.72	0.74	0.73	0.09	0.32	0.01	0.63

[1] Fermented soy protein was contained 0 or 5% in simple and complex diets, respectively. [2] Pooled standard error.

be highly digestible in early weanling period. However, the digestibility results were not always consistent. Some researchers demonstrated that FSP diet had higher DM and N digestibility than the SBM diet (Yun et al., 2005; Feng et al., 2007). These inconsistencies may be due to the differences in the levels of protein sources, weanling ages of pigs as well as the experimental environment.

Some apparent ileal amino acid digestibility such as Met, Val and Ala were higher in FSP treatments, the result was consistent with N digestibility and feed efficiency in the present research. However, the complex diets increased the mean of total essential amino acid digestibility and non-essential amino acid digestibility while soybean meal diet was the lowest. Min (2006) reported that individual essential amino acid digestibility of FSP diet was improved compared with SBM diet because FSP had a high amount of small peptide by microbial fermentation (Kim, 2004) that presented to the mucosa in short-chain peptide bound form (Rerat et al., 1992). Our lab previous studies showed that His and Lys digestibility was increased by FSP supplementation (Cho et al., 2008). Besides, researchers demonstrated that young pigs fed FSP were similar to pigs fed other protein sources (whey protein, fish meal, SPC, ISP and DSM) on the apparent amino acid digestibility (Shon et al., 1994; Yun et al., 2005). These data partially agreed with our research on non essential amino acid digestibility. Interestingly, interactive effect of FSP and diet complexity was observed in AID of Met, Asp and Pro. As has been stated, this is the first research that reported the interaction between FSP and diet complexity. Therefore, no comparisons could be made. It is hypothesized that this may be due to the additive effects of complex diets with FSP which were rich in such amino acid.

In conclusion, the current study indicated the feeding of 5% FSP to nursery pig improved feed efficiency and apparent ileal amino acid digestibility and adding 5% FSP to corn-soybean meal based diets can achieve comparable growth performance, DM, N and amino acid digestibility with a complex weanling diet. In addition, diet complexity did not maximize the pig growth performance in the subsequent phase. These results seem to be able to simplify and lessen the cost of weanling diet.

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