Asian-Aust. J. Anim. Sci.
Vol. 22, No. 8 : 1167 - 1173
August 2009

www.ajas.info

Effects of Fermented Soy Protein on Nitrogen Balance and Apparent Fecal and Ileal Digestibility in Weaned Pigs

J. S. Yoo, H. D. Jang, J. H. Cho, J. H. Lee and I. H. Kim*
Department of Animal Resource and Science, Dankook University
#29 Anseodong, Cheonan, Choognam, 330-714, Korea

ABSTRACT : This study was conducted to evaluate the effects of providing fermented soy protein to weaned pigs on nitrogen balance and apparent fecal and apparent ileal digestibility (AID) of AA. Four weaned ((Yorkshire×Landrace)×Duroc) barrows (BW = 6.58±0.98 kg), surgically fitted with a simple T-cannula approximately 15 cm prior to the ileo-cecal junction, were fed four diets according to 4×4 Latin square design. Diets were a basal diet supplemented with one of the following: 3% SDPP (spray dried plasma protein), 5% RBP (soy protein fermented by *Lactobacillus spp.*), 5% PSP (soy protein fermented by *Aspergillus oryzae* and *Bacillus subtilis*), and 2.5% RPP (2.5% RBP+2.5% PSP). No differences were observed in DM and N intakes among treatments. However, the level of urine excretion was greater in the RPP group than in the PSP group. Additionally, fecal DM excretion, fecal N concentration and fecal N excretion were increased in the RBP, PSP and RPP groups when compared with the SDPP group ($p<0.05$). Furthermore, total excretion was increased in the RPP group when compared with the PSP group ($p<0.05$). In addition, N absorption and the N absorption ratio were higher in the SDPP group than in the RPP group ($p<0.05$). Moreover, the DM and N digestibilities were lower in the RBP, PSP and RPP groups than in the SDPP group ($p<0.05$), and the ash and energy digestibilities were higher in the SDPP and RBP groups than in the PSP and RPP groups ($p<0.05$). However, no significant differences were observed in the DM, N, Ash, Ca, P or ileal digestibilities among treatments, although the energy digestibility was higher in the SDPP group than the RBP group ($p<0.05$). In addition, the apparent ileal digestibilities of essential amino acids (Arg, His, Iso, Leu, Lys, Phe, Thr, and Val) were significantly higher in the SDPP group than in the other groups ($p<0.05$), and the levels of Ala, Cys, Glu and Try were greater in the SDPP treatment group than the RBP, PSP and RPP groups ($p<0.05$). Additionally, the levels of Asp, Gly and Ser were higher in the SDPP group than the PSP and RPP groups, and the level of Pro was higher in the SDPP group than the RPP group ($p<0.05$). Finally, total non-essential amino acid and total amino acid digestibility were higher in the SDPP group than in the other treatments ($p<0.05$). Taken together, the results of this study indicate that animal protein is more bioavailable than plant protein. However, the N absorption ratio and ileal digestibility were found to be similar in the SDPP and RBP groups. (**Key Words :** Fermented Soy Protein, N Balance, Ileal Digestibility, Amino Acid Digestibility, Pigs)

INTRODUCTION

Due to the availability, cost and risk factors associated with diseases from animal protein sources that are traditionally used to feed weaned pigs many studies have been conducted to evaluate alternative plant protein sources (Li, et al., 2003). Plant protein sources are generally cheaper per unit of nutrient than animal protein sources, however, they are also lower in some essential amino acids, energy and minerals, such as phosphorus, when compared with animal protein supplements. Therefore, although the cost of animal protein sources is generally higher than that of plant protein sources, the rate at which animal proteins are consumed is lower. Conversely, by-products of blood and milk are more likely to carry pathogens than refined protein products of plant origin. Soybean meal is a plant protein source that is readily available, relatively inexpensive, and contains a high quantity of proteins, however, due to the presence of anti-nutritional factors such as trypsin inhibitors, haemagglutinins, raffinose and stachyose, its use has result in poor growth performance and low digestibility in weanling pigs (Anderson et al., 1979). Therefore, most processed soy proteins that are widely used as feed are heat treated (Osborne and Mendel, 1917; Hancock et al., 1990), extruded (Burnham et al.,

* Corresponding Author: I. H. Kim. Tel: +82-41-550-3652, Fax: +82-41-553-1618, E-mail: inhokim@dankook.ac.kr
Received May 2, 2008; Accepted March 21, 2009

2000; Shiyan et al., 2003), purified (Hancock et al., 1989) or defatted (Jones et al., 1989) to decrease the concentrations of anti-nutritional factors. However, fermentation may also decrease or eliminate anti-nutritional factors (Mital and Garg, 1990; Hachmeiste and Fung, 1993; Reddy and Pierson, 1994). In East Asia, soybeans have traditionally been prepared for use as foods commonly known as 'Dou-Bian Jiang' in China, 'Miso' in Japan, and 'Duen-Jang' in Korea using the fermentation process. These fermented soy foods are beneficial to human health because they provide benefits such as high digestibility, high calcium content, high vitamin A and B content, laxative effects, and cancer prevention effects (Lee, 1998: Kim et al., 1999).

Therefore, this study was conducted to evaluate the nitrogen balance and apparent fecal and apparent ileal digestibility in weaned pigs fed processed soy protein.

MATERIALS AND METHODS

Fermented soy protein source

The experimental diets evaluated in this study included plant protein sources that were comprised of two types of fermented soy protein, one that was fermented by *Lactobacillus spp.* (RubyZyme®, CTC Bio INC., Korea), and one that was fermented by *Aspergillus oryzae* and *Bacillus subtilis* (PepSoyGen®, Genebiotech, Korea).

Animal and surgery treatment

Four weaned pigs that were 21±3 days of age (Landrace ×Yorkshire×Duroc; barrows) were surgically fitted with simple T-cannulas approximately 15 cm prior to the ileo-cecal junction according to procedures adapted from Sauer et al. (1983). The pigs were fasted for 16 to 20 h prior to surgery. Anesthesia was then induced by injecting the pigs

Table 1. Experimental diet composition

Ingredient (%)	SDPP[1]	RBP[1]	PSP[1]	RPP[1]
Expender corn	45.06	44.32	44.32	44.32
Soybean meal	26.00	26.00	26.00	26.00
Whey	12.50	12.50	12.50	12.50
Spray-dried porcine plasma	3.00			
RBP(RubyZyme)		5.00		2.50
PSP(PepSoyGen)			5.00	2.50
Bakery by-product	5.00	5.00	5.00	5.00
Soy oil	4.00	4.00	4.00	4.00
Limestone	0.20	0.20	0.20	0.20
Monocalcium phosphate	0.95	0.85	0.85	0.85
Zinc oxide	0.30	0.30	0.30	0.30
L-lysine-HCl (78%)	0.26	0.31	0.31	0.31
Salt	0.25	0.25	0.25	0.25
DL-methionine (50%)	0.24	0.22	0.22	0.22
Threonine	0.08	0.12	0.12	0.12
Choline chloride (50%)	0.10	0.10	0.10	0.10
Mineral premix[2]	0.10	0.10	0.10	0.10
Vitamin premix[3]	0.20	0.20	0.20	0.20
Oxytetracycline	0.05	0.05	0.05	0.05
Calculated composition (%)				
ME (Kcal/kg)	3,536	3,530	3,530	3,530
Crude protein	20.78	21.02	21.02	21.02
Calcium	0.75	0.75	0.75	0.75
Phosphorus	0.74	0.74	0.74	0.74
Lysine	1.42	1.42	1.42	1.42
Methionine	0.50	0.51	0.51	0.51
Analyzed composition (%)				
Crude protein	20.82	21.20	21.19	21.10
Calcium	0.78	0.76	0.76	0.76
Phosphorus	0.71	0.75	0.72	0.74
Lysine	1.40	1.41	1.40	1.42
Methionine+cystamine	0.95	0.94	0.95	0.96

[1] SDPP = 3% SDPP; RBP = 5% RubyZyme; PSP = 5% PepSoyGen; RPP = 2.5% RubyZyme+2.5% PepSoyGen.

[2] Provided the following per kilogram of diet: Mn 8 mg, Cu 20 mg, Zn 60 mg, Fe 80 mg, I 0.36 mg, Co 0.4 mg, Se 0.16 mg.

[3] Provided the following per kilogram of diet: Vitamin A 7,800 IU, Vitamin D_3 1,560 IU, Vitamin E 26 IU, Vitamin K 1.95 mg, Thiamin 1.95 mg, Riboflavin 4 mg, Vitamin B_6 1.95 mg, Vitamin B_{12} 26 μg, Niacin 19.5 mg, Pantothenic acid 13 mg, Folic acid 0.65 mg, Biotin 0.13 mg.

Table 2. Essential amino acid profiles of the experiment diet

Item (%)	SDPP[1]	RBP[1]	PSP[1]	RPP[1]
Essential amino acid				
Arginine	1.26	1.27	1.27	1.3
Histidine	0.66	0.64	0.66	0.66
Isoleucine	0.83	0.81	0.84	0.85
Leucine	1.68	1.61	1.69	1.64
Lysine	1.40	1.41	1.40	1.42
Methionine	0.37	0.40	0.54	0.41
Phenylalanine	0.97	0.96	0.98	0.99
Threonine	0.92	0.88	0.88	0.9
Valine	0.91	0.86	0.88	0.89
Non essential amino acid				
Alanine	0.96	0.92	0.96	0.93
Aspartic acid	2.09	2.07	2.1	2.1
Cysteine	0.67	0.57	0.61	0.6
Glutamic acid	4.15	4.14	4.35	4.23
Glycine	0.77	0.78	0.78	0.79
Proline	1.03	1.02	1.11	1.03
Serine	1.03	1.00	1.03	1.01
Tyrosine	0.75	0.72	0.73	0.73

[1] SDPP = 3% SDPP; RBP = 5% RubyZyme; PSP = 5% PepSoyGen; RPP = 2.5% RubyZyme+2.5% PepSoyGen.

with Stresnil™ (Janssen Pharmaceutica, Belgium) and Zoletil 50 (Virbac Lab., Korea). Following surgery, the barrows were individually housed in 1.2×0.6 m stainless steel metabolism crates in a temperature controlled (28°C) room. The pigs were then allowed 12 d of recovery prior to initiation of the experiments and the detailed description of pre- and post- operative care was previously described by Li et al. (1994).

Experimental design and feeding

The experimental treatments evaluated in this study included diets that were supplemented with the following: SDPP (3% SDPP), RBP (5% RubyZyme), PSP (5% PepSoyGen) and RPP (2.5% RubyZyme+2.5% PepSoyGen) (Tables 1 and 2). The experimental design were comprised of 4×4 Latin squares with pigs and periods as the blocking criteria. Pigs were allowed to adjust to the experimental diets for 4 d in each period. Feces and urine were then collected at d 5, and ileal-digested samples were collected from d 6 to 7 during each experimental period. The daily feed allowance was $0.05\times BW^{0.9}$, as proposed by Armstrong and Mitchell (1955). The daily feed was provided as two meals at 12 h intervals (8:00 a.m. and 8:00 p.m.), and water was provided *ad libitum*. Chromic oxide (0.2%) was added to the diet as an indigestible marker to allow digestibility determinations.

Nitrogen balance and apparent fecal digestibility

Feces were collected twice daily and stored at -20°C until further analysis. Urine produced each day was collected into a bottle, which was subsequently acidified by the addition of 25 ml of 6 N HCl. The urine volume was measured daily, filtered through glass wool, and a 5% aliquot was then collected and stored at -20°C until further analysis. For analysis, each fecal sample was thawed and dried at 65°C before being ground using a Wiley mill with a 1-mm screen. The fecal and urine samples were then analyzed for N balance and apparent nutrient digestibility.

Apparent ileal amino acid digestibility

Ileal digesta were collected during the 24 h period between the morning and evening feeding for the last 2 d of each collection period. Ileal digesta were collected into plastic bags attached to the cannulas, with the digesta being emptied into plastic containers and placed on ice every 20 minutes during the collection period.

The collected digesta were pooled and frozen, after which they were lyophilized and ground. The feed, feces and ileal digesta were then analyzed for dry matter, nitrogen, energy, ash, calcium and phosphorus concentration (AOAC, 1994) by determining the amount of chromium present by UV absorption spectrophotometry (Shimadzu, UV-1201, Japan) and then calculating the apparent ileal digestibilities using the indirect method. The amino acids digestibility of the experimental feed was determined, following acid hydrolysis with 6 N 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

Data were analyzed by ANOVA according to a randomized complete block design using GLM procedures

Table 3. Effect of different protein sources on apparent N balance in weaned pigs

Item	SDPP[1]	RBP[1]	PSP[1]	RPP[1]	SE[2]
DM intake (g/d)	397.40	383.40	390.36	391.97	8.47
N intake (g/d)	13.39	12.81	13.27	13.41	0.29
Urine excretion (g/d)	782.60^{ab}	781.20^{ab}	747.60^{b}	860.00^{a}	30.84
Urine N (%)	0.25	0.22	0.25	0.28	0.03
Urine N excretion (g/d)	1.96	1.72	1.87	2.41	0.23
Fecal excretion (g/d)	55.85	71.20	66.63	62.13	4.98
Fecal DM (%)	22.04^{c}	27.22^{b}	28.75^{b}	33.62^{a}	1.57
Fecal DM excretion (g/d)	12.31^{b}	19.38^{a}	19.16^{a}	20.89^{a}	1.73
Fecal N (%)	4.10^{b}	4.49^{a}	4.61^{a}	4.62^{a}	0.07
Fecal N excretion (g/d)	0.50^{b}	0.87^{a}	0.88^{a}	0.97^{a}	0.07
Total excretion (g/d)	838.45^{ab}	852.40^{ab}	814.23^{b}	922.13^{a}	31.83
Total N excretion (g/d)	2.46	2.59	2.75	3.38	0.25
N absorption (g/d)	10.93^{a}	10.22^{ab}	10.52^{ab}	10.03^{b}	0.21
N absorption ratio (%)	81.63^{a}	79.78^{a}	79.28^{ab}	74.79^{b}	1.67

[1] SDPP = 3% SDPP; RBP = 5% RubyZyme; PSP = 5% PepSoyGen; RPP = 2.5% RubyZyme+2.5% PepSoyGen.
[2] Pooled standard error.
[a,b] Means in the same row with different superscripts differ ($p<0.05$).

of SAS (1996). Duncan's multiple range test (Duncan, 1955) was used to determine significant differences among treatments.

RESULTS

Nitrogen balance

The result of the nitrogen balance experiment is shown in Table 3. No differences were observed in the DM and N intake among treatments. However, the urine excretion was greater in the RPP group than in the PSP group ($p<0.05$). In addition, the fecal DM excretion, fecal N concentration, and fecal N excretion were greater in the RBP, PSP and RPP groups than in the SDPP group ($p<0.05$). Furthermore, total excretion was greater in the RPP group than in the PSP group ($p<0.05$). However, the N absorption and the N absorption ratio were higher in the SDPP group than the RPP group ($p<0.05$).

Apparent fecal digestibility

The DM and N digestibilities were lower in the RBP, PSP and RPP groups than in the SDPP group ($p<0.05$, Table 4). The ash and energy digestibilities were higher in the SDPP and RBP groups than in the PSP and RPP groups ($p<0.05$).

Apparent ileal digestibility

No differences were observed in the DM, N, Ca and P

Table 4. Effect of different protein sources on apparent fecal digestibility in weaned pigs

Item (%)	SDPP[1]	RBP[1]	PSP[1]	RPP[1]	SE[2]
Dry matter	96.89^{a}	94.91^{b}	94.78^{b}	94.40^{b}	0.58
Nitrogen	96.27^{a}	93.21^{b}	93.37^{b}	92.77^{b}	0.64
Ash	84.71	84.13	83.73	83.57	0.57
Ca	81.63^{a}	79.77^{a}	74.20^{b}	73.17^{b}	1.42
P	73.90^{a}	68.42^{ab}	64.35^{b}	64.99^{b}	2.14
Energy	90.81^{a}	89.33^{a}	85.97^{b}	86.75^{b}	0.58

[1] SDPP = 3% SDPP; RBP = 5% RubyZyme; PSP = 5% PepSoyGen; RPP = 2.5% RubyZyme+2.5% PepSoyGen.
[2] Pooled standard error.
[a,b] Means in the same row with different superscripts differ ($p<0.05$).

Table 5. Effect of different protein sources on apparent ileal digestibility of nutrient in weaned pigs

Item (%)	SDPP[1]	RBP[1]	PSP[1]	RPP[1]	SE[2]
Dry matter	82.48	80.09	81.05	80.61	0.78
Nitrogen	85.77	84.53	84.48	84.36	0.90
Ash	81.00^{a}	81.00^{a}	77.36^{b}	77.32^{b}	0.57
Ca	74.68	74.10	75.03	74.91	1.06
P	63.92	64.17	61.76	61.64	2.59
Energy	85.54^{a}	83.27^{b}	83.89^{ab}	84.33^{ab}	0.59

[1] SDPP = 3% SDPP; RBP = 5% RubyZyme; PSP = 5% PepSoyGen; RPP = 2.5% RubyZyme+2.5% PepSoyGen.
[2] Pooled standard error.
[a,b] Means in the same row with different superscripts differ ($p<0.05$).

digestibilities among treatments (Table 5). However, the ash digestibility was higher in the SDPP and RBP groups than in the PSP and RPP groups ($p<0.05$). Moreover, energy digestibility was higher in the SDPP group than the RBP group ($p<0.05$).

Apparent ileal amino acid digestibility

The apparent ileal digestibilities of Arg, Iso, Leu, Lys, Phe, Thr and Val were significantly higher in the SDPP group than in the other groups ($p<0.05$). In addition, the His digestibility was higher in the SDPP group than the PSP and RPP groups ($p<0.05$). Additionally, the Ala, Cys, Glu and Try digestibilities were higher in the SDPP treatment group than the RBP, PSP and RPP groups ($p<0.05$). Furthermore, the Asp, Gly and Ser digestibilities were higher in the SDPP group than the PSP and RPP groups, and the Pro digestibility was higher in the SDPP group than the RPP group ($p<0.05$). Finally, the total non-essential amino acids and total amino acid digestibility were higher in the SDPP group than the other groups ($p<0.05$).

DISCUSSION

Generally, supplementation of the diets of weaned pigs with animal protein sources has been found to improve nitrogen availability. For example, Min (2006) reported that N retention was greater in pigs that received fermented soy protein than in those that received dried skim milk, but that it was much greater in pigs fed fish meal. The results of our study showed that the N absorption was not significantly different between the SDPP, RBP and PSP groups.

Makkink (1993) reviewed the digestibility of different protein sources in various trials and concluded that the digestibility of animal protein was superior to that of soybean meal during the post-weaning period, regardless of the method used to process the soybean meal, which is similar to the results of the current study. In addition, Min (2006) showed that DM and N digestibilities were higher in pigs fed a diet containing dried skim milk than in those fed fermented soy protein, which is also in agreement with the results of our study. Furthermore, Yun et al. (2005) reported that the digestibilities of GE, DM and CP in animal protein (whey protein concentrate and fish meal) were higher than those of plant protein (fermented soy protein and rice protein concentrate). This finding is similar to the results of our experiment, which showed that the digestibility of SDPP was higher than that of fermented soy protein. However, no significant differences in ileal digestibility were observed between the SDPP and fermented soy protein groups in our study, which is consistent with the result of a study conducted by Chae et al. (1999).

The digestibility and absorbability of fermented soy beans has been shown to be much higher than that of cooked and autoclaved soybeans (Kiers et al., 2000). However, this effect is not obvious when fermented soy beans are compared with SDPP. It is likely that soy beans fermented with bacteria such as *Lactobacillus* or *Bacillus* can improve feed utilization via extensive hydrolysis of protein, which results in the formation of readily available free amino acids and peptides (Steinkraus, 1996; Sarkar et al., 1997). Previous studies conducted on mice have shown that treatment with SDPP can improve the nitrogen intakes and retention. Additionally, the present study showed that the fecal and urinary nitrogen outputs were similar, which implied that the nitrogen digestibility was greater for the SDPP diet (Thomson et al., 1995). In the present study, fecal excretion was not affected by the SDPP or fermented soy protein treatment, which is similar to the results of the aforementioned study. However, in the current study, fecal nitrogen concentration was decreased and total nitrogen absorption was increased in the SDPP group, which indicates that the retention of nitrogen was increased by treatment with SDPP. Soy beans that were fermented showed increased and the absorbability was much higher in

Table 6. Effect of different protein sources on apparent ileal amino acid digestibility in weaned pigs

Item (%)	SDPP[1]	RBP[1]	PSP[1]	RPP[1]	SE[2]
Essential amino acid					
Arginine	93.13[a]	90.44[b]	88.14[b]	88.95[b]	0.74
Histidine	85.88[a]	79.36[ab]	73.07[b]	76.18[b]	2.56
Isoleucine	90.97[a]	84.65[b]	82.90[b]	83.35[b]	1.55
Leucine	90.29[a]	84.61[b]	82.56[b]	82.41[b]	1.34
Lysine	91.71[a]	87.14[b]	86.39[b]	85.14[b]	1.3
Methionine	93.05	90.63	92.44	90.58	0.98
Phenylalanine	90.95[a]	85.35[b]	83.07[b]	84.00[b]	1.46
Threonine	86.93[a]	81.34[b]	78.53[b]	79.58[b]	1.65
Valine	89.59[a]	81.93[b]	80.04[b]	79.90[b]	1.8
TEAA[3]	89.17[a]	83.94[b]	82.26[b]	81.76[b]	1.45

[1] SDPP = 3% SDPP; RBP = 5% RubyZyme; PSP = 5% PepSoyGen; RPP = 2.5% RubyZyme+2.5% PepSoyGen.

[2] Pooled standard error.

[3] TEAAD = Total essential amino acid; TNEAA = Total non essential amino acid.

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

these beans than in cooked and autoclaved beans (Kiers et al., 2000). However, this effect was not obvious when pigs treated with fermented soy beans were compared with those treated with SDPP. It has been shown that the use of *bacillus* fermented soybeans is likely to improve feed utilization due to the extensive hydrolysis of protein, which produces readily available free amino acids and peptides (Steinkraus, 1996; Sarkar et al., 1997). The urinary output was significantly higher in pigs that receive the RPP treatment than in those that received the PSP treatment, therefore, further studies should be conducted to evaluate the effects of different types of fermented soy protein.

Sohn et al. (1994) reported that processed soybean proteins, such as isolated or concentrated soybean protein, were utilized almost as well as milk protein in 21 to 35 day-old pigs. This indicates that processed soybean protein may have almost the same bioavailability of compounds responsible for the elimination of factors that are detrimental to performance. Previous studies (Giesting and Easter, 1991) have shown that processed soy protein sources result in a digestibility between that of milk proteins and soybean meal.

CONCLUSION

Animal protein has more bioavailability than plant protein. However, fermentation of soybean protein using different methods results in no effects on apparent fecal and ileal amino acid digestibility. Further studies are necessary to determine the compensation quantity of fermented soy protein necessary to replace animal protein.

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