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Effect of Additive on the Chemical Composition of Tra Catfish (*Pangasius hypophthalmus*) By-product Silages and Their Nutritive Value for Pigs

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ABSTRACT : Two experiments were conducted to determine i) the fermentation characteristics of catfish by-product (C) ensiled with rice bran (RB) or sugarcane molasses (M) in different ratios, and ii) the digestibility of the silages in growing pigs. In the ensiling experiment, there were three ratios of C, ensiled with RB or M, of 8:2, 7:3 and 6:4 (wet basis for C and air-dry basis for RB and M). The six treatments were CRB8:2, CRB7:3, CRB6:4, CM8:2, CM7:3 and CM6:4, with 3 replications per treatment and seven sampling times. The pH of CRB7:3 and CRB6:4 decreased ($p<0.05$) from the first week and stayed stable until 8 weeks of ensiling, but the pH did not decrease in CRB8:2. The pH of CM8:2, CM7:3 and CM6:4 decreased rapidly from the first week until week 8. Dry matter (DM) and crude protein (CP) contents were slightly lower when catfish by-product was ensiled with RB than with M. Ammonia content in all treatments increased ($p<0.05$) during ensiling. Lactic acid content in silages with molasses increased from the first week, with the highest value at week 4. However, the lactic acid content in CRB8:2 did not increase with time and had the lowest value of all treatments. Acetic acid proportions of total volatile fatty acids were low at day 0, with the highest value at day 7, decreasing slowly until 8 weeks. The butyric acid proportion was highest at day 0 and decreased up to week 8. The proportion of propionic acid increased during ensiling. The digestibility experiment had a 4×4 Latin-square design and included 4 castrated crossbreed (Yorkshire×Landrace) male pigs fed four diets. The basal diet (BD) included rice bran, broken rice and maize meal, and the other three diets included fish meal (FMD) or catfish by-product ensiled with rice bran (CRBD) or sugarcane molasses (CMD). The silages chosen were those which gave the best results in the ensiling experiment for each additive. The coefficient of total tract apparent digestibility (CTTAD) of DM and organic matter (OM) was not significantly different among treatments. The CTTAD of CP was not different among the silage diets, but was lower in BD ($p<0.01$). The CTTAD of ether extract (EE) was higher in the catfish by-product silage diets than in FMD and BD. There was no significant difference in the digestibility of DM, OM, CP and EE between the fish meal and the by-product silages. In conclusion, catfish by-product can be successfully preserved by ensiling. Moreover, the total tract apparent digestibility of OM, CP and EE in ensiled catfish by-product was comparable with that of fish meal. (**Key Words :** Additives, Catfish By-product, Digestibility, Silage, Pig)

INTRODUCTION

There is a great potential for increased aquaculture production in the Mekong Delta in southern Vietnam (Wilder and Phuong, 2002). Currently, the most commonly cultured fish species in the region are river catfish, especially Tra catfish (*Pangasius hypophthalmus*), the production of which is developing very rapidly because of the high value of the exported fillet products (Phillips, 2002). There are several factories in the Delta that produce *Pangasius* fillets for export, and their processing generates considerable quantities of by-products, including head, skin and bone, that in total represent about 65% of the fish by weight (Thuy et al., 2007). These are potentially valuable protein sources for livestock in the Mekong Delta, especially for pig production (Men et al., 2004). Conventional protein sources such as fishmeal and soya bean meal have become very expensive recently, and it is economically attractive then to use catfish by-products, such as dried catfish meal or ensiled catfish by-product as protein sources. However, the wastes are underutilized, and represent an environmental problem due to their odor and

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casual disposal. Production of dried meal for use as animal feed is technically feasible, but the conventional methods of drying use energy derived from fossil fuels and are expensive (Goddard and Perret, 2005). An additional difficulty is that small-scale processors do not possess the facilities for drying, and generally discard the fish waste into watercourses, adding to the pollution problem. An alternative to drying is ensiling, which results in a rapid drop in pH, and the increasing concentrations of non-dissociated organic acids will inhibit the growth of microorganisms that cause spoilage (Pahlow et al., 2003). Fish silage is usually manufactured and stored in liquid form after processing (Pahlow et al., 2003). Several studies on ensiling protein-rich by-products without starter culture (lactic acid bacteria) have been carried out, for example using sugarcane molasses (M), cassava root meal or rice bran (RB). The resulting silages were of good quality, and for example shrimp by-product silage (Ngoan and Lindberg, 2000), Golden apple snails ensiled with M and RB (Kaensombath and Ogle, 2005) and freshwater fish ensiled with RB and sugar palm syrup (Phiny and Rodríguez, 2001) were successfully fed to growing pigs. However, no studies have been carried out on catfish by-product silage.

The present study was conducted to investigate the chemical composition of catfish by-product and ensiling characteristics with different ratios of by-product to molasses and rice bran, and to determine the most appropriate combinations of catfish by-product with these additives with respect to preservation and digestibility in growing pigs. It is expected that catfish by-product ensiled with either rice bran or sugarcane molasses will have a similar digestibility and nutritive value to that of fish meal.

MATERIALS AND METHODS

Ensiling experiment

Catfish by-product (CBP) includes head, bone, skin and abdominal organs that remain after the two side fillets are removed for export at a number of processing factories in the Mekong Delta. Other factories nearby then purchase the fresh CBP, where it is ground, boiled and the oil removed. The ground CBP was collected from several of these factories and mixed with rice bran (RB) (wet weight of by-product and air-dry weight of rice bran) and sugarcane molasses (M) of 71 degree Brix (wet weight basis) in ratios of 8:2, 7:3 and 6:4. The mixtures were placed in plastic bags and were sealed to prevent air contamination, and then stored at room temperature (29-31°C) for three months. In total there were 126 bags of 2 kg, with 6 treatments, 7 sampling times and 3 replications per treatment. Samples of CBP ensiled with RB or M were taken at 0, 7, 14, 21, 28, 42 and 56 days of ensiling for measurement of pH, and the samples were then stored at -20°C for analysis of dry matter (DM), crude protein (CP), ammonia nitrogen (NH_3-N) and organic acids (lactic, butyric, acetic and propionic acid). The chemical composition of the CBP, RB and M is shown in Table 1.

Digestibility experiment

Animals and experimental design : Four crossbred castrated male (Yorkshire×Landrace) pigs with an average live weight of 35.1±0.48 kg at the start, and 55.3±1.32 kg (mean±SD) at the termination of the experiment were used to determine the total tract apparent digestibility and nitrogen (N) utilization of diets with inclusion of CBP

Table 1. Dry matter content (%), chemical composition and essential amino acids (% in DM) of catfish by-product, silage additives and ingredients in the digestibility experiment (% of DM)

Chemical composition	Catfish by-product	Rice bran	Sugarcane molasses	Fish meal	Maize meal	Broken rice	CRB*	CM*
Dry matter	41.5	81.2	71.0	89.2	86.7	87.5	52.0	48.3
Crude protein	35.1	10.5	1.50	51.0	8.28	7.01	27.4	27.3
Ether extract	19.5	9.22		10.2	0.79	0.64	13.0	15.1
Ash	35.9	7.99		31.5	3.68	3.95	22.1	25.7
Crude fibre	2.10	10.5		3.4	3.70	6.50	4.62	2.28
Acid detergent fiber	1.65	22.0		1.78	2.88	1.82	8.40	1.32
Neutral detergent fiber	3.32	38.1		7.32	8.67	7.10	14.8	2.67
Nitrogen-free extractives	7.40	61.8		3.87	83.6	81.9	32.6	29.6
Essential amino acids								
Lysine	3.92	0.42		4.45	0.31	0.32	2.87	3.07
Methionine	1.12	0.19		1.55	0.18	0.18	0.98	1.25
Threonine	1.33	0.35		2.14	0.39	0.23	1.05	1.31
Phenylalanine	2.41	0.45		1.89	0.53	0.34	1.82	1.49

* CRB: Catfish by-product (70%) ensiled with rice bran (30%); CM: Catfish by-product (80%) ensiled with sugarcane molasses (20%).

silage or fish meal. The pigs were vaccinated against hog cholera and foot and mouth disease and kept in individual metabolism cages in an environmentally controlled house with an average temperature of 30-33°C, where they had free access to water from nipple drinkers. Four experimental diets were introduced to the pigs according to a 4×4 Latin square design, and were fed during four 12 day periods, each consisting of 7 days of adaptation to each diet followed by 5 days of quantitative collection of faeces and urine. During the collection period, faeces and urine were collected and weighed daily at 8:00 and 16:00 h and stored at -20°C. At the end of each period, samples were thawed, mixed within animal and diet, and sub-samples were taken for chemical analysis. Urine was collected in 50 ml of 10% H_2SO_4. The pigs were given two meals per day at 8:00 and 15:00 h, and water was available *ad libitum*. The feed level during collection was set slightly below the maximum level consumed during the preliminary period.

Diets and feeding : Dietary treatments consisted of a basal diet (BD) including broken rice, rice bran and maize meal without any protein source, a fish meal diet (FMD) and diets with catfish by-product ensiled with either rice bran (7:3) (CRBD) or sugar cane molasses (8:2) (CMD). All four diets were supplemented with a standard mixture of vitamins and minerals. The diets were made every week for the 6 weeks of the experiment, so that the time of preservation of the silage was the same. The ingredient and chemical composition of the experimental diets is shown in Table 7.

Chemical analysis

A representative 100 g (fresh matter) sample of silage from each sampling time was macerated with 100 ml of distilled water and stored at 4°C for 2 h. Then the extract was filtered, and the pH of the filtrate measured by electrode. Dry matter was measured by drying the fresh samples at 105°C until dry. Total N was determined on fresh samples by the Kjeldahl method and CP calculated as total N×6.25. Ammonia nitrogen (NH_3-N) was determined by distilling with water and MgO, collecting the distillate in 0.3% H_2BO_3, and then titrating with standard 0.1 N H_2SO_4. Lactic, acetic, butyric and propionic acids were measured by reverse-phase High Performance Liquid Chromatography (HPLC), (DIONEX Pump, P.580, UVD-170S (USA) with Column C18 from Merck Company. The wavelength of the UV-VIS detector was set at 220 nm. The mobile phase consisted of 0.02M KH_2PO_4 and methanol at a 98:2 v/v ratio. pH was adjusted to 2.25 for lactic acid, acetic and propionic acid determination, and to 4.5 for butyric acid. The flow rate was set at 1.0 ml/min (AOAC, 2000).

The chemical composition of feed, faeces and urine was determined using standard methods (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the methods of Van Soest et al. (1991). Amino acids were analyzed according to Spackman et al. (1958) on an ion-exchange column using HPLC.

Calculations

Digestibility of feed ingredients (FM, CRB and CM) was calculated using the following formulae:

Digestibility of FM
= (Digestibility of FMD-(0.88*×Digestibility of BD))
/0.12**

Digestibility of CRB
= (Digestibility of CRBD-(0.76*×Digestibility of BD))
/0.24**

Digestibility of CM
= (Digestibility of CMD-(0.77*×Digestibility of BD))
/0.23**

* Proportion of the basal diet in the experimental diets;
** Proportion of FM, CRB and CM, respectively, in the experimental diets.

Statistical analysis

For the ensiling experiment data were analyzed as a factorial design with treatment and ensiling time as factors using the general linear model in Minitab Statistical Software Version 15, following the statistical model below:

$$Y_{ijk} = \mu+\alpha_I+\beta_j+(\alpha\beta)_{ij}+e_{ijk}$$

Where, Y_{ijk} is the individual observation, μ the general mean, α_i treatment effect (i = 1..,6); β_j ensiling time effect (j = 1..,7); $\alpha\beta_{ij}$ interaction between treatment and ensiling time and e_{ijk} is the residual error (k = 1..,3). When the F-test was significant at p<0.05, pairwise comparisons were performed using the Bonferroni procedure.

For the digestibility experiment, the data were analyzed 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

Chemical composition of catfish by-product, silage additives and dietary ingredients

The chemical composition of catfish by-product (CBP), silage additives and dietary ingredients in the digestibility experiment are shown in Table 1. The DM (41.5%) and CP

Table 2. Effect of treatment and ensiling time on pH, DM (%), CP (% in DM), ammonia (% of total N), lactic acid and total organic acids (g/kg DM), and proportions of acetic, butyric and propionic acids of total VFA (%)

	pH	DM	CP	N-NH_3	Lactic acid	Total organic acids	Proportion of total VFA		
							Acetic acid	Butyric acid	Propionic acid
Ensiling time									
Day 0	6.1^{a}	51.8^{a}	26.2^{a}	0.31^{e}	11.6^{e}	35.5^{d}	22.0^{b}	52.5^{a}	25.5^{e}
Day 7	5.2^{b}	51.4^{ab}	26.2^{a}	0.75^{d}	69.4^{d}	95.2^{c}	26.2^{a}	44.6^{b}	29.2^{d}
Day 14	4.4^{f}	50.8^{abc}	25.9^{ab}	0.83^{d}	110^{c}	137^{b}	20.1^{bc}	32.5^{c}	47.4^{c}
Day 21	4.5^{f}	50.8^{abc}	25.7^{ab}	0.96^{c}	119^{b}	147^{a}	19.8^{c}	25.3^{d}	54.9^{a}
Day 28	4.6^{e}	50.9^{abc}	25.6^{ab}	1.05^{c}	130^{a}	151^{a}	17.2^{d}	31.8^{c}	51.0^{b}
Day 42	4.8^{d}	50.6^{bc}	25.3^{ab}	1.18^{b}	124^{b}	141^{b}	17.5^{d}	33.0^{c}	49.5^{bc}
Day 56	5.1^{c}	50.4^{c}	25.2^{b}	1.30^{a}	120^{b}	135^{b}	17.3^{d}	33.2^{c}	49.5^{bc}
SE	0.020	0.228	0.221	0.021	1.325	1.372	0.494	0.666	0.691
P	0.000	0.001	0.008	0.000	0.000	0.000	0.000	0.000	0.000
Treatment*									
CRB8:2	6.0^{a}	48.1^{d}	29.7^{a}	1.66^{a}	18.8^{e}	53^{e}	24.5^{a}	41.6^{b}	33.9^{e}
CRB7:3	4.9^{b}	52.0^{b}	26.9^{b}	0.87^{b}	125^{a}	151^{a}	25.5^{a}	37.6^{c}	36.9^{d}
CRB6:4	4.9^{b}	56.1^{a}	24.0^{c}	0.59^{d}	92^{d}	120^{d}	15.6^{d}	33.4^{d}	51.0^{b}
CM8:2	4.6^{d}	47.4^{d}	27.3^{b}	0.70^{c}	124^{a}	141^{b}	17.0^{cd}	47.6^{a}	35.4^{d}
CM7:3	4.7^{cd}	49.8^{c}	24.1^{c}	0.71^{c}	116^{b}	130^{c}	18.1^{bc}	37.3^{c}	44.6^{c}
CM6:4	4.7^{c}	52.4^{b}	21.4^{d}	0.95^{b}	111^{c}	126^{c}	19.2^{b}	19.3^{e}	61.5^{a}
SE	0.018	0.211	0.204	0.019	1.226	1.270	0.457	0.616	0.639
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

* CRB8:2; CRB7:3; CRB6:4 = Ratio of catfish by-product to rice bran; CM8:2; CM7:3; CM6:4 = Ratio of catfish by-product to sugarcane molasses.
$^{a, b, c, d, e, f}$ Within columns, values with different superscript letters are different (p<0.05).

(35.1%) contents of CBP were lower than in fish meal (89.2% and 51%, respectively), and the ether extract in CBP (19.5%) was higher than in fish meal (10.2%). The contents of essential amino acids in the fish meal were generally higher than in CBP, with lysine contents of 4.45% and 3.92%, respectively, methionine contents of 1.55% and 1.12%, respectively, and threonine contents of 2.14% and 1.33%, respectively, although the content of phenylalanine was lower in fish meal (1.89%) than in CBP (2.41%). The DM (52%), CF (4.62%), ADF (8.4%), NDF (14.8%) and NFE (32.6%) contents in the CBP ensiled with RB were higher than in CBP ensiled with M (DM, 48.3%; CF, 2.28%; ADF, 1.32%; NDF, 2.67% and NFE, 29.6%). Ether extract, lysine, methionine and threonine contents were higher in CBP ensiled with M than with RB.

Effect of treatment and ensiling time

There were overall differences among treatments in pH, CP, DM, NH_3-N, lactic acid and total organic acids (Table 2). The lowest pH values were after 14-28 days, and silages made with M had lower pH values than silages made from RB (p<0.05). Dry matter and CP were stable until day 28 and then decreased slowly (p<0.05) up to day 56. NH_3-N increased slowly from 0-56 days (p<0.001) with the highest value in CRB8:2. Lactic acid and total organic acids increased from day 0, with the highest value at day 28 in CM8:2. Acetic and butyric acid proportions decreased slowly, and overall the proportion of propionic acid increased during ensiling (p<0.001).

The individual effects of treatment and ensiling time on pH, DM, CP and NH_3-N are shown in Table 3 and 4. The pH in CRB8:2 decreased to only 5.3 after 14 days (p<0.05) and then after 21 days started to increase, reaching a value of 6.3 after 42 days. All silages with M and RB, except CRB8:2, reached a pH of less than 4.7 after 14 days, but at 56 days the pH of CRB6:4 and CRB7:3 had increased to over 5.0. There was an inverse relationship between the proportion of rice bran in the silage and the pH after day 14, but the opposite was the case for the molasses silage. There were no significant differences in DM and CP contents with time (p>0.05). Ammonia contents increased with time (p<0.05) and were highest in CRB8:2 at day 42. There was a decrease in NH_3-N with increasing proportions of RB, whereas it increased with increasing proportions of M in the silage.

Lactic acid contents in all treatments increased to a maximum at day 21-28 (p<0.01, Table 5), and then either remained stable or decreased slightly up to day 56. Concentrations of lactic acid were significantly lower in CRB8:2 than in all other treatments (p<0.05). Total organic acids increased to a maximum at 14-21 days, and were significantly lower in CRB8:2.

The proportion of acetic acid of total volatile fatty acids (VFA) was highest at day 7, and then decreased slowly to day 56 in all treatments. The proportion of butyric acid decreased after the first week, except in CM8:2 and CM7:3,

Table 3. Effect of treatment (catfish by-product ensiled with rice bran or sugarcane molasses) and ensiling time on pH and DM (%) content

Parameter	Treatment*	Ensiling time (days)							SE	P
		0	7	14	21	28	42	56		
pH	CRB8:2	$^{A}6.8^{a}$	$^{A}6.2^{b}$	$^{A}5.3^{c}$	$^{A}5.4^{c}$	$^{A}6.1^{b}$	$^{A}6.3^{b}$	$^{A}6.3^{b}$	0.049	0.000
	CRB7:3	$^{B}6.5^{a}$	$^{B}5.6^{b}$	$^{B}4.7^{cd}$	$^{B}4.4^{e}$	$^{B}4.5^{de}$	$^{C}4.5^{cde}$	$^{D}4.7^{c}$		
	CRB6:4	$^{B}6.3^{a}$	$^{B}5.4^{b}$	$^{C}4.3^{e}$	$^{B}4.5^{de}$	$^{B}4.5^{de}$	$^{BC}4.6^{d}$	$^{B}5.1^{c}$		
	CM8:2	$^{C}6.0^{a}$	$^{C}4.9^{b}$	$^{D}4.0^{e}$	$^{C}4.1^{e}$	$^{C}4.2^{e}$	$^{C}4.4^{d}$	$^{D}4.7^{c}$		
	CM7:3	$^{D}5.7^{a}$	$^{D}4.6^{c}$	$^{CD}4.1^{d}$	$^{BC}4.3^{de}$	$^{BC}4.4^{e}$	$^{B}4.7^{c}$	$^{BC}5.0^{b}$		
	CM6:4	$^{D}5.5^{a}$	$^{D}4.6^{bc}$	$^{C}4.3^{c}$	$^{B}4.3^{c}$	$^{B}4.5^{bc}$	$^{B}4.8^{b}$	$^{CD}4.8^{b}$		
DM	CRB8:2	49.3	48.9	47.6	48.0	48.2	47.8	47.1	0.558	0.993
	CRB7:3	53.3	52.2	51.9	51.4	52.0	51.5	51.7		
	CRB6:4	57.2	56.7	55.8	55.6	55.9	56.0	55.3		
	CM8:2	47.5	48.0	47.2	48.0	47.5	47.0	47.1		
	CM7:3	50.3	49.7	50.0	50.1	50.1	49.5	49.1		
	CM6:4	53.2	52.9	52.4	52.0	52.0	52.0	52.3		

* CRB8:2; CRB7:3; CRB6:4 = Ratio of catfish by-product to rice bran; CM8:2; CM7:3; CM6:4 = Ratio of catfish by-product to sugarcane molasses.

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

$^{A, B, C, D, E}$ Within columns, values with different superscript letters are different ($p<0.05$).

Table 4. Effect of silage additive (rice bran, RB, or sugarcane molasses, M) ratio and ensiling time on the content of crude protein (% of DM) and NH_3-N (% of TN**)

Parameter	Treatment*	Ensiling time							SE	P
		0	7	14	21	28	42	56		
Crude protein	CRB8:2	30.1	30.6	30.6	29.7	29.1	29.1	29.2	0.541	0.991
	CRB7:3	27.5	27.5	27.3	26.7	26.5	26.8	26.6		
	CRB6:4	25.2	25.3	24.8	24.8	25.3	24.2	24.4		
	CM8:2	28.4	27.4	27.4	27.0	27.2	27.2	26.8		
	CM7:3	25.0	24.3	23.8	24.4	24.1	23.9	23.8		
	CM6:4	21.2	22.1	21.8	21.9	21.3	20.8	20.5		
NH_3-N	CRB8:2	$^{A}0.59^{e}$	$^{A}1.29^{d}$	$^{A}1.58^{c}$	$^{A}1.82^{b}$	$^{A}2.09^{a}$	$^{A}2.17^{a}$	$^{A}2.10^{a}$	0.051	0.000
	CRB7:3	$^{C}0.23^{e}$	$^{C}0.66^{d}$	$^{BC}0.75^{d}$	$^{BC}0.90^{c}$	$^{B}1.02^{bc}$	$^{B}1.18^{b}$	$^{B}1.38^{a}$		
	CRB6:4	$^{C}0.13^{e}$	$^{C}0.49^{d}$	$^{C}0.56^{cd}$	$^{D}0.62^{bcd}$	$^{C}0.72^{abc}$	$^{C}0.78^{ab}$	$^{D}0.83^{a}$		
	CM8:2	$^{C}0.22^{c}$	$^{C}0.56^{b}$	$^{C}0.60^{b}$	$^{CD}0.74^{b}$	$^{C}0.70^{b}$	$^{B}1.01^{a}$	$^{C}1.07^{a}$		
	CM7:3	$^{C}0.31^{d}$	$^{C}0.61^{c}$	$^{C}0.60^{c}$	$^{CD}0.73^{bc}$	$^{C}0.75^{bc}$	$^{BC}0.91^{ab}$	$^{C}1.08^{a}$		
	CM6:4	$^{B}0.41^{c}$	$^{B}0.90^{b}$	$^{B}0.93^{b}$	$^{B}0.97^{b}$	$^{B}1.01^{b}$	$^{B}1.05^{b}$	$^{B}1.37^{a}$		

* CRB8:2; CRB7:3; CRB6:4 = Ratio of catfish by-product to rice bran; CM8:2; CM7:3; CM6:4 = Ratio of catfish by-product to sugarcane molasses.

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

$^{A, B, C, D, E}$ Within columns, values with different superscript letters are different ($p<0.05$).

** Total nitrogen.

and then increased slowly after 4 weeks. The proportion of propionic acid increased with time in all RB treatments, although in CM8:2 and CM7:3 it decreased between 14 and 56 days.

Total tract apparent digestibility

The ingredient, chemical and amino acid composition of the experimental diets is shown in Table 7. The daily feed intake and CP intakes were higher ($p<0.05$) in the diet with catfish by-product ensiled with molasses (CMD) and rice bran (CRBD) in comparison with the fish meal (FMD) and the basal diet (BD) (Table 8). There were no differences in the total tract digestibility of DM and organic matter (OM) among diets. The CP digestibility did not differ between the FMD, CRBD and CMD diets, but was significantly lower in BD ($p<0.01$) than in the two silage diets. The EE digestibility was higher ($p<0.05$) in the catfish by-product silage diets than in FMD and BD. Nitrogen retention in BD was lower ($p<0.01$) than in the other diets. However, there was no significant difference in nitrogen utilization among diets.

There was no difference ($p>0.05$) in the calculated digestibility of DM, OM, CP and EE among the dietary ingredients (Table 9).

Table 5. Effect of silage additive (rice bran, RB, or sugarcane molasses, M) ratio and ensiling time on the content of lactic acid and total organic acids (g/kg DM) in silage.

Parameter	Treatment*	Ensiling time (days)							SE	p
		0	7	14	21	28	42	56		
Lactic acid	CRB8:2	$^{B}2.31^{c}$	$^{D}10.5^{bc}$	$^{D}17.7^{ab}$	$^{E}28.9^{a}$	$^{D}25.4^{a}$	$^{D}26.5^{a}$	$^{E}20.0^{a}$	3.245	0.000
	CRB7:3	$^{AB}5.79^{d}$	$^{C}25.9^{c}$	$^{A}160^{b}$	$^{A}163^{ab}$	$^{A}175^{a}$	$^{A}174^{ab}$	$^{A}173^{a}$		
	CRB6:4	$^{AB}7.34^{c}$	$^{B}55.9^{b}$	$^{C}108^{a}$	$^{C}121^{a}$	$^{C}121^{a}$	$^{C}118^{a}$	$^{D}115^{a}$		
	CM8:2	$^{AB}16.1^{d}$	$^{A}105^{c}$	$^{B}130^{b}$	$^{BC}125^{b}$	$^{A}172^{a}$	$^{A}164^{a}$	$^{B}159^{a}$		
	CM7:3	$^{A}19.6^{c}$	$^{A}109^{b}$	$^{C}116^{b}$	$^{D}143^{a}$	$^{B}143^{a}$	$^{B}144^{a}$	$^{C}140^{a}$		
	CM6:4	$^{A}18.9^{e}$	$^{A}110^{d}$	$^{B}131^{bc}$	$^{BD}135^{ab}$	$^{B}147^{a}$	$^{C}119^{cd}$	$^{D}116^{d}$		
Total organic acids	CRB8:2	33.4^{c}	$^{C}44.3^{bc}$	$^{D}51.7^{bc}$	$^{D}70.9^{a}$	$^{C}65.9^{ab}$	$^{E}60.3^{ab}$	$^{E}47.0^{c}$	3.361	0.000
	CRB7:3	30.3^{c}	$^{C}54.6^{b}$	$^{A}188^{a}$	$^{A}193^{a}$	$^{A}199^{a}$	$^{A}198^{a}$	$^{A}196^{a}$		
	CRB6:4	40.1^{e}	$^{B}94.2^{d}$	$^{BC}143^{ab}$	$^{BC}157^{a}$	$^{B}148^{a}$	$^{D}132^{bc}$	$^{D}127^{c}$		
	CM8:2	35.6^{e}	$^{A}126^{d}$	$^{B}153^{bc}$	$^{C}147^{c}$	$^{A}185^{a}$	$^{B}175^{b}$	$^{B}167^{b}$		
	CM7:3	35.9^{c}	$^{A}123^{b}$	$^{C}137^{b}$	$^{B}163^{a}$	$^{B}152^{a}$	$^{C}153^{a}$	$^{C}149^{a}$		
	CM6:4	37.9^{c}	$^{A}129^{b}$	$^{B}154^{a}$	$^{BC}154^{a}$	$^{B}160^{a}$	$^{D}127^{b}$	$^{D}123^{b}$		

* CRB8:2; CRB7:3; CRB6:4 = Ratio of catfish by-product to rice bran; CM8:2; CM7:3; CM6:4 = Ratio of catfish by-product to sugarcane molasses.
$^{a, b, c, d, e}$ Within rows, values with different superscript letters are different ($p<0.05$).
$^{A, B, C, D, E}$ Within columns, values with different superscript letters are different ($p<0.05$).

DISCUSSION

Catfish by-product composition varies according to time of collection and source because it depends on the input material quality (Thuy et al., 2007), and especially the ether extract content is different among factories as a result of different procedures for fat extraction. However, DM and CP contents are always lower than in fish meal. The reduction of CP, EE and amino acid contents in catfish by-product ensiled with RB and M compared with the raw input material was due to the inclusion of RB (30% in air weight) and M (20% in wet weight), both of which have low contents of CP, EE and amino acids. Also the EE in catfish by-product (CBP) ensiled with M was higher than in

Table 6. Effect of treatment (catfish by-product ensiled with rice bran or sugarcane molasses in different ratios) and ensiling time on the proportion of acetic, butyric and propionic acids (% of total volatile fatty acids)

Parameter	Treatment*	Ensiling time (days)							SE	P
		0	7	14	21	28	42	56		
Acetic acid	CRB8:2	$^{B}25.4^{bc}$	$^{A}31.5^{a}$	$^{A}27.5^{ab}$	$^{BC}19.7^{d}$	$^{AB}18.9^{d}$	$^{A}21.8^{cd}$	$^{A}26.7^{abc}$	1.210	0.000
	CRB7:3	$^{C}16.6^{d}$	$^{A}34.9^{a}$	$^{A}29.5^{b}$	$^{A}26.5^{bc}$	$^{A}23.4^{c}$	$^{A}24.2^{c}$	$^{A}23.3^{c}$		
	CRB6:4	$^{D}8.60^{c}$	$^{BC}23.9^{ab}$	$^{A}28.7^{a}$	$^{AB}23.1^{b}$	$^{C}6.00^{c}$	$^{D}9.20^{c}$	$^{C}10.2^{c}$		
	CM8:2	$^{C}19.1^{a}$	$^{C}19.6^{a}$	$^{B}10.2^{c}$	$^{BC}18.7^{a}$	$^{A}21.0^{a}$	$^{CD}12.7^{bc}$	$^{B}17.6^{ab}$		
	CM7:3	$^{AB}29.5^{a}$	$^{BC}21.8^{bc}$	$^{B}10.3^{f}$	$^{D}13.4^{ef}$	$^{AB}18.7^{cd}$	$^{BC}16.7^{cde}$	$^{B}16.5^{de}$		
	CM6:4	$^{A}32.7^{a}$	$^{B}25.6^{b}$	$^{B}14.7^{d}$	$^{CD}17.2^{cd}$	$^{B}14.9^{cd}$	$^{AB}20.0^{c}$	$^{C}9.50^{e}$		
Butyric acid	CRB8:2	$^{A}61.3^{a}$	$^{A}53.5^{b}$	$^{A}47.6^{b}$	$^{A}32.6^{cd}$	$^{C}27.8^{d}$	$^{C}32.1^{cd}$	$^{CD}36.3^{c}$	1.631	0.000
	CRB7:3	$^{AB}55.5^{a}$	$^{C}39.4^{b}$	$^{B}34.7^{bc}$	$^{AB}30.7^{c}$	$^{BC}34.6^{bc}$	$^{C}34.5^{bc}$	$^{D}34.2^{bc}$		
	CRB6:4	$^{AB}57.2^{a}$	$^{BC}46.3^{b}$	$^{B}35.1^{c}$	$^{B}25.8^{d}$	$^{BC}31.5^{cd}$	$^{D}19.5^{ef}$	$^{E}17.8^{f}$		
	CM8:2	$^{A}59.3^{a}$	$^{A}57.7^{ab}$	$^{B}34.3^{de}$	$^{AB}28.8^{e}$	$^{A}47.8^{c}$	$^{A}55.1^{b}$	$^{A}50.6^{b}$		
	CM7:3	$^{B}51.7^{a}$	$^{BC}41.5^{bc}$	$^{C}25.3^{de}$	$^{C}19.6^{e}$	$^{B}37.8^{c}$	$^{B}42.8^{b}$	$^{BC}42.6^{b}$		
	CM6:4	$^{C}29.7^{a}$	$^{D}29.4^{a}$	$^{D}18.0^{bc}$	$^{C}14.4^{bc}$	$^{D}11.4^{c}$	$^{D}14.3^{bc}$	$^{E}17.8^{bc}$		
Propionic acid	CRB8:2	$^{D}13.3^{d}$	$^{D}15.0^{d}$	$^{D}24.9^{c}$	$^{BC}47.7^{a}$	$^{C}53.3^{a}$	$^{B}46.1^{a}$	$^{BC}37.0^{b}$	1.693	0.000
	CRB7:3	$^{B}27.9^{c}$	$^{C}25.7^{c}$	$^{C}35.8^{b}$	$^{C}42.8^{ab}$	$^{D}42.0^{ab}$	$^{B}41.3^{ab}$	$^{B}42.5^{ab}$		
	CRB6:4	$^{AB}34.2^{d}$	$^{BC}29.8^{d}$	$^{C}36.2^{d}$	$^{B}51.1^{c}$	$^{B}62.5^{b}$	$^{A}71.3^{a}$	$^{A}72.0^{a}$		
	CM8:2	$^{BC}21.6^{c}$	$^{C}22.7^{c}$	$^{BC}55.5^{a}$	$^{B}52.5^{a}$	$^{E}31.2^{b}$	$^{C}32.2^{b}$	$^{C}31.8^{b}$		
	CM7:3	$^{C}18.8^{c}$	$^{B}36.7^{b}$	$^{A}64.4^{a}$	$^{A}67.0^{a}$	$^{D}43.5^{b}$	$^{B}40.5^{b}$	$^{B}40.9^{b}$		
	CM6:4	$^{A}37.6^{c}$	$^{A}45.0^{c}$	$^{A}67.3^{ab}$	$^{A}68.4^{ab}$	$^{A}73.7^{a}$	$^{A}65.7^{b}$	$^{A}72.7^{ab}$		

* CRB8:2; CRB7:3; CRB6:4 = Ratio of catfish by-product to rice bran; CM8:2; CM7:3; CM6:4 = Ratio of catfish by-product to sugarcane molasses.
$^{a, b, c, d, e}$ Within rows, values with different superscript letters are different ($p<0.05$).
$^{A, B, C, D, E}$ Within columns, values with different superscript letters are different ($p<0.05$).

Table 7. Ingredient (% of DM), chemical composition (% of DM) and amino acid composition (% of DM) of experimental diets with fish meal and catfish by-product ensiled with rice bran or sugarcane molasses

	Diet*			
	BD	FMD	CRBD	CMD
Ingredients				
Basal diet		88.0	76.0	77.0
Rice bran	39.8			
Maize meal	40.0			
Broken rice	20.0			
Vitamin premix**	0.2			
Fish meal		12.0		
CRB***			24.0	
CM****				23.0
Chemical composition				
OM	94.5	91.9	90.5	89.9
CP	8.89	13.1	13.2	13.2
Ash	5.46	8.06	9.45	10.1
EE	4.13	4.74	6.25	6.65
CF	6.98	6.62	6.41	5.90
ADF	10.3	9.46	9.86	8.25
NDF	20.1	18.8	18.9	16.1
NFE	74.5	67.5	64.6	64.2
Amino acid composition				
Lysine	0.36	0.85	0.96	0.98
Methionine	0.18	0.35	0.38	0.43
Threonine	0.34	0.52	0.5	0.56
Phenylalanine	0.46	0.63	0.79	0.70
Arginine	0.57	0.87	0.70	0.72
Isoleucine	0.40	0.61	0.61	0.58
Leucine	0.84	1.21	1.13	1.13
Histidine	0.28	0.37	0.32	0.32
Valine	0.49	0.66	0.72	0.68

* BD = Basal diet, including broken rice, rice bran and maize meal; FMD = Fish meal diet; CRBD = Catfish by-product ensiled with rice bran diet; CMD = Catfish by-product ensiled with sugarcane molasses diet.

** Per kg complete diet: Vitamins: A 48×105 IU; D 48×104 IU; E 44×102; K3 280 mg; B1 600 mg; B2 200 mg; B6 320 mg; B12 6× 103 mcg; Biotin 104 mcg; Folic acid 160 mg; Nicotinic acid 44×102 mg; Pantothenic acid 24×102 mg. Minerals: Fe 475×102 mg; Cu 315×102 mg; Zn 475×102; I 350 mg; Co 47 mg; Mn 195×102; Se 39 mg.

*** Catfish by-product ensiled with rice bran (7:3). **** Catfish by-product ensiled with sugarcane molasses (8:2).

CBP ensiled with RB because of higher proportion of the by-product in the molasses silage.

The pH was used as an indicator of the course of fermentation, and successful fermentation was obtained in all treatments except for CRB8:2, as evidenced by the rapid pH decline to a stable value of around 4.4 (Zahar et al., 2002). The results from the study show that sugarcane molasses was more effective than rice bran as a silage additive, because silages made with sugarcane molasses were successfully preserved at all the inclusion ratios studied, whereas in the CRB8:2 treatment the pH never fell below 5.3, and the concentration of NH_3-N was significantly higher at all times than in the other treatments. The explanation for this is that molasses has a high water-soluble carbohydrate content (McDonald et al., 2002), and the sugar in molasses is more rapidly fermented than the starch in rice bran (Ngoan and Lindberg, 2000). Several studies have been carried out on using sugarcane molasses as additive to silage made from fish by-products. For example, Zahar et al. (2002) showed that sardine waste ensiled with sugarcane molasses at a ratio of 6:4 at 35°C reached a pH of 4.4 in about 1 week, and according to Samuels et al. (1992), fish waste ensiled with sugarcane molasses as additive rapidly attained a pH of 4.5, with significant increases in lactic acid content. The preservation occurs due to both the anaerobic environment and bacterial fermentation of carbohydrates, which lower pH primarily through the production of lactic and acetic acids (Muck,

Table 8. Total tract apparent digestibility (%) of experimental diets, N metabolism (g/d) and utilization (% of N retained of N digested) in diets with fish meal or catfish by-product ensiled with rice bran or sugarcane molasses

	Diet*				SE	p
	BD	FMD	CRBD	CMD		
Digestibility						
DM	81.1	80.7	80.2	81.0	0.63	0.749
OM	82.5	81.8	81.4	82.1	0.58	0.600
CP	73.4[a]	75.6[ab]	77.5[b]	77.7[b]	0.62	0.009
EE	68.1[a]	70.2[b]	73.1[c]	73.0[c]	0.45	0.001
N metabolism						
N intake	17.6[a]	25.4[b]	26.8[b]	28.8[b]	0.69	0.000
N in urine	4.57[a]	6.27[ab]	6.78[b]	7.40[b]	0.39	0.010
N in faeces	4.68[a]	6.20[b]	6.02[b]	6.46[b]	0.25	0.010
N retention	8.32[a]	13.0[b]	14.0[b]	14.9[b]	0.79	0.004
N utilization, % (N retained/N digested)	64.5	67.2	67.2	66.8	2.64	0.875
Daily DM intake (g/d)	1,213[a]	1,217[a]	1,270[ab]	1,335[b]	24.1	0.035
CP intake (g/d)	110[a]	159[b]	167[bc]	180[c]	4.30	0.000

* BD = Basal diet, including broken rice, rice bran and maize meal; FMD = Fish meal diet; CRBD = Catfish by-product ensiled with rice bran diet; CMD = Catfish by-product ensiled with sugarcane molasses diet.

[a, b, c] Within rows, values with different superscript letters are different (p<0.05).

1988). However, the fermentation process was very slow in CRB8:2, and pH decreased only slightly from the first week to 8 weeks of ensiling as a result of the low proportion of readily fermentable carbohydrates. The addition of increasing amounts of CBP in silage with RB thus adversely affected the fermentation characteristic of silage, whereas the addition of M improved it, which is in agreement with Rassol et al. (1996), who ensiled poultry litter with sugarcane molasses.

The DM and CP contents of silage decreased only slightly with ensiling time, and Cai et al. (1999) found that low pH suppressed DM loss of silage. The reduction in DM content can be explained by the conversion of starch in the RB and sugar in the molasses to volatile fatty acids. Even in the rice bran silage with the highest proportion of by-product (CRB8:2) the decrease in CP content with time was only around one percentage unit, indicating that the breakdown of amino acids by bacteria was limited in spite of the relatively high pH. However, ammonia nitrogen (NH_3-N) did increase slightly during ensiling in all treatments. Ammonia can result from several reactions, such as deamination of amino acids and oxidation of amines by bacterial amino-oxidases (Hassan and Heath, 1987). In the present study, the highest concentration of ammonia was in treatment CRB8:2, as a result of the relatively high pH values, particularly after 28 days. In the molasses additive silages the pH decreased more rapidly, and as a result the NH_3-N contents in the molasses silages were lower than in the rice bran additive treatments. This was in agreement with Samuels et al. (1992), who showed that molasses was very effective in reducing pH and NH_3-N levels in silage. In addition there is the action of clostridia, which in wetter silage also show a higher potential for fermentation to produce ammonia. According to McDonald et al. (2002) clostridia are able to grow at high pH levels and ferment lactic acid to butyric acid, and clostridia can break down amino acids to amines and ammonia, which explains the increased NH_3-N in the silage.

The main objective of using additives when making silage is to obtain a lactic acid fermentation, with a rapid drop in pH, that results in a well preserved silage and prevents secondary fermentation (McDonald, 1981).

Table 9. Total tract apparent digestibility (%) of the experimental feed ingredients

	Feed ingredient*			SE	p
	FM	CRB	CM		
Digestibility					
DM	78.0	77.3	80.7	5.15	0.891
OM	77.2	77.8	80.9	4.76	0.843
CP	91.8	90.5	92.1	4.21	0.963
EE	85.6	88.6	89.1	3.26	0.735

* FM = Fish meal; CRB = Catfish by-product ensiled with rice bran; CM = Catfish by-product ensiled with sugarcane molasses.

According to Rasool and Sial (1998), the highest amounts of lactic acid in silage are formed after active bacterial growth has ceased and the sugar has been depleted. The inhibition of the growth of undesirable bacteria is associated with a high rate of lactic acid production following ensiling, which depends on the initial population of lactic acid bacteria and substrate availability at ensiling. In fact, the lactic acid content in treatment CRB8:2 did not increase as much as in the other treatments, resulting in a poor fermentation. This was most likely due to the low level of carbohydrate added, resulting in a too slow drop in pH and the development of a bacterial population with high content of clostridia and enterobacteria (McDonald et al., 1991). The proportion of acetic acid was higher in silages with rice bran additive than with sugarcane molasses additive. This is in agreement with Pettersson and Lindgren (1990), who found higher contents of acetic acid in many of the low sugar silages. Kung and Shaver (2001) found that acetic acid content was highest after the first week of ensiling then decreased during the ensiling, because microbial fermentation improved the aerobic stability of the silage.

Butyric and propionic acid proportions were higher than the proportions of acetic acid. In particular the proportion of butyric acid was highest in the first week, possibly because of the initial composition of the bacterial population in the material. According to Kung and Shaver (2001) a high concentration of butyric acid indicates the silage has undergone clostridia fermentation, and silage with a high concentration of ammonia is coupled with high butyric acid content. This is more likely to occur in silages with a high moisture content (McDonald et al., 1991), and the DM content of the catfish by-product was only around 40%. However, even though the initial proportion of butyric acid of total VFA was over 50% in all silages except for CM6:4, the proportion fell with ensiling time and silage quality was not affected.

Propionic acid contents increased slowly during ensiling, probably as a result of the actions of bacteria from the propionibacteria family (Kung and Shaver, 2001), which grow well in silage with high protein and moisture contents. According to Tyrce et al. (1991) propionic acid may be produced from sugars through lactate as an intermediate, and propionibacteria are able to utilize lactate as a substrate much more rapidly than glucose.

There were no differences in DM and OM digestibility between FM and catfish by-product ensiled with rice bran and with sugarcane molasses. Similarly, the difference in CP digestibility between the fishmeal diet and the two silage diets was not significant, only between the basal diet and the FMD, CRBD and CMD, so it is not surprising that there was no difference in the digestibility of CP among the protein sources (FM, CRB and CM). The CP content in the basal diet was low compared with the growing pigs' requirements (NRC, 1998), which explains the lower daily N-retention in BD than in the fishmeal and silage diets. However, N-utilization was similar among diets, as a result of their comparable amino acid balance. That the CP digestibility in BD was lower compared to the silage diets was partly due to the lower CP content, but could also have been due to the fermentation processes that occur during ensiling. Hong and Lindberg (2007) showed that fermentation influenced the gut environment and improved the digestibility of some dietary components. Fermented feed contains high concentrations of organic acids, especially lactic acid, and the large number of lactobacilli and low pH all can reduce the microbial activity of harmful species such as *Enterobacteriaceae,* including *Salmonella* and coliforms (Mikkelsen and Jensen, 1997) and change the bacterial ecology of the gastrointestinal tract (Prohaszka et al., 1990), thus improving nutrient digestibility. According to Scholten et al. (1999) fermented feed may stimulate pancreatic secretion and positively influence villus architecture, and these factors may have contributed to the improved digestion and absorption of CP and EE in CMD and CRBD. This is in agreement with Lopez (1989), who concluded that silage made from fish and fish by-products has a nutritional value comparable with that of fish meal.

CONCLUSION

The present data suggests that catfish by-product can be successfully ensiled with 20-40% of sugarcane molasses and 30-40% of rice bran (fresh and air-dry weights, respectively). The total tract apparent digestibility of crude protein and ether extract in ensiled catfish by-product was comparable to that of fish meal.

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