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Effects of Fermented Fish Meal on N Balance and Apparent Total Tract and Ileal Amino Acid Digestibility in Weanling Pigs

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Abstract: Two experiments were conducted to evaluate fermented fish meal as a new animal protein source on N balance, Apparent Total Tract (ATTD) and ileal AA Digestibility (AID) in weanling pigs. In Exp. 1: total 40 [(Landrace×Yorkshire)×Duroc] barrows were randomly allocated 4 treatments (10 pigs per treatment) for a 12 days metabolic trial. The dietary treatments of Exp. 1 and 2 were SFM (small fish), LFM (small fish fermented with *L. acidophilus*), AMFM (Anchovy-Mackerel Fish Meal, LT®) and MFM (Mackerel Fish Meal, BIO-CP®). N concentrations in the feces were found to be greater in pigs fed SFM than pigs fed AMFM ($p<0.05$). The AID of Arg, Val and Cys was greater in pigs fed LFM than in SFM ($p<0.05$). The AID of all AA except Met, Tyr was greater in pigs fed MFM than in SFM ($p<0.05$). In Exp. 2: 16 [(Landrace×Yorkshire)×Duroc] barrows were surgically equipped with a T-cannula in the distal ileum. They were allotted 4 treatments (4 pigs per treatment) for evaluating the AID. The AID of His, Thr and total AA was greater in pigs fed AMFM and MFM than in SFM ($p<0.05$). The AID of Val was greater in pigs fed MFM than in SFM ($p<0.05$). The AID of Asp and Ser was greater in pigs fed MFM than in SFM ($p<0.05$). The AID of Glu was greater in pigs fed AMFM and MFM than in SFM ($p<0.05$). The AID of dispensable and total AA was greater in pigs fed LFM, AMFM and MFM than in SFM ($p<0.05$).

Key words: Fermented fish meal, N balance, ileal AA digestibility, weanling pigs, AID, Korea

INTRODUCTION

Fish meals are traditionally recognized as very digestible proteins that contain greater amounts of AA, vitamins and minerals (Kim and Easter, 2001). Stoner *et al.* (1990) reported that 4-8% of fish meal could replace 10% of the dried whey weanling pigs diets and improve ADG, ADFI and G:F. However, Wiseman *et al.* (1991) indicated that fish meal is highly variable because of the quality of fish and processing factors. Fish meal can increase histamine content depending upon the kind of fish, storage method and time.

Leuschner *et al.* (1998) reported that some micro-organisms (*Brevibacterium linens*, *Staphylococcus carno*, *Geotrichum candidum* and *Micrococcus varians*) which were suitable for food fermentation were also effective in degrading histamine in food. Dapkevicius *et al.* (2000) demonstrated that fish waste fermented by Lactic Acid Bacteria (LAB) decreased the acidic pH value to below 4.5 and degraded histamine levels by 50-54%. This procedure is safe, economically advantageous and environmentally friendly according to Dapkevicius *et al.* (2000). Thus, we hypothesized that fish meal fermented with micro-organisms that decrease histamine would improve growth performance, N retention and nutrient digestibility in weanling pigs compared with non-fermented fish meal and could be used in nursery diets, replacing other high-quality protein fish meals. This study was conducted to evaluate fermented fish meal as a new animal protein source on N balance, Apparent Total Tract (ATTD) and Ileal AA digestibility (AID) in weanling pigs.

MATERIALS AND METHODS

The Animal Care and Use Committee of Dankook University approved all the experimental protocols used in the current study.

Fish meals: Four different fish meals, SFM (small fish; drying temperature: 70°C; Genebiotech. Co. Ltd., Seoul, Korea), LFM (small fish meals fermented with *L. acidophillus*; drying temperature: 60-70°C; Genebiotech. Co. Ltd., Seoul, Korea), AMFM (anchovy and jack mackerel; drying temperature: 70°C; LT®, Hinrichsen Tradnding S.A., Santiaga, Chile) and MFM

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Table 1: Analyzed values of amino acid and histamine in experimental fish meals

	Fish meals			
Item	SFM[1]	LFM[1]	AMFM[1]	MFM[1]
CP (%)	61.29	61.23	68.54	66.24
Lys (%)	5.10	5.28	5.21	5.01
Met (%)	1.50	1.45	1.98	1.81
Cys (%)	0.69	0.71	0.68	0.61
Thr (%)	2.61	2.42	2.97	2.91
Trp (%)	0.65	0.67	0.78	0.74
Ile (%)	2.14	2.34	2.42	2.99
Val (%)	2.89	2.90	3.35	3.52
Histamine (ppm)	540.03	271.00	500 (max)[2]	500 (max)[2]

[1]SFM (Small Fish based Fish Meal) and LFM (SFM fermented with *L. acidophilus*) were supplied by Genebiotech. Co. Ltd (Seoul, Korea). AMFM (Anchovy-Mackerel based Fish Meal: LT®) and MFM (Mackerel based Fish Meal: BIO-CP®) were supplied by Hinrichsen Tradnding S.A (Santiaga, Chile). [2]The values were provided by the manufacturer (Hinrichsen tradnding S.A)

Table 2: Molecular peptide size in experimental fish meals

	Fish meals (%)			
Item (kDa)	SFM[1]	LFM[1]	AMFM[1]	MFM[1]
0-10	-	5	20	60
10-15	-	20	35	30
15-27	-	50	30	10
27-55	30	15	15	-
>55	70	10	-	-

[1]SFM (Small fish based Fish Meal) and LFM (SFM fermented with *L. acidophilus*) were supplied by genebiotech. Co. Ltd (Seoul, Korea). AMFM (Anchovy-Mackerel based Fish Meal: LT®) and MFM (Mackerel based Fish Meal: BIO-CP®) were supplied by Hinrichsen tradnding S.A (Santiaga, Chile)

(mackerel; drying temperature: 70°C; BIO-CP®, Hinrichsen Tradnding S.A. Santiaga, Chile) were evaluated in weanling pigs. The analyzed values of fish meals are shown in Table 1. The *L. acidophillus* was selected from MRS broth agar and Luria-Bertani, Miller (BD, USA), respectively after being selected from fresh or salted fish. The molecular peptide size of fish meals are shown in Table 2.

Exp. 1: Animals, diets, design and sample collection: A total 40 [(Landrace×Yorkshire)×Duroc] barrows (8.88±0.3 kg) were individually housed in housed in stainless steel metabolism cage and randomly allotted to 4 treatment diets in an environmentally controlled room and allowed 7 and 5 days to adapt and collect to cages. There were 10 replications per treatment. Dietary treatments were SFM (Small fish based Fish Meal), LFM (SFM fermented with *L. acidophilus*), AMFM (Anchovy-Mackerel based Fish Meal) and MFM (Mackerel based Fish Meal). Ingredients and nutrient compositions of the diets were shown in Table 3. Experimental diets were formulated to meet or exceed the nutrient requirements recommended by NRC (1998). Pigs were provided a daily quantity of feed that supplied 2.4 times the estimated maintenance requirement for energy

Table 3: Composition of experimental diets (as fed basis)

Item	SFM	LFM	AMFM	MFM
Ingredient (%)				
Corn, ground	45.810	45.810	46.000	47.120
Soybean meal, dehulled (47% CP)	26.000	26.000	26.000	26.000
Dried whey	13.700	13.700	13.750	13.750
Non-fermented fish meal	5.000	-	-	-
Fermented fish meal	-	5.000	-	-
LT	-	-	5.000	-
BIO-CP	-	-	-	5.000
Soy oil	3.900	3.900	3.550	2.500
Fine sugar	3.000	3.000	3.000	3.000
Monocalcium phosphate	1.520	1.520	1.720	1.780
Salt, iodized	0.230	0.230	0.200	0.200
Vitamin/mineral Premix[1]	0.300	0.300	0.300	0.300
L-Lys. HCl (78%)	0.250	0.250	0.220	0.130
DL-Met (99%)	0.190	0.190	0.170	0.130
L-Thr (99%)	0.100	0.100	0.090	0.090
Calculated composition				
CP (%)	21.320	21.320	21.570	21.550
Lys (%)	1.300	1.300	1.300	1.300
Met (%)	0.410	0.410	0.410	0.410
Cys (%)	0.360	0.360	0.360	0.360
Thr (%)	0.840	0.840	0.840	0.840
Try (%)	0.250	0.250	0.250	0.250
Ile (%)	0.830	0.830	0.830	0.830
Val (%)	0.860	0.860	0.860	0.860
Ca (%)	0.840	0.840	0.750	0.750
P (%)	0.750	0.750	0.700	0.730
DE (kcal kg^{-1})	3,300.000	3,300.000	3,300.000	3,300.000

[1]Provides per kg of complete diet: 3,300 IU vitamin A; 440 IU vitamin D_3; 22 IU vitamin E; 3.2 mg vitamin K; 6.6 mg riboflavin; 16.5 mg pantothenic acid; 33 mg niacin; 0.99 mg folic acid; 0.11 mg biotin; 16.5 mg vitamin B_{12}; 2.2 mg vitamin B_6 and 150 mg zinc (as $ZnSO_4$); 120 mg iron (as $FeSO_4 \cdot 7H_2O$); 12 mg copper (as $CuSO_4 \cdot 5H_2O$; 45 mg manganese; 1.5 mg iodine (as KI); 0.3 mg selenium (as $Na_2SeO_3 \cdot 5H_2O$)

(i.e., 106 kcal ME $kg^{-0.75}$; NRC, 1998) during the experiments. Daily feed allotments were offered as two meals fed in 12 h intervals (0600 and 1800). Water was available to the pigs at all times. The beginning and end of the collection period were marked by the addition of 0.25% Chromic oxide (Cr_2O_3) to the morning meal (on day 8 and 13). During the collection periods, the total amounts of feces were collected daily stored in plastic bags and frozen at -20°C until the end of the collection period. Urine collection started immediately after feeding the morning meal on day 8 and ceased immediately after feeding the morning meal on day 13. The total urine volumes were recorded each day and 10% of the total volume was frozen subsequent for analysis of the N concentration until analysis. Retention of N was obtained by subtracting the daily output in urine and feces from the daily intake.

Exp. 2: Animals, diets, design and sample collection: A total 16 [(Landrace×Yorkshire)×Duroc] barrows were surgically fitted with simple T-cannulas approximately 15 cm prior to the ileo-cecal junction. The pigs were fasted for 16-20 h prior to surgery. Anesthesia was induced using Stresnil™ (Janssen Pharmaceutica, Belgium) and

Virbac Zoletil 50 injections (Virbac Laboratory, France). After surgery, the barrows were housed individually in stainless steel metabolism crates in a temperature controlled (28°C) room. The pigs were permitted day 10 of recovery prior to the initiation of the experiments. The pigs were blocked by initial body weight (8.7±0.2 kg) and allocated randomly to one of four dietary treatment groups in a randomized complete block design. The 7 day was a period of adaptation to the experimental diets and ileal digesta were collected on d 8 and 9 (12 h day^{-1}). The daily feed allowance and dietary treatments were same as described in Exp. 1. The ileal digesta were collected between 0600 and 1800 for 2 days by attaching a transparent 100 mL latex collection bag to the cannulas. During the 12 h collection period, digesta were collected every 30 min and immediately frozen at -20°C. The samples were then freeze-dried and finely ground prior to analysis for chromium (Kimura and Miller, 1957).

Chemical analysis: For feed, fecal and ileal digesta samples, the chromium concentration was determined via UV absorption spectrophotometry (Shimadzu, UV-1201, Japan) and the ileal apparent digestibilitiy was calculated via indirect methods. N content was determined by using a Kjeltec 2300 Analyzer (Foss Tecator AB, Hoeganaes, Sweden). Amino acids (excluding tryptophan) were analyzed by dansylation (Beckman Intruments Inc., Fullerton, CA) and HPLC after acid hydrolysis for 24 h in 6 M HCl.

Statistical analyses: In this study, all data were analyzed in accordance with a randomized complete block design using GLM procedures of SAS (1996) with each pen comprising one experimental unit. Also, data was compared according to the means of treatments via Duncan's multiple range test (Duncan, 1955). Variability in the data was expressed as the Standard Error of Mean (SEM) and the selected level of significance was 0.05.

RESULTS AND DISCUSSION

Exp. 1: N balance and Apparent Total Tract Digestibility (ATTD): N balance and biological values are provided in Table 4. N concentrations in the feces were greater in pigs fed SFM than in pigs fed AMFM ($p<0.05$). There were no significant differences in total N excretion, N retention and biological values among treatments ($p>0.05$). The ATTD is shown in Table 5. The ATTD of Arg, Val and Cys was greater in pigs fed LFM than in SFM ($p<0.05$). The ATTD of all AA except Met, Tyr was greater in pigs fed MFM than in SFM ($p<0.05$).

Table 4: N balance in weaning pigs fed each fish meal (Exp. 1)

Item	SFM	LFM	AMFM	MFM	SE[1]
N intake (g day^{-1})	9.08	9.04	9.21	9.23	0.12
Urine excretion (g day^{-1})	835.00	857.00	834.00	826.00	80.00
N concentration in urine (%)	0.24	0.23	0.23	0.25	0.04
N excretion in urine (g day^{-1})	2.00	1.97	1.91	2.06	0.47
Fecal excretion (g day^{-1})	77.00	70.00	69.00	61.00	7.00
DM concentration in feces (%)	28.10	28.80	27.80	29.10	1.22
DM excretion in feces (g day^{-1})	21.60	20.20	19.20	17.80	2.11
N concentration in feces (%)	4.41[a]	4.17[ab]	4.00[b]	4.25[ab]	0.12
N excretion in feces (g day^{-1})	0.95	0.84	0.78	0.75	0.10
Total fecal and urine excretion (g day^{-1})	912.00	927.00	903.00	887.00	81.00
Total N excretion (g day^{-1})	2.95	2.81	2.69	2.81	0.46
N retention (g day^{-1})	6.13	6.23	6.52	6.42	0.44
N retention (% of N intake)	67.50	68.90	70.80	69.60	4.82
Biological value[2] (%)	74.98	80.10	77.75	74.34	5.32

[a,b]Means in the same row with different superscripts differ ($p<0.05$). [1]Standard error. [2](N intake-urinary N excretion-fecal N excretion)/(N intake-fecal N excretion)×100

Table 5: Apparent Total Tract Digestibility (ATTD) of AA in weanling pigs fed each fish meal (Exp. 1)

Item (%)	SFM	LFM	AMFM	MFM	SE[1]
Indispensable AA					
Arg	82.70[b]	86.96[a]	87.22[a]	88.24[a]	1.29
His	68.66[b]	72.18[ab]	75.31[ab]	77.77[a]	2.14
Ile	69.55[b]	75.99[ab]	76.77[a]	78.03[a]	2.36
Leu	73.98[b]	78.65[ab]	79.65[ab]	81.00[a]	1.19
Lys	79.49[b]	82.43[b]	83.60[ab]	87.57[a]	1.54
Met	79.76	81.45	84.67	85.66	1.90
Phe	74.33[b]	78.97[ab]	79.13[ab]	80.89[a]	1.95
Thr	73.03[b]	77.88[ab]	78.45[ab]	80.83[a]	2.00
Val	66.02[b]	73.73[a]	74.77[a]	76.40[a]	2.54
Mean	74.19[b]	78.69[ab]	79.95[ab]	81.82[a]	1.19
Dispensable AA					
Ala	63.47[b]	69.12[ab]	72.62[a]	74.99[a]	2.65
Asp	75.52[b]	79.73[ab]	80.92[a]	82.26[a]	1.76
Cys	65.90[b]	74.02[a]	71.92[ab]	75.27[a]	2.20
Glu	80.99[b]	84.33[ab]	84.79[ab]	85.88[a]	1.33
Gly	66.85[b]	72.82[ab]	73.91[ab]	75.86[a]	2.35
Pro	74.88[b]	80.29[ab]	79.32[ab]	82.17[a]	1.83
Ser	76.65[b]	80.38[ab]	80.63[ab]	82.42[a]	1.59
Tyr	73.80	77.43	78.21	79.55	2.07
Mean	72.26[b]	77.26[ab]	77.79[ab]	79.80[a]	1.92
Total	73.28[b]	78.02[ab]	78.93[ab]	80.87[a]	1.91

[a,b]Means in the same row with different superscripts differ ($p<0.05$); [1]Standard error

Exp. 2: Apparent Ileal Digestibility (AID): The AID is shown in Table 6. With regards to the AID of indispensable AA, the AID of His, Thr and total AA was greater in pigs fed AMFM and MFM than in SFM ($p<0.05$). The AID of Val was greater in pigs fed MFM than in SFM ($p<0.05$). With respect to the AID of dispensable AA, the AID of Asp and Ser was greater in pigs fed MFM than in SFM ($p<0.05$). The AID of Glu was greater in pigs fed AMFM and MFM than in SFM ($p<0.05$). The AID of dispensable and total AA was greater in pigs fed LFM, AMFM and MFM than in SFM ($p<0.05$).

Fermented foods have been consumed by humans in Southeast Asian countries but food fermentation technology remains limited with regard to its application

Table 6: Apparent ileal digestibility of AA in weanling pigs fed each fish meal (Exp. 2)

Item (%)	SFM	LFM	AMFM	MFM	SE[1]
Indispensable AA					
Arg	71.66	73.95	76.13	76.64	2.78
His	56.43^{b}	60.73^{ab}	64.18^{a}	65.96^{a}	1.18
Ile	72.90	72.71	75.50	75.51	2.17
Leu	68.29	70.26	72.77	74.09	2.06
Lys	72.11	76.39	75.92	77.14	2.15
Met	71.04	70.79	70.87	73.60	1.51
Phe	70.93	75.62	76.81	77.73	2.51
Thr	63.06^{b}	66.60^{ab}	75.10^{a}	75.06^{a}	3.31
Val	66.13^{b}	72.01^{ab}	72.91^{ab}	76.84^{a}	2.81
Mean	68.06^{b}	71.01^{ab}	73.38^{a}	74.73^{a}	1.21
Dispensable AA					
Ala	68.39	72.53	70.98	73.97	2.00
Asp	71.82^{b}	74.89^{ab}	75.49^{ab}	77.81^{a}	1.63
Cys	59.09	62.34	65.62	61.37	2.16
Glu	66.27^{b}	73.44^{ab}	77.07^{a}	78.04^{a}	3.35
Gly	62.48	67.42	69.24	69.22	3.30
Pro	66.26	71.08	70.87	71.26	1.82
Ser	63.69^{b}	70.22^{ab}	69.70^{ab}	73.13^{a}	2.58
Tyr	72.68	73.80	74.95	75.68	3.24
Mean	66.34^{b}	70.72^{a}	71.74^{a}	72.56^{a}	1.19
Total	67.25^{b}	70.87^{a}	72.61^{a}	73.71^{a}	0.93

^{a,b}Means in the same row with different superscripts differ ($p<0.05$); [1]Standard error

in animal production sector. Currently, there is little information on feeding pigs fermented fish meal. However, Hong *et al*. (2004) found that fermentation of SBM by *A. oryzae* decreased the size of peptides and most of the peptides in fermented SBM were smaller than 10 kDa. In this study, fermentation of SFM by *L. acidophilus* decreased the size of peptides. Although, 70% of peptides in SFM were larger than 55 kDa, 75% of peptides in LFM were smaller than 27 kDa. The fermentation process decreased histamine of SFM that is allergenic compound. Fish and fish products contain high level histamine which is a biogenic amine and occurs from the microbial decarboxylation of His (Lehane and Olley, 2000). Biogenic amines can be degraded by Diamine Oxidase (DAO). Dapkevicius *et al*. (2000) demonstrated that fish waste can be advantageously upgraded into animal feed by fermentation with Lactic Acid Bacteria (LAB). Therefore, decrease in size of peptides and histamine in FM can affect utilization of protein source for weanling pigs.

In Exp. 1, pigs fed SFM diet evidenced 10% higher fecal nitrogen concentration than that of pigs fed the AMFM diet and had reduced the ATTD of AA compared with pigs fed LFM (Arg, Val and Cys), AMFM (Arg, Ile, Val, Ala and Asp) and MFM (most of AA). In Exp. 2, the AID of dispensable and total AA was greater in pigs fed LFM, AMFM and MFM than in pigs fed SFM. However, with regards to the ATTD and AID of AA except Lys (Exp. 1), pigs fed LFM did not show significant differences as compared with pigs fed high-quality fish meals (AMFM and MFM). This result can be attributed to the increased availability of fermented fish meal and also to the fact that it contains small peptides which are readily absorbed via fermentation with *L. acidophilus*. Microbial fermentation may result in a high amount of small peptides (Kim, 2004). Generally, small peptides in the mucosal membranes are more easily absorbed by the gastrointestinal tract (Rerat *et al*., 1992); protein products adsorbed in the small intestine are primarily in the form of small peptides rather than AA (Ganapathy and Leibach, 1999).

Fermented diets may be an alternative for the prophylactic use of antimicrobial growth promoters in pig diets (Scholten *et al*., 1999). Feeding a fermented diet minimizes the time available for the gastrointestinal microflora to decarboxylate free amino acids present in the diet which has shown to improve growth performance in pigs (Scholten, 2001; Pedersen *et al*., 2002; Pedersen, 2006). A promising area for future research would be analyzing the effects of feeding liquid feed containing fermented liquid cereal grains as a means of avoiding microbial decarboxylation of free amino acids and increasing feed intake by improving palatability (Canibe *et al*., 2007).

MFM is a high quality fish meal after a hydrolysis process, it may have more short-chained peptides which could have positive effects on the AA absorption. Husby (1991) reported that a salmon protein hydrolysate has been found to improve growth when fed to nursery pigs at up to 10% of the diet. Folador *et al*. (2006) reported that 10% salmon protein hydrolysate increased the feed intake of dogs. In addition within studies of fish populations, feeds containing hydrolysates have been shown to improve growth and digestive system development (Day *et al*., 1997; Zmbonino *et al*., 1997; Cahu *et al*., 1999).

Although, peptides are considered a protein source in livestock industry, it may be important to balance the protein: peptide ratio in diets provided to pigs. Taken together, the results of this study revealed that N retention and the ATTD and AID of total AA were similar in pigs fed fermented fish meal using *L. acidophillus* and those fed hydrolysated fish meal. These findings suggest that fermented fish meal (by *L. acidophillus*) could potentially be used to replace imported fish meal for weanling pigs.

CONCLUSION

In this study, fermented fish meal with *L. acidophilus* improves the AID of total AA compared with non-fermented fish meal and provides N balance and the ATTD and AID of total AA similar to recognized high quality fish meals. It should therefore be considered as a potential ingredient for weanling pigs.

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