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Partial replacement of fish meal by porcine meat meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*)

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Abstract

In this study, it evaluated the growth performance of the Pacific white shrimp *Litopenaeus vannamei* in response to the replacement of fish meal with rendered porcine meat meal (PMM) in its diet. Six isolipidic and isonitrogenous diets were formulated with 0, 25, 35, 45, 55 or 65% replacement of fish meal with PMM on a protein basis. Shrimp grew from 0.55 g to >3.6 g during the 41-day experimental period. Specific growth rate (SGR) was significantly lower when PMM inclusion was 26.18% or greater, replacing more than 45% fish meal protein. A significant negative relationship was observed between growth response and the level of fish meal protein replacement with PMM protein. Methionine content decreased as PMM inclusion levels increased, consequently compromising growth performance. Dry feed intake (DFI) and the feed conversion ratio (FCR) were unaffected by fish meal replacement levels. The protein efficiency ratio (PER) was highest at the lowest PMM inclusion level. Apparent protein digestibility coefficient (APDC) for PPM was 66.2%. Experimental diets D-0 and D-25 had apparent dry matter digestibility (ADMDs) ranging from 77–81% and ADPs from 82–85%, while the diets with higher PMM inclusion (D-35 to D-65) had a significantly lower ADMD range (70–72%) and APD range (73–78%). It is concluded that porcine meat meal is an acceptable alternative animal protein source that can replace up to 35% of fish meal protein in shrimp diets without significant adverse effects on growth, survival, FCR, PER and body composition.

Keywords: Rendered protein; Digestibility; Amino-acids; Growth

1. Introduction

Mexico has become the third largest producer of cultured shrimp in the western hemisphere (Wurmann et al., 2004). In northwest Mexico alone a total of 51,059 ha are dedicated to shrimp farming, of which 37,390 ha are located along the coast of Sinaloa state (CESASIN, 2007). Under aquaculture conditions shrimp are fed manufactured balanced diets which typically contain approximately 25–35% fish meal (Tacon and Barg, 1998). A steadily increasing proportion of global fish meal production is being utilized in aquatic animal feed production; in 2003 about 53% went to produce fish and shrimp feeds (FAO, 2006).

Fish meal's high cost and concern about the reliability of future supplies from traditional sources (Europe and Western South America) have prompted efforts to identify and develop novel ingredients to function as fish meal substitutes (Tacon, 1997; Forster et al., 2004; Samocha et al., 2004; Hardy, 2006). The increasing price of feed is considered one of the most important factors limiting profitability in shrimp culture. The high cost of shrimp feed mainly responds to the cost of fish meal, and therefore finding a relatively lower cost alternative ingredient has been an ongoing research goal (Forster et al., 2003; FAO, 2006).

Production of functional shrimp feed that relies less on fish meal requires accurate information on the nutritive value of lower cost protein sources. Rendered products have been used in animal feeding since the mid 20th Century, and poultry by-products, feather meal and meat and bone meal or meat meal can be successfully

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used as protein sources for growing animals (New and Csavas, 1995; Tacon, 1997). In comparison to fish meal, however, these products are of only limited utility because they vary in protein quality (amino acid profile) and digestibility (Bureau et al., 2000).

These factors often affect overall productivity, particularly when an animal by-product is used as the main protein source in shrimp or fish feeds (Hegedius et al., 1990; Davies et al., 1991; Hardy, 1996; Robinson and Li, 1996; Bureau et al., 1999; Millamena, 2002). Complete replacement of fish meal with other protein sources can produce low growth rates, especially in crustaceans and carnivorous fish. This may be due to the poor digestibility and variable quality of these protein sources.

The quality of these terrestrial animal protein sources depends on both raw material quality and processing. Use of more adequate processing technologies, particularly drying techniques, has helped to produce more defined and selected products for formulating shrimp and fish diets (Bureau et al., 1999, 2000). For example, coextrusion and flash drying are now used to produce high quality meat and bone, and poultry by-products. Practical diets including these ingredients have been shown to positively effect growth performance in the Pacific white shrimp *Litopenaeus vannamei* (Lawrence and Castille, 1991; Beiping et al., 2000; Davis and Arnold, 2000; Foster et al., 2003; Samocha et al., 2004; Tan et al., 2005; Cruz-Suarez et al., 2007). The study objective was to evaluate the effectiveness of porcine meat meal (PMM) as a fish meal replacement in practical diets for Pacific white shrimp *L. vannamei*.

Table 1
Proximate composition and amino acid profile of sardine fish meal (FM) and pork meat meal (PMM)

	FM	PMM
Proximate analysis (% in dry matter)		
Crude protein	67.4	53.7
Crude fat	7.7	10.5
Ash	12.8	24.1
ADC crude protein	87.0[1]	66.2
Amino acid (AA %/100 gr of protein)		
Alanine	7.5	8.9
Arginine*	6.6	8.8
Aspartic acid	9.2	8.4
Glutamic acid	16.6	14.5
Glycine	11.5	17.3
Histidine*	3.5	1.8
Isoleucine*	5.7	3.2
Leucine*	8.0	6.3
Lysine*	6.5	5.8
Methionine*	2.2	1.7
Phenylalanine*	4.9	3.2
Serine	4.6	3.6
Threonine*	2.3	4.3
Tyrosine	3.1	6.4
Valine*	6.8	4.5

*Essential amino acid for shrimp (Lim and Persyn, 1989; Forster et al., 2003). Tryptophan was not determined.
[1]Determined by *in vitro* digestion with diluted pepsin (AOAC, 1990). Manufacturer data.

2. Materials and methods

2.1. Experimental ingredients and diets

Porcine meat meal (PMM) was obtained from a rendering plant (National By-product, Des Moines, IA, USA), raw material are ground, thermally pasteurized and dehydrated, and the meat and bone solids separated from the fat, and all other ingredients were obtained from a local feed manufacturer (maltaCleyton Feed Mills, Culiacán, Sinaloa, México). Based on the chemical compositions and amino acid profiles of the protein sources (Table 1), five experimental practical diets and a control diet were formulated to be isolipidic and isonitrogenous. All diets were formulated to provide approximately 90 g kg^{-1} lipids and 360 g kg^{-1} crude protein, on a dry matter basis (Table 2), levels reported as adequate for juvenile Pacific white shrimp (Smith et al., 1985). The experimental diets contained 145.5, 203.6, 261.8, 320.0 or 378.2 g kg^{-1} PMM (Table 2) in replacement of 25, 35, 45, 55 or 65%, respectively, of the fish meal protein contained in the control diet (0 g kg^{-1} PMM). As diet fish meal content decreased with increases in added PMM, minor adjustments were made to the fish oil and starch contents to balance the formulations (Table 2).

All major dry ingredients were mixed for 15 min in a Hobart food mixer. The oil, lecithin and cholesterol were blended in a Kitchen Aid mixer, then added to the mash and mixed for an additional 15 min. Hot water (approximately 60 °C) was mixed into the mash to provide a consistency appropriate for pelleting and this is mixed for another 15 min. The resulting mash was passed through a meat grinder equipped with a 1.6 mm diameter die to produce pellets. The pellets were dried in a forced air oven for 16 h at 38±2 °C. A sample of each diet was retained and stored in plastic bags at −20 °C until determination of proximate and amino acid composition. The diets were supplemented with chromic oxide (0.5%) as an external digestibility marker.

2.2. Chemical analysis

Feed ingredients, formulated diets, and pre- and post-experiment carcass samples were analyzed in triplicate using standard methods (AOAC, 1990). Ingredients and diets were analyzed for crude protein content (total nitrogen × 6.25) using a LECO FP-528 nitrogen analyzer (Method 990.03, AOAC). Crude fat concentrations were determined by petroleum ether extraction in a Goldfisch apparatus (Method 920.39, AOAC). Ash content was obtained by incinerating samples in a muffle furnace at 550 °C for 12 h (Method 942.05, AOAC). Dry matter was determined by drying the sample in an oven at 105 °C for 16 h (Method 934.01, AOAC) and weighing to the nearest 0.1 mg.

Amino acid composition of ingredients and diets was determined using samples (1.0 mg) hydrolyzed with 6 N HCL for 6 h. Sodium thioglycolate was added to samples to prevent oxidation. The hydrolysates were suspended in sodium citrate buffer (pH 2.2) and derivatized with *o*-phathaldialdehyde (OPA). Ten milliliters of the hydrolyzed sample was injected into a solvent delivery system (Varian 9012) equipped with a fluorescent detector with a 340–380 nm excitation filter and a 460 nm emission filter. The Star Chromatography Work-Station version 6.0 program was used. The precolumn was a Microsorb (4.5 × 30 mm) packed with octadecylsilane and the column was a 3 m (4.6 × 100 mm) Microsorb Short C18. Amino acid standards were used and α-aminobutyric acid added as an internal standard. Flow rate was 1.5 ml/min at 25–29 °C. The amino acids were completely eluted at 20 min and the column equilibrated for 10 min (Vazquez-Ortiz et al., 1995). Chromic oxide content of the feed and fecal samples was estimated using the acid digestion technique (Furukawa and Tsukahara, 1966).

2.3. Shrimp and experimental procedures

The feeding experiment was carried out in a closed recirculating seawater, which included settling tanks, a bubble bead biological filter and heating-pumping tank. System located at the CIAD (Mazatlán, Sinaloa, México) with 25-l circular plastic tanks, each containing 15 shrimp with an initial mean weight (±SD) of 0.55 g (±0.01 g). Each tank was supplied with aerated seawater at a rate of 1.5 l min^{-1} under natural lighting conditions. Water temperature was maintained at 28±1 °C, dissolved oxygen ranged between 7.0 and 8.0 mg L^{-1} and salinity was 34±1 g l^{-1}. Levels of NH_3+NH_4 (0.06–0.3 mg l^{-1}), NO_2 (0.016 mg l^{-1}) and NO_3 (0.32 mg l^{-1}) were recorded weekly following the methods of Spotte (1979).

Table 2
Composition of experimental diets for Pacific white shrimp *L. vannamei* containing pork meat meal as a substitute for fish meal

	Diet [a]					
	D-0	D-25	D-35	D-45	D-55	D-65
Ingredients (g kg^{-1} dry weight)						
Fish meal [b]	422	305	258	211	164	117
Pork meat meal [c]	0	145.5	203.6	261.8	320	378.2
Soybean meal [d]	64.4	64.4	64.4	64.4	64.4	64.4
Fish oil [e]	28	28	28	29	28	28
Soybean lecithin [f]	17.5	17.5	17.5	17.5	17.5	17.5
Squid liver meal	20	20	20	20	20	20
Binder(Na alginate) [g]	20	20	20	20	20	20
Corn starch	126.1	97.7	86.5	74.3	64.1	52.9
Wheat	260	260	260	260	260	260
Mineral premix [f]	15	15	15	15	15	15
Vitamin premix [f]	15	15	15	15	15	15
Vitamin C [f]	2	2	2	2	2	2
Cholesterol [g]	5	5	5	5	5	5
Chromic oxide [h]	5	5	5	5	5	5
Proximate analysis (% dry basis)						
Moisture	8.8	7.9	8.6	8.8	9.4	9.8
Crude protein	35.6	35.5	34.6	34.3	34.6	33.5
Crude fat	9.3	8.0	7.5	7.6	8.3	8.4
Ash	10.8	12.8	13.1	14.4	14.7	15.8
NFE [i]	44.3	43.7	44.8	43.7	42.4	42.3
Gross energy [j] (kcal/100 g)	450.8	436.7	430.0	426.9	427.3	425.9
LDM % [k]	8.13	8.12	8.10	8.00	8.11	8.01

[a] Number in the diet identifier indicates replacement level of fish meal protein with PMM protein (e.g. D-35=35%).
[b] Obtained from Selecta de Guaymas, S.A. de C.V., Guaymas, Sonora, México.
[c] Provided by National Renderers Association (NRA,USA).
[d] Solvent extracted soybean meal (local supplier).
[e] Droguería Cosmopolita, S.A. de C.V. México, D.F., México.
[f] maltaCleyton.
[g] Sigma Chemical.
[h] J.T. Baker.
[i] Nitrogen-free extract with fiber included (calculated by difference).
[j] Gross energy (kcal/g) was calculated based on the physiological values for protein, 5 kcal/g; fat, 9 kcal/g; and N-Free Extract, 4 kcal/g (Shiau and Chou, 1991).
[k] Loss of dry matter (LDM %) of the experimental diets were determined according to Cruz-Suárez et al. (2001).

The 41-day long experiment was designed to determine the effect of the diets on growth performance and feeding efficiency. The diets were randomly assigned to the tanks. The shrimp were initially fed at 10% of the biomass of each tank divided into three feedings each day (0800, 1300 and 1700 h), and the ration was adjusted based upon limiting the amount of unconsumed feed.

The shrimp were weighed individually every seven days to calculate mean body weight per tank. Response variables were determined using the equations:

$$\text{Survival (\%)} = 100 \times (\text{final count})/(\text{initial count})$$

$$\text{Weight gain (g)} = (\text{final weight} - \text{initial weight})/(\text{initial weight})$$

$$\text{Specific growth rate } (\text{SGR\% d}^{-1}) = [\text{Ln (final weight)} - \text{Ln (initial weight)}] \times 100/\text{time (days)}$$

Dry feed intake (DFI) was estimated based on the sum of average daily food intake per tank:

$\text{DFI} = \Sigma_i^{41}$ [(intake on *i*th day)/(number of shrimp on *i*th day)]. A value adjusted for the pre-prandial loss of dietary dry matter was calculated as follows: DFI adj. = DFI * (1 − LDM/100)

%LDM = [(Weight of feed (dry wt) before leaching − weight of feed (dry wt) after leaching)/weight of feed (dry wt) before leaching] * 100 (Cruz-Suárez et al. (2001).

Feed conversion ratio (FCR) = DFI adj./individual weight gain
Protein efficiency ratio (PER) = individual weight gain/protein intake
Apparent N utilization (ANU) = 100(carcass N deposition/N intake)

2.4. Digestibility determination

Chromic oxide (0.5%) was added as an external indicator to determine the apparent digestibility coefficient (ADC) for dry matter, crude protein and crude lipids (PMM and experimental diets). A reference diet based on fish meal alone was formulated with 360 g kg^{-1} protein (Smith et al., 1985). The porcine meat meal protein ADC was evaluated using the ingredient inclusion method. An experimental diet was formulated with 70% of the reference diet and 30% PMM. Digestibility trial conditions were similar to those of the growth trial. Juvenile *L. vannamei* were obtained from a commercial shrimp farm and acclimated to laboratory conditions for one week in a 1000-l circular fiberglass tank. During this period, they received a commercial shrimp feed. Digestibility was determined for each of the six diets (D-0, D-25, D-35, D-45, D-55 and D-65), with three replicates (tanks) per treatment (diet) and 7 shrimp (5.2±0.2 g initial mean weight) per replicate. Shrimp were adapted to the experimental diets for 7 days before feces collection was begun. They were fed twice a day at a fixed daily ration (10% biomass) and all unconsumed feed removed from the tank 60 min after each feeding. Immediately after collection, fecal strands were siphoned onto a mesh using a pipette, gently rinsed with distilled water several times, transferred to conic tubes and stored at −20 °C. Samples were then oven dried at 60 °C, ground and stored again at −20 °C until analysis. Sample collection was done over a 21-day period and samples pooled by replicate for each treatment.

Crude protein ADC for the PMM was calculated according to the substitution principle, using the method of Cho and Slinger (1979) and mathematical expressions in agreement with Forster (1999):

$$\text{NAD} = \frac{\left[\left(70\% \times \text{Nutr}_{\text{basal}} + \text{Nutr}_{\text{ing}} \times \text{AD}_{\text{test}}\right) - \left(70\% \times \text{Nutr}_{\text{basal}} \times \text{AD}_{\text{basal}}\right)\right]}{\text{Nutr}_{\text{ing}} \times 30\%}$$

Where, NAD=digestibility of a given nutrient from the test ingredient included in the test diet at 30%; AD_{test}=apparent digestibility of test diet; AD_{basal}=apparent digestibility of basal diet, which represents 70% of test diet; Nutr_{ing} and $\text{Nutr}_{\text{basal}}$ are the ingredient nutrient levels in the test diet and basal diet, respectively (Forster, 1999).

Table 3
Essential amino acid composition (%/100 g of protein) in diets containing increasing proportions of porcine meat meal protein

	R [a]	D-0	D-25	D-35	D-45	D-55	D-65
Arginine [b]	5.8	5.9	5.9	5.9	6.1	5.9	5.9
Histidine [b]	2.1	2.8	2.5	2.8	3.5	2.6	2.3
Isoleucine [b]	3.5	3.5	3.4	3.5	3.7	3.6	3.9
Leucine [b]	5.4	6.2	6.2	6.6	6.6	6.1	6.7
Lysine [b c]	4.7	5.6	4.0	5.0	5.9	5.2	6.1
Methionine [b]	2.4	2.4	2.3	2.3	1.8	1.8	2.0
Phenylalanine	4.0	4.3	4.3	3.4	3.5	3.3	3.4
Alanine	–	6.1	6.1	7.2	6.5	6.3	6.7
Aspartic acid	–	7.5	7.5	7.9	7.9	7.7	8.1
Glutamic acid	–	14.4	14.7	16.0	16.1	16.5	15.7
Glycine	–	6.6	7.3	9.6	10.3	10.9	10.5
Serine	–	2.3	2.7	3.0	3.7	2.9	3.0
Threonine [b]	3.6	3.8	3.6	3.8	4.0	3.2	3.5
Tyrosine	–	2.4	3.4	3.0	2.5	2.4	4.4
Valine [b]	4.0	4.4	4.5	4.7	3.8	4.0	4.8

[a] Recommended requirements for shrimp (%/100 g of protein) (Akiyama et al., 1991).
[b] Essential amino acid for shrimp (Akiyama et al., 1991). Tryptophan not determined.
[c] Fox et al. (1995).

Table 4
Apparent digestibility coefficients of dry matter, protein, lipid and energy for diets containing an increasing proportion of porcine meat meal protein fed to juvenile *L. vannamei* (mean initial weight 5.2±0.2 g)

Diet[1]	Dry matter	Protein	Lipid	Energy
D-0	81.92^{a}	85.34^{a}	93.56^{a}	87.65^{a}
D-25	77.77^{ab}	82.26^{ab}	88.77^{b}	84.42^{ab}
D-35	72.61^{bc}	78.48^{bc}	86.17^{b}	82.18^{b}
D-45	67.31^{c}	73.15^{c}	84.07^{c}	79.33^{b}
D-55	70.35^{c}	74.27^{c}	83.87^{c}	79.51^{b}
D-65	70.37^{c}	73.47^{c}	83.76^{c}	79.18^{b}
SEM	0.002	0.002	0.031	0.01

[1]Values in the same column with the same superscript are not significantly different ($P>0.05$).

Apparent digestibility coefficients (ADCs) of the experimental diets were calculated according to (Mainard and Loosli, 1969).

$$\text{ADC dry matter (\%)} = 100 - [(100\text{Cr}_2\text{O}_3 \text{ in feed}/\%\text{Cr}_2\text{O}_3 \text{ in feces}) \times 100];$$

$$\text{ADC nutrients (\%)} = 100 - 100[(\%\text{Cr}_2\text{O}_3 \text{ in feed}/\%\text{Cr}_2\text{O}_3 \text{ in feces}) \times (\%\text{ nutrient in feces}/\%\text{ nutrient in feed})].$$

2.5. Statistical analysis

Results were processed with a one-way analysis of variance (ANOVA). All percentage and ratio data were arc-sin transformed prior to analysis. Before the ANOVA, all data were tested for normality and heterogeneity of variances. Differences between experimental diets were identified with a Tukey HSD test (Zar, 1984) and differences considered significant at a 5% probability level. Given that the experiment had a dose–response design, responses to PMM inclusion were examined with a regression analysis. All statistical procedures were run with SigmaStat ver.3 software package.

3. Results

3.1. Proximate and amino acid composition of experimental diets

Amino acid composition of the fish meal (FM) and PMM varied slightly (Table 1). Some essential amino acids (expressed as % of 100 g protein) were lower in the PMM than in FM, particularly isoleucine, lysine, methionine and valine. Apparent protein digestibility for PMM was very low compared to FM (Table 1), and PMM ash content (24.1%) was very high compared to FM (12.8%).

Although the diets were formulated to be isolipidic and isonitrogenous, proximate analysis showed there to be slight differences in crude protein and crude fat contents (Table 2). Ash content increased as PMM inclusion levels increased (Table 2). The essential amino acid (EAA) profile showed methionine content to progressively decrease as PMM inclusion levels increased (Table 3).

3.2. Digestibility determination

The ADC ranged from 70 to 81% for dry matter, 73 to 85% for protein and 87 to 92% for energy (Table 4). Diet ADCs tended to be lower in diets with higher ash content (i.e. higher PMM inclusion). The dietary protein, dry matter and energy ADCs for the D-25 and D-35 diets were not significantly affected by PMM replacement of FM.

3.3. Growth, survival and feed conversion efficiency

Survival was higher than 90% in all treatments and not statistically different ($P>0.05$) between them (Table 5). Growth performance was highest in the D-0, D-25 and D-35 treatments, with significantly higher average final body weight, weight gain and SGR; these treatments were not significantly different (Table 5). In contrast, growth response in the D-45, D-55 and D-65 diets (i.e. in those with >26.18% PMM) was significantly lower than in the control diet (D-0).

Regression analysis showed a significant negative relationship of weight gain and SGR to PMM inclusion level (Tables 5 and 6), such that weight gain fell gradually as PMM inclusion level increased ($P<0.05$).

Feed intake exhibited no significant differences between treatments (Table 5). The FCR, in contrast, increased from 1.4 to 1.8 as PMM inclusion level increased, although differences between treatments were not significant. A significant negative relationship to PMM inclusion level was observed in the regression analysis. No significant differences were observed between the treatments for PER and ANU, although both parameters had a significant negative relationship to PMM inclusion.

Whole body composition and moisture content did not vary significantly between treatments (Table 7). Animals in the D-25 treatment had higher ($P<0.05$) crude protein content and lower ($P<0.05$) ash content than those in the other treatments.

4. Discussion

Replacement of up to 35% fish meal protein with rendered pork meat meal (PMM) produced growth rates statistically similar to the control treatment (fish meal-based diets). Decreases in growth response at high FM replacement levels, however, were directly related to increasing PMM inclusion levels.

The juvenile shrimp readily accepted the diets in all treatments, as shown by the similar DFI and FCR (between 1.4 and 1.8) between treatments. This agrees with other studies in which diets incorporating 30% to 70% meat meal as a substitute for fish meal

Table 5
Mean growth response and feed utilization of shrimp fed diets with increasing levels of porcine meat meal protein during a 41-day trial ($n=4$)

Diet	Final mean weight (g)	WG (g)	SGR (% d^{-1})	Survival (%)	DFI (g/shrimp)	FCR	PER	UAN (%)
D-0	4.5^{a}	3.9^{a}	5.1^{a}	90^{a}	5.55^{a}	1.43^{a}	1.97^{a}	35.36^{a}
D-25	4.2^{ab}	3.6^{ab}	4.9^{ab}	95^{a}	5.45^{a}	1.52^{a}	1.86^{a}	36.85^{a}
D-35	4.0^{abc}	3.5^{abc}	4.8^{abc}	93^{a}	5.30^{a}	1.53^{a}	1.91^{a}	36.87^{a}
D-45	3.8^{bc}	3.2^{bc}	4.6^{bc}	91^{a}	5.47^{a}	1.72^{a}	1.72^{a}	32.59^{a}
D-55	3.6^{bc}	3.0^{bc}	4.5^{bc}	93^{a}	5.62^{a}	1.84^{a}	1.58^{a}	30.25^{a}
D-65	3.6^{c}	3.0^{c}	4.5^{c}	95^{a}	5.45^{a}	1.82^{a}	1.64^{a}	31.58^{a}
SEM	0.327	0.492	0.0009	0.031	0.047	0.037	0.097	0.003

[1]Values in the same column with the same superscript are not significantly different ($P>0.05$).

Table 6
Linear regression analysis ($Y=a+bX$) results with percentage of fish meal protein replaced by porcine meat meal protein as independent variable and shrimp response parameters as dependent variables

Dependent variables	Intercept (a)	Slope (b)	P-value	Correlation coefficient	R^2	Standard error
Final weight (g)	7.997	0.0076	0.308	+0.217	0.470	0.790
Weight gain (g)	3.927	−0.0149	<0.001	−0.803	0.645	0.245
SGR (% d^{-1})	3.702	−0.0054	0.007	+0.609	0.372	0.135
DFI (g shrimp)	5.961	+0.0001	0.972	+0.007	0.0005	0.482
FCR	1.5035	+0.008	0.0006	+0.647	0.418	0.200
PER	1.843	−0.0055	0.004	+0.570	0.325	0.175
UAN (%)	34.799	+0.093	0.007	+0.536	0.287	3.252
Survival (%)	91.137	+0.062	0.417	+0.174	0.0302	6.407

were accepted by both omnivorous and carnivorous shrimp and fish (Davies et al., 1991; Shimeno et al., 1993; Watanabe et al., 1993; Kikuchi et al., 1997; Williams et al., 1997; Yang et al., 2004; Cruz-Suárez et al., 2007).

Both PER and UAN were affected by increasing FM replacement. The significant negative relationship of these parameters to FM replacement level is a result of the relative contribution of PMM protein that increase and affects overall digestibility. The ADC crude protein of the PMM (66.2%) observed here was markedly lower than the fish meal and slightly below to that reported (77.1%) by Cruz-Suárez et al. (2006). The present results also coincide with Williams et al. (1997), who reported that the apparent digestibility values of meat meals from different origins are generally lower than those for fish meal for prawns. Part of the variability in ADCs of protein reported could be attributed to chemical composition and quality variability (Bureau et al., 1999).

Lee and Lawrence (1997) stated that feedstuffs with high ash or fiber content increased the impact of lower digestibility, when its level inclusion in the diet is increased. Considering this, diets D-45, D-55 and D-65 with high levels of porcine meat meal may have contained high levels of indigestible fiber (tendons and bones) that lowered their protein digestibility by reducing gut transit time and physically protecting protein in the digesta from enzyme degradation (Brunson et al., 1997). This author reported poor protein digestibility for meat and bone meal in *P. setiferus*, suggesting a limited ability to digest the fiber component of this ingredient.

Table 7
Carcass proximate composition (%, wet weight basis) of juvenile shrimp *L. vannamei* fed diets containing different levels of porcine meat meal after 41 days

Diet [1]	Moisture	Crude protein	Crude fat	Ash
Initial	77.6	14.4	2.2	3.2
D-0	75.8^{a}	16.1^{b}	2.1^{a}	3.1^{a}
D-25	75.0^{a}	17.1^{a}	2.1^{a}	2.9^{a}
D-35	75.1^{a}	16.5^{b}	1.8^{a}	3.1^{a}
D-45	75.1^{a}	16.4^{c}	2.0^{a}	3.1^{a}
D-55	75.5^{a}	16.2^{b}	1.8^{b}	2.8^{b}
D-65	75.7^{a}	16.2^{b}	1.8^{a}	3.0^{a}
±SE	0.00	0.00	1.06	0.00

[1] Values in the same column with the same superscript are not significantly different ($P>0.05$).

L. vannamei inefficiently digested PMM at high inclusion levels. Yang et al. (2004) reported similar results in a study of fish meal replacement with meat and bone meal (MBM) at 500 g kg^{-1} protein in diets for gibel carp, however, their result were attributed to the high ash contents of MBM or poultry by-products meal (PBM), which can reduce protein digestibility (Watanabe and Pongmaneerat, 1991; Alexis, 1997; Robaina et al., 1997; Kureshy et al., 2000). In the present study apparent dry matter digestibility and gross energy decreased significantly, as did apparent protein digestibility for diets D-45, D-55 and D-65, however, the reduction in digestibility would appear to be more negatively impacted by the high levels of porcine protein than the increasing levels of ash in the experimental diets (10.8–15.8%).

Attempts to replace fish meal with meat meal in cultured crustaceans have met with varying degrees of success. Possible reasons for this could be the low protein digestibility and deficient essential amino acids profile (mostly methionine) compared with fish meal (Tan et al., 2005).

The amino acids profile of the experimental diets had methionine levels lower than those required by *L. vannamei* (Akiyama et al., 1991; Fox et al., 1995). This was especially pronounced in the diets with higher PMM inclusion levels (i.e. D-45, D-55 and D-65), which may partially explain the reduced growth performance in these treatments. Tan et al. (2005) observed a similar response in *L. vannamei* fed diets with high MBM levels and determined that the reduced growth was mainly due to deficiencies in dietary methionine. The growth reductions observed in the present study mainly could have been related to methionine deficiency in the diets with high PMM inclusion levels. In a study of the nutritional quality of rendered meat and bone meals (MBM) as a feed ingredient in diets for juvenile *L. vannamei* under experimental intensive rearing conditions, Foster et al. (2003) concluded that MBM could effectively replace between 25 and 75% of fish meal in 35% protein diets, depending on MBM source; a general decrease in growth above 25% replacement was observed for all MBM sources. In this study MBM containing 90% beef had the highest content of essential amino acids and was most apt for use in replacement of fish meal because it provided the best nutritional quality in shrimp diets. The PMM used in the present study was high protein content but had lower histidine, isoleucine, leucine, valine and methionine levels (all essential amino acids) than the 90% beef MBM used by Foster et al. (2003).

In theory, the replacement level of fish meal by alternative proteins in shrimp feeds is partially dependent on the amount of fish meal used in the basal feed (Wang et al., 2005), in addition to experimental conditions. In a raceway pond experiment, Smith et al. (2000) evaluated inclusion of a 56% protein, low-ash (9%), low-fat meat meal (MM) at 150 g kg^{-1} and 300 g kg^{-1} to partially replace fish meal in the basal diet. Inclusion at 300 g kg^{-1} represented a replacement of 41% total crude protein in the diet. Growth rates in shrimp fed the MM diets were not significantly different from those in the basal diet or a commercial *P. monodon* diet. Cruz-Suárez et al. (2007) reported similar responses with practical diets in white shrimp, using a maximum of 50% fish meal (menhaden fish meal) substituted by flash-dried poultry meat and bone meal (PBM) with low-ash and high nutrient quality and availability.

Given the above, the possible reasons for reduced growth performance and feed efficiency observed for *L. vannamei* fed diets at increasing porcine protein inclusion may be due to deficiencies in essential nutrient such as essential amino acids, which is related to origin and chemical of raw material composition of PMM.

L. vannamei seems to be able to utilize good quality pig meat meal, making it a promising alternative protein source in shrimp culture. The low performance reported here in response to this particular ingredient likely could be improved by reducing indigestible materials contends and combined it with other protein and nutrient sources (marine by-products are promising) to complement its amino acids profile and subsequent protein digestibility it could be used to replace up to 65% of fish meal protein without negatively affecting shrimp growth. From the economic standpoint, replacement of fish meal with cheaper animal by-product meal in a practical diet for shrimp can alleviate the problem of low fish meal availability and high cost. The processed by-products can be delivered in the local market at MP (Mexican Peso) $ 4–5 (US$ 0.4)/kg while the present cost of most commercial fish meals is MP $ 15–18 (US$ 1.5–1.8)/kg. However, further research will be needed to determine optimum fish meal replacement levels with pork meat meal in shrimp diets.

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