



Digestibility and Nitrogen Balance of Diets that Include Marine Fish Meal, Catfish (*Pangasius hypophthalmus*) By-product Meal and Silage, and Processing Waste Water in Growing Pigs

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ABSTRACT : Ileal and total tract digestibility and nitrogen (N) balance of diets with four different protein sources were determined in growing pigs. The diets were based on rice bran, broken rice and maize meal and contained Tra catfish by-product (CBP), processed using three different methods, and marine fish meal (FM). The CBP diets consisted of the by-product in meal form, ensiled with molasses, and CBP waste water (WWBD). The four diets were fed to four growing pigs fitted with post-valve T-cecum (PVTC) cannulas in a 4×4 Latin Square design. All experimental diets included Cr₂O₃ at 5 g/kg feed as an indigestible marker. The ileal apparent digestibility of organic matter and ether extract was higher on diet WWBD than on the other three diets ($p < 0.05$), and the total tract apparent digestibility was higher on diet WWBD than on the FM diet ($p < 0.05$). The ileal and total tract apparent digestibility of crude protein and amino acids was not significantly different among diets ($p > 0.05$). No significant effects of diet were found on N-retention and N utilization. In conclusion, the catfish by-product meal, ensiled catfish by-product and processing waste water diets and the fish meal diet had similar ileal and total tract apparent digestibility, and similar N utilization in growing pigs. **(Key Words** : Tra Catfish, Catfish By-product Meal, Processing Waste Water, Ileal Digestibility, Total Tract Digestibility, Growing Pigs)

INTRODUCTION

Pig production is the dominant form of livestock production in Vietnam and accounts for around 80% of the total meat consumption of the country (Livestock Department, 2006). Conventional protein supplements for commercial pigs, such as fish meal and soya bean meal have become relatively expensive. The resulting imbalance between feed and animal production prices has reduced profitability for pig farmers. Catfish production for export has increased rapidly in recent years in the Mekong Delta, and significant amounts of head, bone, scrap meat and skin by-products (710-900 tons/d; Thuy et al., 2007) are produced from fillet processing. By-products account for approximately 65% of the whole catfish (Nortvedt, 2007). Catfish by-product meal (CBM), ensiled by-product (CBE) and processing waste water (WWB) from the CBM

processing are potentially valuable alternative protein sources in pig rations to conventional protein supplements such as soya bean meal and fish meal. However, there are no published data on the digestibility of these by-products, especially ileal amino acid digestibility in pigs. The aim of this study was to determine the nitrogen retention and the apparent digestibility of dietary components and amino acids in diets containing catfish by-product meal, ensiled by-product and processing waste water resulting from catfish processing.

MATERIAL AND METHODS

Animals and experimental design

Four crossbred castrated male (Yorkshire×Landrace) pigs with an average live weight of 35.5±0.41 kg at the start, and 60.1±1.31 kg (mean±SD) at the termination of the experiment, were used to determine the coefficient of apparent ileal (CIAD) and total tract digestibility (CTTAD) of the experimental diets. The pigs were treated against intestinal parasites and vaccinated against hog cholera and foot and mouth disease during the initial 7 days, then surgically fitted with post-valve T-cecum cannulas (Van

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Leeuwen et al., 1991) to allow collection of ileal digesta. The pigs were housed in individual metabolism cages in an environmentally controlled house with an average temperature of 30-33°C. Urine and feces were collected separately, and water from nipple drinkers was freely available.

Four experimental diets were introduced to the pigs two weeks after surgery according to a 4×4 Latin Square design. Experimental periods were 12 days, consisting of 5 days of adaptation to each diet followed by 4 days of collection of feces and urine, one day of ileal digesta collection, one day of rest and finally a second day of ileal digesta collection. Dietary treatments consisted of broken rice, rice bran and maize meal as energy sources with four different test protein sources: A fish meal diet (FMD) as control, and diets including catfish by-product meal (CBMD), ensiled catfish by-product (CBED) and processing waste water (WWBD). All diets were supplemented with a standard mixture of vitamins and minerals, and chromium oxide was added as an indigestible marker at 0.5% of the diet.

Fish meal was bought from factories which specifically process small marine fish. The CBM was bought from catfish by-product meal factories which purchase the CBP from fillet exporting companies, and includes the head, bone, skin and abdominal organs that remain after the two side fillets have been removed. This residue is then ground fresh, boiled and the oil removed, and then dried to produce CBM. The ground boiled CBP was collected from CBM factories, then mixed with molasses at a ratio of 8:2 (wet basis). The mixture was placed in plastic bags and sealed to prevent air contamination and then ensiled for 3-4 weeks. A new batch of the ensiled product was made every week and then mixed daily with the basal ingredients. The WWB was collected each week, preserved with acetic acid and then stored before being mixed daily with the basal ingredients. The FM and CBM were mixed with the basal ingredients initially and stored for the whole period of data collection. The feeding level during the collection period was set slightly below the maximum level consumed during the adaptation period. The pigs were given two meals per day at 7:00 and 17:00 h, and water was available *ad libitum*.

Digesta, feces and urine collection and calculations

On each day of ileal digesta collection, samples were taken after every 2 h during the 12 h period between the morning and afternoon feedings, with a 1 h adjustment between days to cover every hour during the 12 h period. At each sample collection, digesta were quantitatively collected for 1 h in a plastic bag through a soft plastic tube connected to the ileal cannula. The plastic bags were then removed, new bags installed, and the samples stored in a refrigerator. The 6 samples (bags) per day of collection, in total 12 samples of digesta from each pig from 2 days of

ileal digesta collection in each experimental period, were mixed together and kept frozen at -18°C. During each collection period, feces were also quantitatively collected daily at 8:00 and 16:00 h and representative samples stored in a refrigerator. After 4 days of feces collection, the samples were mixed and representative samples were kept frozen at -20°C for analysis. Urine was collected in 50 ml of 10% H₂SO₄. Finally, prior to chemical analysis, individual samples of ileal digesta and feces were thawed and pooled within pigs and periods, and then dried at 60°C before analysis.

The digestibility of the diets at each sampling site was calculated using the indicator technique according to the following equation:

$$CAD_D = 1 - \frac{(DC_F \times I_D)}{(DC_D \times I_F)}$$

Where: CAD_D is the coefficient of apparent digestibility of dietary components in the assay diet; DC_F: Dietary component concentration in ileal digesta or feces (g/kg); I_D: Indicator concentration in the assay diet (g/kg); DC_D: Dietary component concentration in the assay diet (g/kg); I_F: Indicator concentration in ileal digesta or feces (g/kg).

Chemical analysis

The chemical composition of feed, feces and urine was determined using the following Association of Official Analytical Chemists methods (AOAC, 1990): Dry matter (DM) was measured by drying the fresh samples at 105°C until dry. Crude protein was determined by the Kjeldahl method. Total ash was the residue after ashing the samples at 550°C and organic matter (OM) was calculated by difference. The ether extract (EE) was determined by Soxhlet extraction. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the methods of Van Soest et al. (1991). Amino acids (AA) were analyzed on an ion-exchange column using an HPLC (Spackman et al., 1958). Chromium was measured by atomic absorption spectrophotometer after ashing and digesting the sample in a mixture containing perchloric and nitric acid (Fenton and Fenton, 1979).

Statistical analysis

Analysis of variance was performed according to a 4×4 Latin Square arrangement, using the General Linear Model of Minitab Statistical Software Version 15. Tukey pair-wise comparisons were used to determine differences between treatment means at p<0.05.

RESULTS

The experiment was carried out without any problems

Table 1. Dry matter content (%), and crude protein (CP), ether extract (EE), crude fiber (CF), NFE, NDF and ADF (% of DM) contents of diet ingredients

Ingredient	DM	CP	EE	Ash	CF	NFE	ADF	NDF
Rice bran	82.5	9.9	9.2	8.2	9.2	63.5	20.2	37.5
Maize meal	85.4	8.5	1.0	4.2	3.6	82.6	2.7	8.6
Broken rice	88.0	7.2	0.8	4.1	6.8	81.1	1.8	7.1
FM ¹	82.3	47.7	10.3	33.2	2.5	6.3	1.8	8.3
CBM ² (meat, scraps, head and bone)	83.7	41.9	15.6	31.4	3.7	7.4	1.7	8.8
CBE ⁽³⁾	52.0	29.2	14.2	28.5	2.1	26.0	5.3	14.5
WWB ⁽⁴⁾	24.2	32.5	19.8	29.5	2.6	15.6	1.6	6.5
Catfish by-product (head and bone)	43.5	37.5	19.8	33.8	2.1	6.7	1.6	3.1

¹ Fish meal. ² Catfish by-product meal. ³ Catfish by-product ensiled with molasses at a ratio of 8:2. ⁴ Waste water from catfish by-product meal processing.

regarding illness or behavior of the animals. All diets offered to the pigs were completely consumed at every meal. The chemical composition of the ingredients is shown in Table 1. The DM, CP and EE contents were different between CBP (43.5%, 37.5% and 19.8%, respectively), CBM (83.7%, 41.9% and 15.6%, respectively) and CBE (52.0%, 29.2% and 14.2%, respectively), and the contents in WWB (24.2%, 32.5% and 19.8%, respectively) and FM (82.3%, 47.7% and 10.3%, respectively) were different. The amino acid composition of the experimental ingredients is shown in Table 2, and of the diets in Table 4. Lysine and total essential amino acids contents were similar in FM

(51.1 and 253 g/kg, respectively) and CBM (49.0 and 216 g/kg, respectively), and were also similar in CBE (33.5 and 156 g/kg, respectively) and WWB (36.3 and 155 g/kg, respectively). The concentration of lysine in the FMD was lowest, while CBED had higher lysine, leucine and methionine concentrations than the other diets. The ingredient and chemical composition of the experimental diets is shown in Table 3. All experimental diets had the same level of CP (13.3-13.4%). The DM was lowest in WWBD, but there were no significant differences in OM, CF, ADF, NDF and NFE among FMD, CBMD, CBED and WWBD. The EE content in FMD was lower (4.71%) than

Table 2. Amino acid composition of the experimental feed ingredients (g/kg DM)

	Ingredients*			
	FM	CBM	CBE	WWB
Essential amino acids (EAA)				
Arginine	28.8	32.8	16.5	14.0
Isoleucine	20.1	11.1	11.0	11.6
Leucine	30.1	23.2	23.5	21.4
Lysine	51.1	49.0	33.5	36.3
Histidine	13.5	13.5	5.9	6.2
Methionine	18.9	16.8	11.5	10.6
Phenylalanine	17.8	13.1	11.6	12.9
Threonine	18.9	12.9	10.7	12.1
Tyrosine	26.6	24.0	14.7	11.2
Valine	27.7	20.1	16.9	19.0
Total EAA	253	216	156	155
Non-essential amino acids				
Alanine	40.6	36.1	21.5	30.9
Aspartic acid	1.5	1.6	3.2	4.5
Glutamic acid	61.0	53.0	34.7	24.5
Glycine	46.7	32.8	29.2	31.9
Proline	49.8	36.0	30.9	29.5
Serine	34.0	31.6	17.6	14.5
Sum of amino acids (SAA)	487	408	293	291

* FM = Fish meal; CBM = Catfish by-product meal; CBE = Ensiled catfish by-product; WWB = Processing waste water.

Table 3. Ingredient (% of DM) and chemical composition (% of DM) of the experimental diets

	Diet*			
	FMD	CBMD	CBED	WWBD
Ingredients (%)				
Rice bran	32.0	32.0	30.0	32.0
Maize meal	35.3	33.3	26.3	27.3
Broken rice	20.0	20.0	20.0	20.0
Fish meal	12.0			
CBM		14.0		
CBE			23.0	
WWB				20.0
Vitamin-mineral premix ¹	0.2	0.2	0.2	0.2
Cr ₂ O ₃	0.5	0.5	0.5	0.5
Chemical composition (%)				
Organic matter	91.1	90.0	89.0	89.5
Crude protein	13.3	13.3	13.4	13.4
Ether extract	4.7	5.6	6.5	7.3
Ash	8.9	10.0	11.0	10.5
Crude fiber	5.9	6.0	5.5	5.8
ADF	8.0	7.9	8.3	7.5
NDF	17.5	17.5	16.4	18.4
NFE	66.4	65.8	62.9	62.2
ME (MJ/kg DM)	13.1	13.2	13.1	13.2

¹ Per kg complete diet: Vitamins: A 48×10^3 UI; D 48×10^4 IU; E 44×10^2 ; K₃ 280 mg; B₁ 600 mg; B₂ 200 mg; B₆ 320 mg; B₁₂ 6×10^3 mcg; Biotin 10^4 mcg; Folic acid 160 mg; Nicotinic acid 44×10^2 mg; Pantothenic acid 24×10^2 mg. Minerals: Fe 475×10^2 mg; Cu 315×10^2 mg; Zn 475×10^2 ; I 350 mg; Co 47 mg; Mn 195×10^2 ; Se 39 mg.

* FMD = Fish meal diet; CBMD = Catfish by-product meal diet; CBED = Ensiled catfish by-product diet; WWBD = Processing waste water diet.

Table 4. Amino acid composition of the experimental diets (g/kg DM)

	Diet*			
	FMD	CBMD	CBED	WWBD
Essential amino acids (EAA)				
Arginine	10.3	11.3	9.9	9.2
Isoleucine	5.8	4.9	5.6	5.5
Leucine	10.9	10.2	11.7	10.8
Lysine	9.5	10.2	10.7	10.4
Histidine	4.2	4.4	3.6	3.6
Methionine	3.9	3.9	4.0	3.6
Phenylalanine	5.3	4.9	5.3	5.4
Threonine	4.9	4.3	4.7	4.8
Tyrosine	6.0	6.0	5.8	4.8
Valine	7.5	6.9	7.6	7.6
Total EAA	68.2	67.0	68.9	65.6
Non-essential amino acids				
Alanine	9.7	9.0	8.5	9.9
Aspartic acid	1.0	1.1	1.6	1.8
Glutamic acid	13.0	13.0	13.0	10.2
Glycine	9.0	7.9	9.7	9.5
Proline	10.7	9.6	11.2	10.1
Serine	9.6	8.6	7.9	6.8
Sum of amino acids (SAA)	121	116	121	114

* FMD = Fish meal diet; CBMD = Catfish by-product meal diet; CBED = Ensiled catfish by-product diet; WWBD = Processing waste water diet.

Table 5. Ileal and total tract apparent digestibility (%), and nitrogen balance of the experimental diets

	Diet*				SE	p
	FMD	CBMD	CBED	WWBD		
Ileal digestibility (%)						
OM	78.4 ^a	77.0 ^a	78.4 ^a	79.8 ^b	0.28	0.03
CP	73.2	73.1	74.9	72.5	0.56	0.09
EE	64.0 ^a	67.0 ^{ab}	67.8 ^b	68.4 ^b	0.70	0.02
Total tract digestibility (%)						
OM	84.2 ^a	84.4 ^a	83.5 ^b	84.8 ^a	0.15	0.01
CP	77.2	77.8	78.8	76.8	0.43	0.08
EE	70.1 ^a	71.9 ^{ab}	72.0 ^{ab}	73.2 ^b	0.48	0.02
Daily DM intake (g/d)	1,278	1,272	1,267	1,218	75.8	0.87
N intake (g/d)	27.2	27.1	27.6	26.8	1.67	0.98
N in feces (g/d)	6.6	5.8	6.1	6.5	0.99	0.92
N in urine (g/d)	8.2	9.1	7.8	8.0	1.21	0.88
N-balance (g/d)	12.3	12.4	13.7	12.3	2.51	0.97
N utilization (%)	59.9	53.2	63.2	57.4	6.73	0.31

* FMD = Fish meal diet; CBMD = Catfish by-product meal diet; CBED = Ensiled catfish by-product diet; WWBD = Processing waste water diet.

^{a,b} Within rows, values with different superscript letters are different ($p < 0.05$).

in CBMD (5.6%), CBED (6.5%) and WWBD (7.3%). The daily DM and N intake and N-retention and utilization were not different among diets (Table 5).

The coefficient of ileal apparent digestibility (CIAD) of OM in the WWBD was higher than in the other three diets ($p < 0.05$), while the CIAD of EE was lower ($p < 0.05$) in the FMD compared with CBED and WWBD (Table 5). The coefficient of total tract apparent digestibility (CTTAD) of

OM was lower ($p < 0.05$) in CBED than in the other diets, while the CTTAD of EE was lower ($p < 0.05$) in FMD compared with WWBD. There was a trend ($p < 0.1$) towards higher CP digestibility at both ileal and total tract level in CBED than in the other diets. The CIAD and CTTAD of amino acids (Table 6) were not different among diets ($p > 0.05$). In all diets, the highest CIAD of essential AA (EAA) was for leucine (74.3%), lysine (75.6%) and

Table 6. Ileal and total tract apparent digestibility (%) of amino acids in pigs fed diets containing fish meal and different catfish by-products*

	Ileal digestibility				SE	p	Total tract digestibility				SE	p
	FMD	CBMD	CBED	WWBD			FMD	CBMD	CBED	WWBD		
Essential amino acids												
Arginine	73.7	73.5	74.2	74.5	1.48	0.96	77.5	77.3	78.5	76.3	0.78	0.35
Isoleucine	72.4	71.2	73.1	72.7	0.84	0.50	75.4	74.2	75.4	75.6	1.37	0.89
Leucine	73.2	74.6	74.3	72.7	0.60	0.20	76.7	77.8	77.4	76.9	0.25	0.07
Lysine	74.5	74.1	75.6	74.8	1.32	0.86	78.0	78.9	80.2	77.6	0.90	0.28
Histidine	72.7	73.0	73.8	71.6	1.13	0.60	76.6	76.4	78.4	76.1	1.91	0.82
Methionine	73.8	72.9	73.8	73.2	1.36	0.94	76.1	77.6	78.7	77.0	1.11	0.45
Phenylalanine	72.5	73.2	73.9	72.2	1.32	0.80	77.0	77.7	77.8	75.5	0.93	0.39
Threonine	70.9	70.3	71.4	69.7	1.08	0.70	73.2	72.6	72.2	71.6	1.30	0.83
Tyrosine	72.7	73.0	71.1	71.3	0.71	0.25	77.9	78.6	77.6	76.5	0.45	0.08
Valine	73.3	73.4	73.2	72.8	0.83	0.95	79.1	78.8	79.8	78.8	0.34	0.88
Non-essential amino acids												
Alanine	71.4	69.2	69.5	70.8	0.54	0.07	76.9	75.5	75.3	75.4	0.37	0.06
Aspartic acid	70.1	70.5	69.3	69.7	0.84	0.76	75.9	77.7	75.1	76.7	0.61	0.09
Glutamic acid	72.4	72.7	71.3	72.7	0.53	0.31	78.4	78.5	78.6	78.8	0.17	0.49
Glycine	70.4	69.6	70.8	70.6	0.84	0.78	74.0	73.2	74.5	74.3	0.49	0.34
Proline	72.1	72.1	74.3	73.4	0.80	0.24	75.0	75.0	75.3	74.2	1.18	0.91
Serine	72.8	71.2	72.3	71.8	0.85	0.62	77.1	78.7	78.8	77.2	0.57	0.14

* FMD = Fish meal diet; CBMD = Catfish by-product meal diet; CBED = Ensiled catfish by-product diet; WWBD = Processing waste water diet.

methionine (73.8%) in CBED, and the highest CTTAD of EAA was for arginine (78.5%), lysine (80.2%), methionine (78.7%) and valine (79.8%), also in CBED.

DISCUSSION

The catfish by-product meals bought from the different CBM processing factories had different DM, CP and EE contents. This can be explained by the different proportions between the scrap meat, skin, head and bone added, as well as by differences in the average weights of the fish processed, and also different procedures for oil extraction between factories. Generally, meals that have a high scrap meat proportion are high in CP content (Thuy et al., 2007). The CBP used for ensiling was in fresh form and consisted of head and bone by-product, which explains the low DM and CP contents compared with CBM. The sugarcane molasses was used as carbohydrate silage additive because it is very effective in reducing pH, and the silage rapidly attained a pH of 4.5, with significant increases in lactic acid content. The low DM content in WWB was a result of the water added before cooking and oil extraction, and the high fat content of WWB was probably due to an inefficient oil extraction process. The slightly higher lysine, methionine and leucine concentration in CBED was probably a result of the high proportion of CBE in the diet (23%). The fish meal used in the experiment was produced from very small, whole marine fish, so the CP content was low and ash content high.

The ileal and total tract apparent digestibility of amino acids was similar in all diets, irrespective of protein source. Moreover, nitrogen retention and N utilization were similar among diets. This indicates similarity in amino acid utilization among diets, as there were only minor differences in essential amino acid composition among diets. However, amino acid digestibility in the experimental diets was low in comparison with previous studies by Jørgensen et al. (1984) and Knabe et al. (1989) on fish meal. There could be several explanations for this, such as a high ash content in the products, heating during processing and diet CP content. The results from Noblet and Perez (1993) showed that diets with a higher ash content had lower fecal apparent digestibility of amino acids. Moreover, the fact that the fish meal was dried at a temperature of $>80^{\circ}\text{C}$ and catfish by-product was boiled at a temperature of $>100^{\circ}\text{C}$ would also have contributed to reduce amino acid digestibility. Opstvedt et al. (2003) showed that fish meal produced at processing temperatures below $70\text{--}80^{\circ}\text{C}$ had higher CP digestibility than when the meal was processed at temperatures above 100°C . Ohh et al. (2002) also concluded that the quality of fish meal may show considerable variation depending on the quality of raw

material and the processing method used in manufacturing. Similarly, Wang and Parsons (1998) found that high processing temperatures of meat and bone meal generally resulted in lower amino acid digestibility than did low processing temperatures. Finally, Fan et al. (1994) showed that there was a quadratic increase in ileal apparent amino acid digestibility as dietary CP content was increased from 4 to 24%, and reduction of dietary CP content was shown to decrease the ileal apparent digestibility of most amino acids in weaning pigs (Htoo et al., 2007).

As expected, the ileal digestibilities of OM, CP and EE in all diets were lower than the total tract digestibilities, and were of a similar order of magnitude to the values reported by Ngoan and Lindberg (2001). The trend towards increased ileal and total tract CP digestibility in CBED may be due to the impact of microbial activity in the diet prior to feeding. Bacterial lactic acid fermentation of the feed has been shown to enhance digestibility (Hong and Lindberg, 2007). The resulting low pH and high acid content has also been shown to stimulate the pancreatic secretion and can improve the digestion and absorption of nutrients (Scholten et al., 1999). The improved feed efficiency that can be obtained by feeding fermented liquid feed is due to changes in the gastro-intestinal tract environment, reduced pH in the stomach and the lower number of enterobacteria (Moon et al., 2004).

The digestibility of EE was low in all diets. This could be explained by the feed processing, as the total tract digestibility of dietary nutrients in pigs can be influenced by the characteristics of the feed, such as chemical composition and processing treatment (Le Goff and Noblet, 2001). Moreover, the digestibility of EE in FMD was lower than in the other diets, which can be explained by a low fat content in this diet as compared with the other diets. This is in agreement with Noblet and Perez (1993), who showed that the lower EE digestibility obtained when dietary fat content was low was due to the concomitant high proportion of fecal endogenous fat, so the amount of digestible EE was relatively and positively related to the dietary EE content. In addition, catfish oil is very high in unsaturated fatty acids (Thuy et al., 2007), with concentrations of between 67.7% (Sathivel et al., 2003) to 75% (Men et al., 2007) of poly-unsaturated fatty acids, which are more efficiently digested and absorbed than saturated fatty acids. This was also demonstrated by Duran-Montge et al. (2007), who found that individual fatty acid digestibility increases with increasing unsaturation, and that digestibility of fat sources is a function of fatty acid content.

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