



# Fishmeal replacement with feather-enzymatic hydrolysates co-extruded with soya-bean meal in practical diets for the pacific-white shrimp (*Litopenaeus vannamei*)

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## Abstract

A feeding experiment was conducted to examine the potential of a commercial steam-processed-feather meal (SPFM) and feathers enzymatically hydrolysed for 60 or 120 min (EHF60 and EHF120) as substitutes for fishmeal (FM) in diets for white-shrimp juveniles. Enzymatically hydrolysed feathers or SPFM were blended through an extruder with soyabean meal (SBM) in a 1:1 ratio (EHF-SBM, SPFM-SBM). Isoproteic and isolipidic diets were formulated to contain 9% EHF60-SBM, 9% EHF120-SBM and 18% EHF60-SBM. These diets were compared with a diet containing 13.7% SPFM-SBM and a control diet designed to contain 18.4% FM and no feather. Quadruplicate groups of 15 shrimp (0.33 g initial-body weight) were fed twice a day on each diet for 4 weeks. The weight gain of shrimp fed on the three EHF-SBM diets did not differ from that of shrimp fed on the FM-control diet; however, shrimp fed on the SPFM-SBM diet gained less weight. The EHF60 and EHF120 coextruded with SBM in a 2:1 ratio were evaluated in a commercial rearing pond. Both ingredients included at 20% in the test diets were compared with a control diet containing 17.8% FM. Triplicate groups of juvenile shrimp (3.4 g initial-mean weight), randomly allocated in 1 m<sup>3</sup> plastic cages, were fed with the test diets during 30 days. Growth (weight gain, specific-growth rate (SGR)) and nutritional value of the diets, food conversion ratio (FCR), protein-efficiency ratio (PER), digestibility were similar. In summary, these results indicate that white shrimp can be fed with a practical diet containing 20% EHF-SBM (2:1) without impairing growth or food conversion. The use of 20% EHF-SBM (2:1) allowed the fish-meal portion to be reduced by nearly by 55%.

**KEY WORDS:** extrusion, feather hydrolyzates, fishmeal, recycling, shrimp, soya-bean meal

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## Introduction

Shrimp diets have long been formulated with fisheries by-products as the primary source of dietary protein (Hughes 1990). Whilst there is no doubt of the high-nutritional value of fishmeal (FM), the best quality meals are expensive, and according to some projections their availability is expected to decline and the price will increase greatly (Dong *et al.* 1993; Rumsey 1993). This shortage of quality FM in the foreseen future has made it necessary to increase the use of other unconventional-protein sources in practical feed.

In Mexico, a large number of indigenous-raw materials such as by-products derived from the poultry industry, are widely available. However, large amounts of these by-products are inefficiently utilized or not utilized at all for any production purposes. A potential source of animal protein for use in shrimp feed can be derived from poultry-feather waste. The volume of this poultry-industry waste represents a serious rendering or disposal challenge, and discarding this material is becoming more difficult owing to restrictive laws that have been enacted to eliminate the current practices of landfill dumps and incineration. At the present, despite the wide utilization of feather meal in feeds for different animal production industries, it has not been used in shrimp feeds. This is because of the paucity of information on nutritional data, and inadequate-economical methods of handling, storage and conversion into acceptable-feed ingredient. However, encouraging results in

several countries under laboratory and practical conditions have shown that it is possible to replace fishmeal in aquatic animals with different types of feather and poultry by-product meals (Koops *et al.* 1982; Fowler 1990; Hughes 1990; Steffens 1994; Bishop *et al.* 1995).

Farming marine shrimp is presently a very fast-growing venture, so the economical processing of this waste and its incorporation into shrimp feed would offer a tremendous potential for both animal processors and producers. However, at present there are but few examples in the literature concerning the successful formulation of diets containing varying percentages of feather meal for crustaceans (Boghen & Castell 1981). Among the reasons for the limited use of feather meal in diets for aquatic animals are its poor digestibility and essential-amino acid profile, attributed to the natural structure of keratin and to commercial steam pressure-processing conditions. Therefore, appraisal of alternative-processing methodologies should be explored. With this regard, waste-treatment systems based on enzymatic hydrolysis and dry extrusion now offer a cheaper small-scale alternative to conventional rendering (Lyons 1992; Tadiyanant *et al.* 1993; Woodroffe 1993).

In this context, the present investigation was designed to determine the suitability of using enzyme-hydrolysed feathers coextruded with soya-bean meal to partially replace FM in a practical-diet formulation for the Pacific-white shrimp *Litopenaeus vannamei*.

## Materials and methods

### Steam-processed feather meal (SPFM)

The feather meal was commercially processed by treating the slaughter wastes in a horizontal dry rendering cooker, steam jacketed and equipped with an agitator (APELSA<sup>®</sup>, Monterrey, Mexico). A mean-operating pressure of 60 psi and a mean temperature of 120 °C were applied during 30 min.

### Enzymatically hydrolysed feather (EHF)

Feathers were obtained from the by-product line of a chicken-processing plant (Rastro S.A. de C.V.). Fresh feathers, without water, were ground in a meat grinder and blended in a vertical mixer with INSTA-PRO<sup>®</sup> (Des Moines IA, USA) proteolytic-enzyme premix (Enzyme premix Number 1955) on a 5.13% wt wt<sup>-1</sup> basis, for 30, 60 and 120 min (EHF30, EHF60 and EHF120). In order to stop the hydrolysis after each time, the enzymatically treated feathers (EMF), with a soup-like consistency, were blended in the

mixer with soya-bean meal (48% crude protein) in a 1:1 or 2:1 ratio (wt wt<sup>-1</sup> basis).

### Degree of hydrolysis

An amount of 10 mL of Tris (0.005 M) NaCl (0.0085 M) pH = 7.5 were used to dissolve 1 g of sample, the mixture was intermittently agitated, using a vortex, during 90 min. After this period the mixture was centrifuged during 15 min at 5000 r.p.m. and the supernatant was separated. In order to determine non-protein nitrogen 10% of cold (4 °C) trichloroacetic acid (TCA) was added to the supernatant (Stone *et al.* 1989). After agitation, the mixture was filtrated on a No. 2 Whatman paper and the rate of hydrolysis of denatured proteins by the enzymatic mixture in the filtrate was measured by the increase in absorption at 280 nm. The final concentration was expressed in mg mL<sup>-1</sup>.

### Extrusion

Moisture content of the material to be extruded was in the range of 20–22%. Extrusion was performed using a single-screw model 600JR INSTA-PRO<sup>®</sup> extruder. Extruder specifications were as follows: 0.5 inch orifice size; 30–60 r.p.m. screw speed; 150–200 kg h<sup>-1</sup> feed rate. A steamlock configuration: 3<sup>5</sup>/<sub>8</sub>, 3<sup>3</sup>/<sub>4</sub>, 3<sup>3</sup>/<sub>4</sub>, 3<sup>3</sup>/<sub>4</sub> was used. The internal temperature of the extruder barrel ranged from 138 to 148 °C at the point of extrusion. For each extrusion mixture the extruder was first run using whole soyabeans in order to stabilize the temperature inside the extrusion barrel. The extruded products were collected into a polystyrene pan, spread on cardboard sheets on the floor, and air dried at room temperature (28 °C) overnight.

### Chemical composition of feed ingredients and experimental diets

Proximate analysis of feed ingredients and diets were conducted by standard AOAC methods (A.O.A.C. & Williams 1984). Moisture was determined gravimetrically considering thermic elimination of water (# 920.36). Crude protein was estimated by a micro-Kjeldahl method (# 984.13; Tecator 1987). Crude lipids were ether extracted by the Soxhlet method (Tecator 1983). Crude fibre was obtained in a fat-free-material sample by dilute acid and alkali treatment (# 962.09), ash content was determined in a muffle furnace by heating at 550 °C for 3 h (# 942.05), and nitrogen-free extract (NFE) was calculated by difference.

### *Pellet water stability*

The stability of the diets in water was evaluated by estimating the loss of dry matter of triplicates (10 g) of pelleted feed after 1 h of intermittent immersion in sea water (28 °C, 35 ppt) according to the methodology described by Aquacop (1978).

### *In vitro digestibility*

*In vitro* digestibility of ingredients was evaluated using a modification of the Lazo single-enzyme assay with porcine trypsin (Lazo *et al.* 1998). The main variation consisted in adding a more concentrated porcine-trypsin solution (3.1 mg mL<sup>-1</sup>) to each substrate mixture in order to meet the saturating conditions of the assay.

### *Feeds and feeding*

The test diets were formulated so that feather (EHF or SPFM) coextruded with soya-bean meal could be added at the expense of different percentages of the fish meal in the control ration.

The EHF60 and EHF120 coextruded with soya-bean meal in a 1:1 ratio (EHF60-SBM and EHF120-SBM) were included in the diet at 9%; EHF60-SBM was also included at 18%. The SPFM-SBM was included at 13.7%. A control diet was designed to contain nearly 18% FM and no feather.

Feed ingredients were ground, sieved and mixed. Minerals and vitamins mixtures were added equally to each diet. The mixture was pelleted with a meat grinder equipped with a 1.5-mm die. Pelleted diets were dried at 80 °C for 1 h, placed in dark plastic bags, and stored at 4 °C until required.

The daily ration (10% of the biomass) was given in two-equal portions at 08:00 and 16:00 h, and, each morning before feeding, faeces and other detritus in the aquarium were siphoned out and mortality was recorded.

### *Laboratory growth trial (expt 1)*

*Litopenaeus vannamei* post-larvae for growth studies were obtained from Genesis S.A. de C.V. shrimp hatchery in Sonora, Mexico. After transportation, shrimp were held in a 500-L fibre-glass tank furnished with synthetic-sea water where they were acclimatized to the experimental conditions and control diet prior to the initiation of the experiment.

Following a fasting period of 24 h, the animals were individually weighed to register the initial weight. The animals were blotted dry before being weighed on a

Sartorius balance to the nearest 0.01 g. At the start of the growth, trial shrimp were selected according to the uniformity of their weight as stated by a one-way ANOVA. Shrimp with a mean-initial weight of  $0.33 \pm 0.0018$  g were randomly assigned to 20 60 L fibre-glass tanks provided with synthetic-marine water in a closed-recirculating system. The tanks were continuously aerated throughout the experiment by an air compressor.

Each of the four-test diets as well as the control diet were fed to four-replicate tanks of shrimp (15 shrimp per tank) over an experimental period of 4 weeks, using a completely random design. Experimental individuals were weighed in bulk on the 15th day to adjust feed allowances. At the end of the study, animals were taken from each tank and were again individually weighed.

### *Field trial (expt 2)*

In order to confirm the results obtained in the laboratory bioassay (expt 1), an experiment under commercial conditions was carried out. The experiment consisted of placing 15 *L. vannamei* juveniles ( $3.40 \pm 0.769$  g mean weight) in a 1-m<sup>3</sup> cage (according to the density currently used in the farm). The food was proffered on a tray at 3.5% of the biomass twice a day for 4 weeks. Three replicates were performed for each treatment. Taking into account the protein content, cost and performance in expt 1, EHF60-SBM and EHF120-SBM (2:1) were included in the diet at 20%. A diet containing nearly 20% FM and no feather was used as a control. The three diets as well as the animals were randomly allocated to the cages.

### *Calculation of diet performance*

Diet performance was evaluated by determining weight gain (WG) =  $100 [(final\ weight - initial\ weight) (initial\ weight)^{-1}]$ ; specific growth rate (SGR) =  $100 [(\ln\ average-final\ weight - \ln\ average-initial\ weight) (No.\ of\ days)^{-1}]$ ; feed-conversion ratio (FCR) = (dry-weight feed (g)) (wet-weight gain (g))<sup>-1</sup>; protein-efficiency ratio (PER) = (weight gain (g)) (protein fed (g))<sup>-1</sup>; survival =  $100 (\text{number of individuals at the end of the experiment}) (\text{number of individuals at the beginning of the experiment})^{-1}$ ; feed consumption = (feed supplied (g) - uneaten feed collected (g)) (number of shrimp in a tank)<sup>-1</sup>.

### *Statistical analysis*

The growth differences among the shrimps reared on the various diets were analysed by a one-way ANOVA with four

**Table 1** Proximate analysis of experimental ingredients (g kg<sup>-1</sup>)

Experimental ingredients	Crude				Nitrogen	
	protein	lipid	Fibre	Ash	free extract	Moisture
EHF30-SBM (1:1)	615	17	25	109	234	105
EHF60-SBM (1:1)	609	14	25	99	253	88
EHF60-SBM (2:1)	602	14	34	91	259	95
EHF120-SBM (1:1)	600	14	25	105	256	89
EHF120-SBM (2:1)	610	11	40	107	232	99
SPFM-SBM (1:1)	503	161	19	45	272	8

and three replicates per-treatment combination for expts 1 and 2, respectively. The new Duncan multiple-range test (Steel & Torrie 1980) was used to identify differences among mean values at the 0.05 level. For comparison of mortalities between treatment values, percentage mortality was subjected to arcsin transformation (Zar 1974) and the resulting data were subjected to ANOVA as above.

## Results

### Experimental ingredients

Proximate analysis of feather ingredients revealed some differences, the SPFM-SBM displayed a higher-lipid content and a lower-protein level when compared with the coextruded-feather hydrolysates (Table 1).

### Degree of hydrolysis

The solubility of nitrogen compounds (free amino-acids and small peptides) as an indicator of the degree of protein hydrolysis showed that the mean-soluble protein of EHF30-SBM although significantly higher than that registered for ground feathers, was lower ( $F = 96.97$ ; 6.63 d.f.,  $P < 0.05$ )

than that determined for the rest of the ingredients (Fig. 1). Thus, this ingredient was discarded from the experimental treatments.

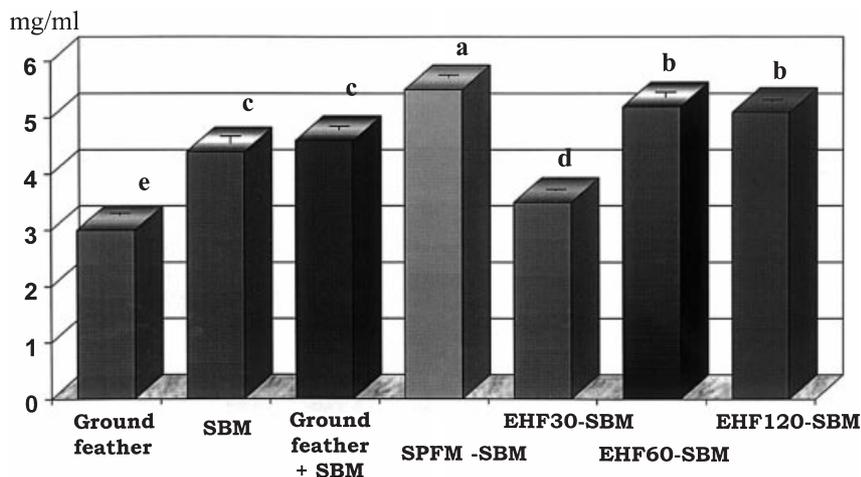
### Experimental diets

Proximate analysis showed that all diets used in expt 1 were nearly isonitrogenous and isolipidic (Table 2a). The slight, but significant differences in the protein ( $F = 13.5$ ; 4.20 d.f.,  $P < 0.05$ ) and lipid content ( $F = 5.8$ ; 4.1 d.f.,  $P < 0.05$ ) of the test ingredients made necessary to adjust the inclusion level of some of the ingredients such as wheat meal, soya-bean meal, cellulose, fish oil, methionine and mono-sodium phosphate. No differences in the loss of dry matter among the test diets were registered. The diets formulated for the field bioassay were isonitrogenous and isolipidic (Table 2b).

### Growth trials

The effects of dietary treatment on shrimp growth and feed-utilization efficiency in laboratory and field experiments are shown in Tables 3a and 3b, respectively.

When EHF-SBM protein replaced FM protein in the diet, there were no significant differences in growth (%WG, SGR) in the animals fed with different diets as compared with those fed with the control diet in the experiments (expt 1:  $F = 0.73$ ; 4.10 d.f.,  $P > 0.05$ ; expt 2:  $F = 0.09$ ; 2.6 d.f.,  $P > 0.05$ ). In spite of the absence of significant differences the control diet showed the lower FCR values, followed by EHF60-SBM 18% and EHF120-SBM 9% in expt 1. In the same way, the higher results concerning the PER were registered for these diets. These results were confirmed in expt 2 where a similarity in growth performance and feed utilization could



**Figure 1** Mean concentration of soluble protein (mg mL<sup>-1</sup>) of experimental ingredients. Mean values of triplicates group values with different superscripts are significantly different ( $P = 0.05$ ).

**Table 2a** Composition and proximate analysis of experimental diets used in experiment 1

Composition (g kg <sup>-1</sup> )	Control diet	EHF60-SBM 9%	EHF60-SBM 18%	EHF120-SBM 9%	SPFM-SBM 13.7%
Crude protein	315	309	296	310	317
Lipids	61	60	61	64	73
Fibre	13	14	15	14	14
Ash	52	53	53	53	50
NFE	526	525	530	523	511
Moisture	33	40	45	36	35
Dry-matter loss (1 h)	76	52	86	69	64
Ingredients					
EHF60-SBM	–	90	180	–	–
EHF120-SBM	–	–	–	90	–
SPFM-SBM	–	–	–	–	137
Soya-bean meal	170	123	78	123	123
Fishmeal	184	146	105	146	146
Shrimp meal	43	43	43	43	43
Wheat meal	483	476	471	475	454
Wheat gluten	40	40	40	40	40
Cellulose	19.9	19.9	19.9	19.8	19.9
Fish (Menhaden) oil	25	26	26	26	10
Soya-bean lecithin	20	20	20	20	12
Etoxiquin	0.2	0.2	0.2	0.2	0.2
Cholesterol	5.4	5.9	5.9	5.9	5.9
Inositol	0.31	0.31	0.3	0.31	0.3
NaH <sub>2</sub> PO <sub>3</sub>	4.0	4.6	6.0	4.6	6.0
DL-Methionin	2.0	3.0	2.2	3.0	2.2
Choline chloride	0.76	0.76	0.76	0.76	0.76
Vitamin C	0.25	0.25	0.25	0.25	0.25
Vitamin mix	2.2	2.2	2.2	2.2	2.2

**Table 2b** Composition and proximate analysis of experimental diets used in experiment 2

Composition (g kg <sup>-1</sup> )	Control diet	EHF60-SBM (2:1)	EHF120-SBM (2:1)
Crude protein	312.3	328.7	318.8
Lipids	61.3	55.5	52.5
Fibre	14.5	14.9	14.6
Ash	62.2	57.6	58.5
NFE	458.9	451.7	463.9
Moisture	90.9	91.8	91.6
Ingredients			
EHF60-SBM (2:1)	0.00	200	0.00
EHF120-SBM (2:1)	0.00	0.00	200
Soya-bean meal	170	50	50
Fishmeal	178.4	102.1	100
Shrimp meal	43	43	43
Wheat meal	488.9	495	485
Wheat gluten	40	40	40
Fish (menhaden) oil	38.7	39.6	40.5
Soya-bean lecithin	20	20	20
Etoxiquin	0.2	0.2	0.2
DL-Methionin	1.1	2.6	2.6
NaH <sub>2</sub> PO <sub>3</sub>	15.8	6.1	7.2
Choline chloride (60%)	0.8	0.8	0.8
Inositol	0.3	0.3	0.3
Vitamin C (Stay C)	0.3	0.3	0.3
Vitamin mix	2.2	2.2	2.2

also be appreciated between the test diets and the control diet.

In both experiments, in all dietary groups tested shrimp fed actively through the experimental period, however, feed intake results in the laboratory trial revealed a better acceptance of EHF60-SBM and SPFM-SBM diets compared with the rest of the test diets experiments (expt 1:  $F = 4.3$ ; 4.1 d.f.,  $P = 0.0157$ ; expt 2:  $F = 0.9$ ; 2.6 d.f.,  $P = 0.4356$ ).

Survival rates did not differ significantly between treatments in any of the experiments.

## Discussion

The most relevant fact in the present study was the lack of significant differences on growth performance and feed utilization between the FM-based-control diet and those diets containing EHF-SBM.

Approximately 85–90% of the protein from the feathers comes from keratin (Moran *et al.* 1966), but owing to its structure, this type of material must be hydrolysed as a prerequisite to be made digestible for the animal, because in its natural state it has little nutritive value. Indeed, the high-cysteine content of keratin (8.8% of the protein) is of

Diet	Control	EHF60-SBM 9%	EHF60-SBM 18%	EHF120-SBM 9%	SPFM-SBM 13.7%
Initial weight	0.33 ± 0.10	0.33 ± 0.09	0.33 ± 0.09	0.33 ± 0.09	0.33 ± 0.09
Final weight (27 days)	1.33 ± 0.32	1.34 ± 0.30	1.41 ± 0.34	1.38 ± 0.41	1.32 ± 0.37
Gain (%)	302	303	323	319	296
SGR	5.4 ± 0.5	5.1 ± 0.09	5.3 ± 0.36	5.1 ± 0.30	5.3 ± 0.20
FCR	1.6 ± 0.1	1.8 ± 0.0	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1
PER	1.9 ± 0.2	1.6 ± 0.1	2.0 ± 0.2	1.9 ± 0.1	1.8 ± 0.1
<i>In vitro</i> digestibility	68 ± 4.99	59 ± 2.27	78 ± 3.54	76 ± 1.45	66 ± 13.65
Feed intake	1.7 ± 0.1 <sup>a,b,c</sup>	1.8 ± 0.0 <sup>a</sup>	1.7 ± 0.0 <sup>b,c</sup>	1.6 ± 0.0 <sup>c</sup>	1.8 ± 0.0 <sup>a,b</sup>
Survival	96 ± 4	88 ± 8	88 ± 6	89 ± 3	96 ± 4

Mean of triplicates group values in the same row with different superscripts are significantly different ( $P = 0.05$ ).

**Table 3b** Growth and feed-utilization efficiency data for shrimp fed with the test diets in experiment 2

Diet	Control	EHF60-SBM 20%	EHF120-SBM 20%
Initial weight	3.53 ± 0.15	3.31 ± 0.27	3.36 ± 0.24
Final weight (27 days)	7.55 ± 0.72	7.18 ± 0.04	7.25 ± 0.56
Gain (%)	113.9	116.9	115.8
SGR	2.8 ± 0.21	2.8 ± 0.29	2.8 ± 0.05
FCR	1.2 ± 0.04	1.7 ± 0.56	1.3 ± 0.34
PER	2.5 ± 0.08	1.8 ± 0.57	2.3 ± 0.57
<i>In vitro</i> digestibility	50 ± 4.46	54 ± 16.04	63 ± 7.25
Feed intake	2.96 ± 0.12	2.76 ± 0.22	2.8 ± 0.18
Survival	93 ± 6	82 ± 16	91 ± 10

structural importance by virtue of the disulphide bonds, which apparently stabilize the aggregation of cylindrical units into cables. In this regard, the degree of hydrolysis, as indicated by the soluble-protein concentration, after exposing the feathers to the enzyme mixture was raised significantly with time. The highly selective action of proteases on inter- and intra-peptide disulphide bonds may have resulted in a group of peptides of uniform size with a great similarity in their structural properties. This is likely to be the reason of the better growth performance of animals fed with EHF-SBM diets compared with SPFM-SBM control diet. By contrast, it has been reported that the excessive and non-selective hydrolysis underwent by the feathers as a result of the combination of high pressure (60 psi) and temperature (120 °C, 60 min) usually leads to the formation of an important amount of free-amino acids and peptides of different size (Menassa 1982), which would explain the highest concentration of soluble protein of SPFM-SBM (5.478 mg mL<sup>-1</sup>).

Similarly, experiments in rainbow trout have shown that partially hydrolysed material is superior in nutritional value

**Table 3a** Growth and feed utilization-efficiency data for shrimp fed with the test diets in experiment 1

than that attained when proteolysis was allowed to continue, producing high levels of free-amino acids (Dong *et al.* 1993; Hardy *et al.* 1983). This is explained by the high amount of free amino acids in fully hydrolysed material which causes a premature absorption of certain essential-amino acids present in the free form relative to the absorption of other essential-amino acids present as polypeptides or intact proteins. These essential-amino acids, if prematurely absorbed, may be irreversibly further metabolized and will not be available for protein synthesis. On the contrary, the gradual liberation of amino acids from intact proteins and peptides by digestive enzymes ensures that amino acids are available for growth over a prolonged-time period. As a result, protein utilization and growth are more efficient when the correct proportion of all essential-amino acids are simultaneously available to the tissues (Hardy 1991). Considering this, the presence of peptides of an adequate size may have contributed to a good growth performance and nutritional value (PER, FCR and digestibility) of the EHF-SBM diets.

To this should be added that the regular commercial-processing method of feather has a significant effect on its nutritive value, namely in an extensive-cysteine degradation (McCasland & Richardson 1966; Moran *et al.* 1966), which leads to the formation of unnatural-amino acids, particularly lanthionin, so the enzymatic attack necessarily associated with digestion process is hindered (Papadopoulos *et al.* 1985). In the case of the EHF-SMB ingredients, less damage is caused to the nutrients because the material passing through an extruder is subjected to heat only for a very limited time (less than 30 s).

The blending of hydrolysed feathers with SBM was advantageous from the functional and nutritional standpoint. On one hand, at the functional level, the addition of a dry ingredient such as SBM (7% moisture) to the feather hydrolysates contributed to stop partially the enzymatic

hydrolysis reaction, by diminishing the quantity of water (Harvey 1992) and by providing an excess of substrate for the enzymes. Moreover, it was possible to achieve a moisture content of less than 22% which is a requisite for materials being fed to the extruder (Woodrofe & Kerns 1993).

On the other hand a major advantage from the nutritional standpoint, that may have resulted from the blending of SBM with feather hydrolysates, is the complementation of the amino-acids profile of both protein sources. This is because keratin is generally low in lysine, but high in sulphur-amino acids (cystine, cysteine and methionine), while SBM although poor in sulphur amino acids is rich in lysine. The combination of these materials could have been therefore synergistic. Although attempts have been made to improve the nutritive value of feather meal-based diets for some aquatic species by adding certain amino acids (Tiews *et al.* 1979; Hughes 1990; Steffens 1994), in the present study a vegetal protein source used as a substitute for amino acid deficiencies instead of individual amino acids supplements was considered as a more practical and more cost-effective alternative.

In addition, the process of extrusion offered several benefits when recycling feather hydrolysates with SBM. First of all, the enzymatic reaction was completely stopped by the temperature reached during the extrusion process. Secondly, it made it possible to obtain a single ingredient with a better amino-acid profile and a nutritive-value enhancement (digestibility, PER, FCR). Thirdly, the final moisture of the ingredients was only of 3.5–4.5%, which allowed storing over a long period at room temperature. A further advantage of extrusion is the sterilization of the product, which is a requisite when recycling animal by-products.

The ingredients resulting from the coextrusion of feather hydrolysates and SBM showed not only an acceptable level of protein (54.5–61.5%), but also a low lipid level (1.0–1.6%). This turned into an advantage while formulating because such a low quantity allowed the introduction of unsaturated C20 and C22 fatty acids from marine-oil sources (fish) that are essential for the growth performance of shrimp. On the contrary, the SPFM-SBM resulted in a lower-protein content (49%) and a higher-lipid level (16.1) than the rest of the experimental ingredients. This was probably a consequence of the commercial processing of a raw material constituted mostly by chicken feathers but also by turkey feathers. Besides of the different processing conditions of feathers, i.e. steam cooked or enzymatically hydrolysed, differences in nutrient composition between feather meals have often been attributed to the inclusion of variable amounts of non-feather residues, e.g. blood (Wessels 1972). This different composition could explain the lower-growth

rates exhibited by the shrimps fed with the SPFM-SBM diet. Our results concur with those of Gallagher & Degani (1988), in this aspect, who observed that eels fed with diets containing poultry oil or a mixture of poultry oil and fish oil had a significantly lower growth than eels fed with diets containing fish oil.

The performance of diets containing EHF-SBM in both experiments (Tables 3a and 3b) could be attributed to the combined effect of the selective-enzyme hydrolysis and the effect of extrusion on proteins. Such a 'pre-digestive' action might have facilitated the digestion and absorption of the resulting peptides and amino acids.

In general the diet formulation ensured that the essential-nutrient requirements were met with proper growth or body maintenance as shown by survivorship that was not significantly different among treatments in both experiments.

Water stability of experimental feeds and the control diet was similar albeit the former containing feather hydrolysates. Water stability through 8 h with or without binders has been reported for the extrusion of cooked sinking-aquatic feeds (Kearns *et al.* 1988) – additionally the wheat flour and wheat gluten at the level included have enough inherent binding ability to have good water stability without added binders.

The incorporation of EHF-SBM ingredients in the diet compares favourably with FM as all diets showed a good acceptability. This, in spite of the known increase in attractability and palatability of feeds caused by the use of fishmeal (Davis *et al.* 1995) also contributed to the low-feed intake associated with the materials used to produce the EHF-SBM ingredients. Indeed, lower-feed consumption have been pointed out when animals are fed with different types of poultry by-products, which may be related to a higher digestible-energy content through the addition of too-high a level of fish oil to compensate for unsaturated essential-fatty acids; excess-digestible energy in the diet would result in a reduced food and protein intake. (Fowler 1991). In the same way reduced-feed consumption of diets containing soya-bean meal have been well documented for different species of fish (Reigh & Ellis 1992; Pongmaneerat & Watanabe 1993; Davis *et al.* 1995), and it has also been reported that some species of shrimp find soya-bean products unpalatable (Lim & Dominy 1990). The acceptability of test diets could be caused by the leakage of free-amino acids and peptides from the diets and to the fact that some spoilage may have occurred during the processing of feathers which could have led to the formation of biogenic amines known to act as feed incitants in crustaceans (Mendoza *et al.* 1997).

A simple-cost analysis suggested better economic efficiency, based on a difference in price of 15%, when 55% of

FM is replaced with the EHF-SBM in a 2:1 ratio. This is considered as a Mexican regular FM made from mackerel press cake and solubles, mixed and dried in a direct-flame dryer. However, it is very likely that the percentage of substitution would be lower if a premium Chilean or Norwegian FM were to be replaced, yet considering their higher price it could still be advantageous to include the EHF-SBM ingredient. Moreover, it should be taken into account that poultry feathers and soya-bean meal are available from many sources during periods when FM supply are limited, and which are always less expensive than FM. Their incorporation into shrimp diets would be beneficial especially in the region where there is a need for good-quality FM because imported FM is expensive. Added value of diets to which EHF-SBM is incorporated comes from a good acceptability, improved-water stability, nutritive complementation, formula-cost saving and enhanced handling properties of a shelf-stable product. Other advantages include the partial elimination of a waste-disposal problem, a supply of useable materials at low cost and good-environmental practice.

In conclusion, the observed-growth rates suggest that resulting ingredient used as supplement can comprise 20% of a practical diet for *L. vannamei* by concurrent reduction of the FM content by 55% without compromising performance. In this way, the experiment verifies, that EHF-SBM is a satisfactory proteinaceous ingredient and may be recommended as a suitable and FM sparing ingredient in diets for shrimp.

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