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Nitrogen balance and ileal nutrient digestibility in weanling pigs fed spray dried plasma protein and fermented fish meals¤

Balance de nitrógeno y digestibilidad ileal de nutrientes en cerdos destetos alimentados con proteína de plasma seco y harinas de pescado fermentadas

Balance do nitrogênio e digestibilidade ileal de nutrientes em suínos desmamados, alimentados com proteína de plasma seco e farinas de peixe fermentadas

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Summary

This experiment was conducted to evaluate the effects of spray dried plasma protein (SDPP) and fermented (L. acidophilus and B. licheniformis) fish meals on nitrogen (N) balance and ileal nutrient digestibility of weanling pigs. Sixteen crossbred [(Landrace × Yorkshire) × Duroc] nursery pigs (8.58 ± 0.32 kg) were surgically fitted with a T-cannula at the distal ileum. The dietary treatments were: 1) FM (5% unfermented fish meal), 2) FFA (5% fermented fish meal with L. acidophilus), 3) FFL (5% fermented fish meal with B. licheniformis), and 4) SDPP (3% Spray dried plasma protein, AP920®, APC, Inc, Ames, IA). The N retention was greater in the SDPP treatment than in other treatments (p<0.05), and biological value was greater in the SDPP treatment than in the FM and FFL treatments (p<0.05). Dry matter (DM) and N digestibility were higher in the SDPP treatment than in other treatments (p<0.05). When calcium (Ca) digestibility was evaluated, it was found to be higher in the SDPP treatment than in the FM and FFA treatments (p<0.05). The FFA and FFL treatments had a higher calcium (Ca) digestibility than the FM treatment (p<0.05). Arginine, histidine and isoleucine digestibility were higher in the SDPP treatment than in the FM and FFL treatments (p<0.05). Lysine digestibility was higher in the SDPP treatment than in the FFL treatment (p<0.05). Compared to all dietary treatments, phenylalanine and valine digestibility were the greatest in the SDPP group (p<0.05). Glutamic acid and tyrosine digestibility were higher in the SDPP treatment than in the FM and FFL treatments (p<0.05). In conclusion, compared with feeding 3% SDPP, no positive effects were

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observed on N retention or nutrient digestibility in weanling pigs on the 5% fermented fish meal diets. Thus, SDPP provides a better nutrient digestibility outcome than the use of fermented fish meal.

Key words: *fermentation, fish meal, ileal amino acid digestibility, nitrogen balance, weanling pigs.*

Resumen

Este experimento se realizó para evaluar los efectos de la proteína de plasma seco (SDPP) y harinas de pescado fermentadas (L. acidophilus y B. licheniformis) sobre el balance de nitorgeno (N) y la digestibilidad ileal de nutrientes en cerdos al destete. Dieciséis cerdos mestizos [(Landrace × Yorkshire) × Duroc] destetos (8.58 ± 0.32kg) fueron quirúrgicamente equipados con cánulas-T en el íleon distal. Los tratamientos dietarios fueron: 1) FM (5% harina de pescado no fermentada), 2) FFA (5% harina de pescado fermentada con L. acidophilus), 3) FFL (5% de harina de pescado fermentada con B. licheniformis), y 4) SDPP (3% proteína de plasma seco, AP920®, APC, Inc, Ames, IA). La retención de N fue mayor en el tratamiento SDPP que en los otros tratamientos (p <0.05) y el valor biológico fue mayor en el tratamiento SDPP que en la FM y los tratamientos FFL (p <0.05). La digestibilidad de la materia seca (MS) y del N fue mayor en el tratamiento SDPP que en los otros tratamientos (p <0.05). Cuando se evaluó la digestibilidad del calcio (Ca), se encontró que era mayor en el tratamiento SDPP que en FM y FFA (p <0.05). Los tratamientos FFA y FFL tuvieron mayor digestibilidad del calcio que el tratamiento FM (p <0.05). La digestibilidad de la arginina, histidina e isoleucina fue mayor en el tratamiento SDPP que en los tratamientos FM y FFL (p <0.05). La digestibilidad de la lisina fue mayor en el tratamiento SDPP que en el tratamiento FFL (p <0.05). En comparación con todos los tratamientos dietarios, la digestibilidad de la fenilalanina y valina fue mayor en el grupo SDPP (p <0.05). La digestibilidad del ácido glutámico y de la tirosina fueron mayores en el tratamiento SDPP que en los tratamientos FM y FFL (p <0.05). En conclusión, en comparación con la inclusión del 3% de SDPP, no se observaron efectos positivos en la retención de N o la digestibilidad de nutrientes en cerdos destetos alimentados con 5% de harina de pescado fermentada. Por lo tanto, el SDPP proporciona una mejor digestibilidad de nutrientes que el uso de harina de pescado fermentada.

Palabras clave: *balance de nitrógeno, cerdos destetos, digestibilidad ileal de aminoácidos, harina de pescado.*

Resumo

O experimento foi realizado para avaliar os efeitos da proteína do plasma seco (SDPP) e de farinas de peixe fermentadas (L. acidophilus e B. licheniformis) sobre o balance do nitrogênio (N) e a digestibilidade ileal de nutrientes em suínos desmamados. Dezesseis suínos mestiços [(Landrace × Yorkshire) × Duroc] desmamados (8.58 ± 0.32kg) foram cirurgicamente equipados com cânulas-T no ileo distal. Os tratamentos foram: 1) FM (5% farina de peixe no fermentada), 2) FFA (5% farina de peixe fermentada com L. acidophilus), 3) FFL (5% de farina de peixe fermentada com B. licheniformis), e 4) SDPP (3% proteína de plasma seco, AP920®, APC, Inc, Ames, IA). A retenção de N foi maior no tratamento SDPP (p <0.05) e o valor biológico foi maior no SDPP que na FM e os tratamentos FFL (p <0.05). A digistibilidade da matéria seca (MS) e do N foi maior o tratamento SDPP (p <0.05). Quando foi avaliado a digestibilidade do cálcio (Ca), encontrou-se que foi maior no tratamento SDPP que em FM e FFA (p <0.05). Os tratamentos FFA e FFL tiveram maior digestibilidade do Ca que o tratamento FM (p <0.05). A digestibilidade da arginina, histidina e isoleucina foi maior no tratamento SDPP que nos tratamentos FM e FFL (p <0.05). A digestibilidade da lisina foi maior no tratamento SDPP que no tratamento FFL (p<0.05). A digestibilidade da fenilalanina e valina foi maior no grupo SDPP (p <0.05). A digestibilidade do ácido glutâmico e da tirosina foram maiores no tratamento SDPP que nos tratamentos FM e FFL (p <0.05). Em conclusão, a inclusão de 3% de SDPP, não tem efeito positivo na retenção de N ou a digestibiidade de nutrientes em suínos desmamados, alimentados com 5% de farina de peixe fermentada, portanto, o SDPP proporciona uma maior digestibilidade de nutrientes que o uso de farina de peixe fermentada.

Palavras chave: *balance de nitrogênio, digestibilidade ileal de aminoácidos, farina de peixe, suinos.*

Introduction

Spray dried plasma protein (SDPP) is a protein source that has a balanced amino acid profile and immunoglobulins. When young pigs consume a diet containing SDPP, the results include increased growth performance (daily gain, feed intake and feed efficiency) (Gatnau and Zimmerman, 1990; Hansen *et al*., 1993; Kats *et al*., 1994) and reductions in the extent and severity of diarrhea (Coffey and Cromwell, 2001; van Dijk *et al*., 2001). However, these protein sources are expensive, and consequently must improve growth performance appreciably in order to be cost effective.

Fish meal is traditionally recognized as a very digestible protein that has a high concentration of amino acids, vitamins, and minerals (Kim and Easter, 2001). Stoner *et al*. (1990) reported that replacing a portion of the soybean meal in a starter diet with select menhaden fish meal resulted in improved average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio. Certain low quality fish meals may contain high levels of histamine, a biogenic amine attributed to food poisoning in poultry (KoÈ se, 1993). Shifrine *et al*. (1959) demonstrated that high histamine levels resulted in reduced growth rates and depression among chicks.

Dapkevicius *et al*. (2000) demonstrated that fish waste could be reprocessed into animal feed by fermentation with lactic acid bacteria (LAB). Leuschner *et al*. (1998) reported that microorganisms, (*Brevibacterium linens, Staphylococcus carno, Geotrichum candidum,* and *Micrococcus varians)* which were suitable for food fermentation were also effective in degrading histamine in food. We hypothesized that fermented fish meal would serve as an optimal protein source for weanling pigs. Therefore, the current study was designed to evaluate the use of SDPP and fermented (*L. acidophillus* and *B. licheniformis*) fish meals on N balance and ileal nutrient digestibility in weaned pigs.

Materials and methods

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

Experimental design, animals, and diets

Sixteen crossbred [(Landrace × Yorkshire) × Duroc] pigs (8.5 ± 0.3 kg) were fasted for 16 hours and then surgically fitted with T-cannulas approximately 15 cm proximal of the ileo-cecal junction. Anesthesia was induced using injected Stresnil™ (Janssen Pharmaceutica, Belgium) and Virbac Zoletil 50 (Virbac Laboratory, France). Following surgery, the barrows were housed individually in stainless steel metabolism crates within a temperature controlled (28 °C) room. The pigs were then permitted 14 d of recovery prior to the initiation of the experiment. The pigs were blocked based on their initial body weight and then randomly allocated to one of four dietary treatments in a randomized complete block design that included four replications per treatment. The daily feed allowance, which was 0.05 ´ $BW^{0.9}$, was fed in two meals at 12 h intervals (0600 and 1800). The experimental period also included 7 d of adjustment to the experimental diets and 2 d (12 h/d) of ileal digesta collection.

Dietary treatments were as follows: 1) FM (5% unfermented fish meal), 2) FFA (5% fermented fish meal with *L. acidophilus*), 3) FFL (5% fermented fish meal with *B. licheniformis*) and 4) SDPP (3% Spray-dried plasma protein, AP920®, APC, Inc, Ames, IA). The ingredients and nutrient compositions of the diets are provided in table 1. The diets were formulated to meet or exceed the nutrient requirements recommended by the NRC (1998).

Table 1. Formulation and chemical composition of the experimental diet.

Ingredient, %	FM[1], FFA[1] and FFL[1]	SDPP[2]
Corn	46.49	47.98
Soybean meal (46% local)	26.00	26.00
Whey (Lactalis France)	13.70	14.34
SDPP[2]	-	3.00
Fish meal[1]	5.00	-
Soy oil	3.90	4.00
Fine Sugar	3.00	2.00
Mono-calcium phosphate	0.44	0.95
Limestone	-	0.20
Salt	0.23	0.25
Zinc oxide	0.30	0.30
Vitamin/Mineral premix[3]	0.30	0.30
HCl-L-Lysine	0.25	0.26
DL-Methionine	0.19	0.24
L-Threonine	0.10	0.08
Choline chloride	0.10	0.10
Chemical composition[4]		
Net energy, MJ/kg	11.07	11.07
CP, %	21.32	21.22
Ca, %	0.84	0.80
P, %	0.75	0.74
Lys, %	1.30	1.30
Met, %	0.51	0.50
Met + Cys, %	0.80	0.80

[1] FM: small fish based fish meal; FFA: FM fermented with ***L. acidophilus***; FFL: FM fermented with ***B. licheniformis.***

[2] SDPP (spray dried plasma protein) product was supplied by APC, Inc (Ames, IA).

[3] 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; 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 $Na_2SeO_3 \cdot 5H_2O$).

[4] Calculated values were derived from the NRC (1998).

Sampling and Analysis

To evaluate the ileal nutrient digestibility, chromic oxide (Cr_2O_3) was added at a level of 0.20% of the diet as an indigestible marker from the initial day of the experiment. Fresh fecal samples were collected from the crates for 2 days (on day 10 and 11), with the samples being dried and pooled for subsequent analysis following collection. In addition, urine samples were collected by placing a bucket under the crates, added with 100 ml of 10% HCL. The total urine volumes were recorded each day, and 10% of the urine was frozen for later analysis to determine the N concentration. The ileal digesta was collected between 0600 and 1800 for 2 days (on d 8 and 9) continuously 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 then immediately frozen. At the conclusion of the experimental collection period, the digesta collected from each pig during the respective periods were homogenized and a 200-g subsample was obtained. Feed, feces and ileal samples were then freeze-dried and finely (1 mm) ground prior to analysis for chromium (AOAC, 1995). The chromium concentration was determined via UV absorption spectrophotometry (Shimadzu, UV-1201, Japan), and the apparent digestibility was then calculated via indirect methods. In addition, the amino acid digestibility of the experimental feed was determined after 24 hours of acid hydrolysis with 6N HCl at 110 °C using an amino acid analyzer (Biochrom 20, Pharmacia Biotech, England). The sulfur-containing amino acids were analyzed following cold performic acid oxidation overnight and subsequent hydrolysis. The gross energy was analyzed using an oxygen bomb calorimeter (Parr, 6100, USA).

Fish Meals

Three types of fish meal were prepared for this experiment: FM, FFA and FFL. FM (small fish; drying temperature: 80 °C), FFA and FFL (fermented small fish; drying temperature: 60 °C) were evaluated in weanling pigs. The analyzed values of amino acid and histamine of fish meals are shown in table 2. The *L. acidophillus* and *B. licheniformis* were selected from MRS broth agar and Luria-Bertani, Miller (BD, USA), respectively, after being selected from fresh or salted fish.

Table 2. Analyzed values of amino acids in experimental fish meal (as-feed basis).

Item	FM[1]	FFA[1]	FFL[1]	SDPP[2]
Arginine	3.41	3.97	4.01	4.62
Histidine	2.45	2.53	2.98	2.14
Isoleucine	2.14	2.94	2.65	3.68
Leucine	4.12	4.65	4.28	7.37
Lysine	5.10	5.28	5.15	7.79
Methionine	1.10	1.45	1.95	0.74
Phenylalanine	2.89	2.81	2.88	3.94
Threonine	2.61	2.42	2.89	2.57
Valine	2.84	3.25	2.98	4.73
Alanine	3.98	4.12	3.87	4.86
Asparatic acid	5.31	5.98	6.11	6.05
Cystine	0.49	0.71	0.72	2.61
Glutamic acid	7.56	8.14	7.95	11.00
Glycine	4.91	4.56	4.28	2.73
Proline	3.10	2.45	2.88	3.53
Serine	2.78	2.12	2.65	4.85
Tyrosine	2.15	1.98	2.14	3.69
Histamine, ppm	242.62	160.26	62.60	-

[1] FM: small fish based fish meal; FFA: FM fermented with ***L. acidophilus***; FFL: FM fermented with ***B. licheniformis.***

[2] SDPP (spray dried plasma protein) product was supplied by APC, Inc (Ames, IA).

Statistical Analysis

In this experiment, all data were analyzed using a randomized complete block design following GLM procedures of SAS (SAS Inst. Inc., Cary, NC), with each pen being used as the experimental unit. The treatment means were compared by using Duncan's multiple range test (Duncan, 1955). Variability in the data was expressed as the SE of the mean and the selected level of significance was set at 0.05.

Results

Nitrogen Balance

N balance and biological values are provided in table 3. N concentration in urine was the lowest in the SDPP treatment among treatments ($p<0.05$). N excretion in urine and DM excretion in fecal were lower in the SDPP treatment than the FM and FFL treatments ($p<0.05$). The SDPP treatment had a lower N concentration and excretion in the feces than the FM and FFL treatments ($P<0.05$). The FFA treatment had a lower N concentration and excretion in the feces than the FM treatment ($p<0.05$). When the total N excretion was evaluated, it was found to be lower in the SDPP treatment compared to the other treatments ($p<0.05$). N retention was greater in the SDPP treatment than in other treatments ($p<0.05$) and the biological value was greater in the SDPA treatment than in the FM and FFL treatments ($p<0.05$).

Table 3. Nitrogen balance in weaning pigs fed each protein source.

Items	FM[1]	FFA[1]	FFL[1]	SDPP[2]	SE[3]
N intake, g/d	10.78	10.77	10.78	10.76	0.04
Urine excretion, g/d	857	843	834	788	73
N concentration in urine, %	0.27[a]	0.26[a]	0.30[a]	0.14[b]	0.03
N excretion in urine, g/d	2.35[a]	2.14[ab]	2.49[a]	1.10[b]	0.36
Fecal excretion, g/d	70.75	67.25	65.75	57.50	5.86
DM concentration in fecal, %	34.75	33.50	34.88	32.00	1.38
DM excretion in fecal, g/d	25.82[a]	22.25[ab]	23.50[a]	18.25[b]	1.65
N concentration in fecal, %	4.56[a]	4.25[bc]	4.40[ab]	4.11[c]	0.08
N excretion in fecal, g/d	1.18[a]	0.95[bc]	1.02[ab]	0.75[c]	0.06
Total fecal and urine excretion, g/d	936	910	899	845	73
Total N excretion, g/d	3.53[a]	3.09[a]	3.51[a]	1.85[b]	0.38
N retention, g/d	7.25[a]	7.68[a]	7.27[a]	8.91[b]	0.37
N retention, % of N intake	67.30[a]	71.33[a]	67.45[a]	82.80[b]	0.48
Biological value[4], %	75.48[a]	78.03[ab]	74.48[a]	88.98[b]	3.73

[1] FM: small fish based fish meal; FFA: FM fermented with ***L. acidophilus***; FFL: FM fermented with ***B. licheniformis.***

[2] SDPP (spray dried plasma protein) product was supplied by APC, Inc (Ames, IA).

[3] Pooled standard error.

[4] (N intake - urinary N excretion - fecal N excretion) / (N intake – fecal N excretion) × 100.

[a, b, c] Different superscripts reflect significant differences among means in the same row ($p<0.05$).

Apparent Total Tract Digestibility of Nutrients and Ileal Amino Acid Digestibility

Apparent total tract nutrient digestibility is shown in table 4. DM and N digestibility were higher in the SDPP treatment than in other treatments ($p<0.05$). When Ca digestibility was evaluated, it was found to be higher in the SDPP treatment than in the FM and FFA treatments ($p<0.05$). The FFA and FFL treatments had a higher Ca digestibility than FM treatment ($p<0.05$).

Table 4. Apparent total tract digestibility in weaning pigs fed each protein source.

Items, %	FM[1]	FFA[1]	FFL[1]	SDPP[2]	SE[3]
DM	84.93[a]	83.85[a]	84.47[a]	88.93[b]	1.42
N	83.55[a]	84.05[a]	83.22[a]	86.93[b]	1.12
Ash	54.00	55.02	54.06	54.92	1.79
Ca	51.70[a]	54.98[b]	56.39[ab]	58.20[c]	0.82
P	58.40	58.96	58.90	58.62	1.34
GE	74.98	76.29	75.04	74.71	1.94

[1] FM: small fish based fish meal; FFA: FM fermented with ***L. acidophilus***; FFL: FM fermented with ***B. licheniformis.***

[2] SDPP (spray dried plasma protein) product was supplied by APC, Inc (Ames, IA).

[3] Pooled standard error.

[a, b] Different superscripts reflect significant differences among means in the same row ($p<0.05$).

Apparent ileal amino acid digestibility is provided in table 5. Arginine, histidine and isoleucine digestibility were higher in the SDPP treatment than in the FM and FFL treatments ($p<0.05$). Lysine digestibility was higher in the SDPP treatment than in the FFL treatment ($p<0.05$). Phenylalanine and valine digestibility were the greatest in the SDPP treatment among all treatments ($p<0.05$). Glutamic acid and tyrosine digestibility were higher in the SDPP treatment than in the FM and FFL treatments ($p<0.05$).

Table 5. Apparent ileal amino acid digestibility in weaning pigs fed each protein source.

Items, %	FM[1]	FFA[1]	FFL[1]	SDPP[2]	SE[3]
Essential amino acid					
Arginine	64.24[a]	67.12[ab]	66.06[a]	68.93[b]	1.06
Histidine	74.69[a]	77.63[ab]	72.67[a]	81.14[b]	1.78
Isoleucine	57.05[a]	58.70[ab]	57.14[a]	60.45[b]	1.09
Leucine	67.18	68.09	67.71	68.20	2.31
Lysine	76.58[ab]	76.97[ab]	75.74[a]	77.99[b]	0.76
Methionine	80.27	78.03	75.50	77.57	1.71
Phenylalanine	62.61[a]	65.04[a]	63.48[a]	67.34[b]	0.67
Threonine	57.19	56.06	56.00	57.84	1.00
Valine	65.30[a]	65.55[a]	64.69[a]	68.38[b]	2.47
Non essential amino acid					
Alanine	64.84	66.14	64.84	67.43	1.33
Aspartic acid	51.43	52.93	50.38	52.85	2.70
Cysteine	64.32	67.95	65.78	66.21	3.02
Glutamic acid	64.15[a]	67.30[ab]	65.85[a]	69.93[b]	1.06
Glycine	54.26	53.54	58.64	54.78	1.55
Proline	64.00	63.02	61.83	65.03	2.08
Serine	64.29	65.52	65.59	67.85	1.81
Tyrosine	60.13[a]	63.67[ab]	60.67[a]	65.36[b]	1.15

[1] FM: small fish based fish meal; FFA: FM fermented with ***L. acidophilus***; FFL: FM fermented with ***B. licheniformis.***

[2] SDPP (spray dried plasma protein) product was supplied by APC, Inc (Ames, IA).

[3] Pooled standard error.

[a, b] Different superscripts reflect significant differences among means in the same row ($p<0.05$).

Discussion

Fermented foods have been consumed by humans in Southeast Asian countries, but food fermentation technology remains limited with regard to its application in animal production sector. Fermented diets might improve daily gain, feed intake, and the feed conversion ratio in pigs in comparison with non-fermented diets. Fermented diets contain high levels of organic acids and 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). Pedersen and Lindberg (2003) also reported an improvement in the in vitro digestibility of organic matter and crude protein due to fermentation. Hong and Lindberg (2007) demonstrated that the total tract apparent digestibility of crude protein was higher ($p<0.05$) in a fermented diet than in raw and cooked diets. 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).

Also, fish waste could be upgraded into animal feed by fermentation with lactic acid bacteria (Dapkevicius *et al*., 2000). The authors hypothesized that fish meal containing a high level of histamine would improve the nutritional value for weaned pigs by fermentation. Although fermentation reduced histamine concentration in experimental fish meals, the present study could not find the enhanced benefits of fermented fish meal relative to feeding SDPP, because SDPP is a protein source that has a balanced amino acid profile (Table 2) compared with experimental fish meals. In conclusion, compared to feeding 3% SDPP, no positive effects were observed on N retention or nutrient digestibility in weanling pigs on 5% fermented fish meal diets. Thus, SDPP provides a better nutrient digestibility outcome than the use of fermented fish meal.

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