

Research Article

Replacement of fishmeal by hydrolyzed feather meal in diets of juvenile *Macrobrachium tenellum* (river prawns) and its effect on muscle fatty acids

Daniel Badillo-Zapata^{1,3} , Fernando Vega-Villasante¹ , Héctor Nolasco-Soria⁴ 
Lus López-Acuña² , Manuel Alejandro Vargas-Ceballos¹  & Fernando Barreto-Curiel² 

¹Laboratorio de Calidad de Agua y Acuicultura Experimental, Departamento de Ciencias Biológicas
Centro Universitario de la Costa, Universidad de Guadalajara, Puerto Vallarta, Jalisco, México

²Facultad de Ciencias Marinas, Universidad Autónoma de Baja California (UABC)
Ensenada, Baja California, México

³Investigadoras e Investigadores por México, Consejo Nacional de Humanidades
Ciencia y Tecnología (CONAHCYT), Ciudad de México, México

⁴Laboratorio de Fisiología Comparada y Genómica Funcional. Centro de Investigaciones
Biológicas del Noroeste, S.C., La Paz, B.C.S., México

Corresponding author: Fernando Barreto-Curiel (fbarreto8@uabc.edu.mx)

ABSTRACT. In recent years, the partial and total substitution of fishmeal (FM) with by-product meals has reduced feed manufactory and aquaculture industry production costs. The present work aimed to determine the effects of the substitution of FM by hydrolyzed feather meal (HFM) in the diet of *Macrobrachium tenellum*. Four isoproteic and isolipidic diets were prepared, substituting 0, 33, 67, and 100% of FM with HFM. These diets were used to feed *M. tenellum* juveniles for 60 days. The results of the biological indices, in terms of weight, final length, and specific growth rate, did not show significant differences between the treatments ($P > 0.05$). The lowest survival index (35 ± 13) is observed in the 100HFM treatment, showing significant differences from the 33HFM treatment (65 ± 13). However, the 67HFM and 100HFM treatments, associated with the lowest values of the biological indices, indicated a decreasing trend. No significant differences were observed in the proximate composition of the muscle between the four treatments. The average values of the four treatments were 33.5% protein, 3.7% lipid, and 18.2% ash. However, the fatty acid profile observed significant differences between the treatments. As the content of HFM increased, the concentration of some fatty acids, such as C14:0, C16:0, C20:5n3, and C22:6n3, decreased, while the content of C18:1n9 and C18:2n6 increased. The results showed that it is feasible to substitute between 33 and 67% of FM with HFM in feed diets for river prawns.

Keywords: *Macrobrachium tenellum*; profile of fatty acids and amino acids; feather meal; prawns river

INTRODUCTION

One of the most widely used ingredients to increase the protein content of aquaculture feed is fishmeal (FM). Its unique characteristics include high digestibility, palatability, amino acid content, and lipid quality (Tacon & Metian 2008, Tangendjaja 2015, Yanti et al. 2019, Meng et al. 2020). Thus, FM is a valuable ingredient in the aquatic feed industry, but it is costly

and has limited availability (Tacon et al. 2011, Subedi et al. 2019). In recent decades, many studies have focused on finding alternative sources of proteins and lipids to substitute FM and fish oil, which have been the main ingredients in the formulation of aquaculture feeds (Gatlin et al. 2007, Tacon & Metian 2008, Meng et al. 2020). Since their price is very competitive, soybean meal and its concentrates are among the most widely used ingredients to replace FMs. However,

these ingredients are associated with various physiological and pathological problems, such as anti-nutritional factors, deficits of essential amino acids, low digestibility, non-soluble carbohydrates, cases of irritable bowel syndrome, and enteritis. They are also relatively costly since they must be imported from several countries (Cheng et al. 2004, Wang et al. 2009, 2017, Li et al. 2020). Several authors have reported that the partial or total replacement of FM by soy flour or concentrate did not have any effect on species such as *Cherax quadricarinatus* (Campaña-Torres et al. 2005), *Macrobrachium tenellum* (García-Ulloa et al. 2008), *M. rosenbergii* (Hasanuzzaman et al. 2009), *Oncorhynchus mykiss* (Cruz-Castro et al. 2011), *Oreochromis niloticus* (Lin & Luo 2011), *Lutjanus guttatus* (Silva-Carrillo et al. 2012), *Sebastes schlegelii* (Lee et al. 2016), and *Dormitator latifrons* (Badillo-Zapata et al. 2021). Other alternative protein sources are the by-products of different food or industrial processes, such as poultry by-product meal, bone meal, meat meal, hydrolyzed feather meal, and blood meal. These are viable alternatives due to their high protein content, excellent amino acid profile, and competitive price, and have been shown to improve the nutritional quality of aquaculture feeds, leading to higher growth rates and lower production costs (Bureau et al. 2000, Durazo et al. 2003, Gatlin et al. 2007, Badillo-Zapata et al. 2014, Pares-Sierra et al. 2014, Wang et al. 2017).

Hydrolyzed feather meal (HFM) contains 83% crude protein, of which keratin can represent up to 90% of the total protein (Alzamora et al. 2018). Keratin is a protein that contains disulfide-cystine bonds, which give it the characteristic of being structurally resistant, insoluble in water, and highly stable (Gallardo et al. 2015). However, in its native form, the utilization of this by-product is very complicated, so industries have developed methods to hydrolyze feather meal and increase its digestibility up to 98% (Fakhfakh et al. 2011). On the other hand, HFM primarily shows a deficiency in methionine and histidine. Still, depending on the extraction method, it can decrease other essential amino acids such as lysine and tryptophan (Florida 2019).

Around 13.7 million metric tons (MT) of chicken will be produced worldwide in 2023 (US\$ 2023). The by-product of feathers generated accounts for approximately 7 to 10% of the total live weight of birds (Webster et al. 1996), resulting in an availability of HFM of 1.3 MT and a protein proportion of 1.1 million MT. In Mexico specifically, the price of HFM ranges from US\$ 900.00 to 1,000.00 per ton. With this premise, a significant protein contribution could partially meet the protein demand in aquaculture feeds.

The prawn river is a food product with a high protein content (Vega-Villasante et al. 2014). This prawn species, unlike most others, has appropriate characteristics for cultivation. They do not exhibit cannibalism or aggressiveness and can be cultivated in high densities (Ponce-Palafox et al. 2002). It also tolerates a wide and fluctuating range of temperatures, from 16 to 32°C, and salinities from 0 to 20 (Vega-Villasante et al. 2014). Nonetheless, the nutritional requirements of this species have been little studied, as has been the effect of using alternative protein sources. For these reasons, the present study aimed to evaluate the substitution of FM by hydrolyzed bird feather meal (as a protein source) in the diet of *M. tenellum* juveniles and its effect on muscle fatty acids.

MATERIALS AND METHODS

Experimental design

Five hundred juveniles of longarm river prawn (*M. tenellum*) were obtained from the hatchery section of the Water Quality and Experimental Aquaculture Laboratory in Puerto Vallarta, Jalisco, Mexico. They had an average weight of 0.04 ± 0.01 g and an average length of 9.4 ± 0.1 mm. The collected specimens were taken to the Water Quality and Experimental Aquaculture Laboratory (LACUIC, by its Spanish acronym) at the Coast University Center of the University of Guadalajara in Puerto Vallarta, Jalisco, Mexico. The organisms were distributed in four tanks of 500 L each. An acclimatization was carried out for 15 days to discard any effect due to the environmental change. During this period, the organisms were fed a commercial shrimp formula/feed (Purina®) with 30% protein and 5% fat at 3% of wet weight. Water temperature was monitored daily using a multiparameter probe (YSI 550 A). The average temperature was 28.5 ± 1.0 °C, with a photoperiod of 14:10 (light:dark).

The experimental unit (EU) consisted of 12 glass tanks with a capacity of 40 L. The tanks were filled to 50% of their capacity (20 L). A Hush 35 cascade filter (Elite®, 75 to 135 L) was used in each tank. The air supply was continuous, provided through a diffuser stone. An oxygen concentration of 5.5 mg L^{-1} was maintained in each tank. After acclimatization, 240 organisms (0.05 ± 0.02 g) were randomly selected and distributed with a density of 20 ind tank^{-1} ($20 \times 4 \times 3$). Each experimental treatment was performed in triplicate. The individuals were fed 5% of their biomass thrice daily (10:00, 13:00 and 18:00 h). The experimental assay was maintained for 60 days.

Biometric parameters were measured every 30 days to monitor the growth rate and the increase in biomass. The food accumulated in the bottom was removed daily from the tanks. The survival rate was measured daily.

The biological indices were calculated as follows:

$$\text{Weight gain (g d}^{-1}\text{)} = \text{final weight} - \text{initial weight} / \text{number of study days}$$

$$\% \text{ Weight increase (WI\%)} = [(\text{final weight} - \text{initial weight}) / \text{initial weight}] \times 100.$$

$$\text{Specific growth rate (SGR)} = [(\text{final weight} - \text{initial weight}) / \text{number of days}] \times 100.$$

$$\text{Survival (\%)} = 100 - (\text{initial} - \text{final number of individuals} / \text{initial number of individuals}) \times 100.$$

Experimental diets

The proximal composition, amino acids (AA), and fatty acids of HFM are shown in Tables 1-2. Compared to FM, poultry oil was added to compensate for the lower oil content in HFM. All ingredients were ground (250 µm) and mixed using a 3-L blender (KitchenAid). The resulting mixture was passed through a meat grinder (Torrey®) with a hole diameter of less than 1.0 mm to make pellets. The pellets were dried at 60°C for 24 h, using a convection drying. (NOVATECH). Afterward, the feed was cooled to room temperature (28°C). The experimental diets were placed in hermetically sealed plastic bags and stored at -20°C for later use as feed. The diets' AA and fatty acid profiles are shown (Tables 3-4).

Four experimental diets were prepared (Tables 3-4, all isoproteic (30% crude protein, CP) and isolipidic (8% crude fat, CF). FM was replaced by HFM at four inclusion percentages: 0, 33, 67, and 100%. These values were selected according to Montoya-Martínez et al. (2018a) since it is shown that the prawns have a high digestibility for the HFM, and a wide range of inclusion was opened. Therefore, this study explored the full range of substitution with equidistant inclusions of 33% to observe the effect of 100% FM replacement. On the other hand, the experimental capacity available in the LACUIC is limited, allowing for the validation of only four treatments with their respective replications.

Proximate analysis

The experimental diets and the muscle tissue extracted from the organisms under study were subjected to proximate analysis, crude protein (method 960.52), crude lipid (method 920.97), ash (method 942.05), and moisture (method 925.10), following standard methods (AOAC 1995). Free nitrogen extract (NFE) was determined by difference (Jobling 2001).

Table 1. Ingredient (g kg⁻¹), proximate composition (% dry weight basis), and amino acid composition (% dry weight basis) of hydrolyzed feather meal (HFM) were utilized for the formulation of the experimental treatments. Obtained from Proteínas Marinas y Agropecuarias SA de CV. Guadalajara. México. NFE: free nitrogen extract.

Proximate composition (% of dry matter) HFM	
Moisture	4
Crude protein	80.7
Crude fat	13
Ash	1.9
NFE	0.4
Amino acids HFM	
Essential amino acids	
HIS	1.4
ARG	8.6
THR	4.1
VAL	3.9
MET	0.6
LYS	2.7
ILE	3.6
LEU	7.9
PHE	4.5
Subtotal	37.4
Non-essential amino acids	
ASP	3.8
SER	11.0
GLU	8.9
GLY	9.6
ALA	4.9
PRO	2.3
TYR	2.9
Subtotal	41.0
Total	80.7

Amino acid (AA) profiles

The AA content of each diet and muscle tissue was determined in the ground, homogenized, and defatted samples (using Soxhlet extraction, according to AOAC (1995)). Four hundred microliters of HCl 6 N were added to 10 mg of each sample. The mixture was then digested for 24 h at 110°C in a closed vial under a nitrogen atmosphere to prevent oxidation. The digested samples were diluted to 25 mL with deionized water. One milliliter of 2.5 mM α-aminobutyric acid (AABA) was added as an internal standard. The samples were then filtered (0.45 µm Teflon filter) and stored under nitrogen at -30°C. AA samples were derivatized (Waters AccQ Tag™ Kit, Cat. N°186003836, Milford, MA, USA) (Cohen & De Antonis, 1994) and injected into an HPLC (Waters), using a C-18 column (3.9×150 mm; Waters) and a water-acetonitrile gradient, at 37.5°C

Table 2. Fatty acid (FA) composition (% of total fatty acids) of the hydrolyzed feather meal (HFM) obtained from Proteínas Marinas y Agropecuarias SA de CV, Guadalajara, México. Nd: non detected.

FA	HFM
C12:0	0.7
C13:0	8.6
C14:0	0.7
C16:0	24.3
C17:0	0.6
C18:0	8.4
C20:0	0.7
C21:0	0.4
C24:0	0.5
Σ SFA	44.8
C16:1	5.6
C18:1n7	1.3
C18:1n9	32.3
C20:1	0.5
C24:1	0.7
Σ MUFA	40.4
C18:2n6	13.3
C18:3n3	0.5
C18:4n6	0.3
C20:3	0.5
Σ PUFA	14.6
EPA+DHA	nd
Σn3/n6	0.1

for elution. Following the supplier's recommendations (Waters AccQ Tag™), the samples were monitored using a fluorescence detector (Waters 474 series, Milford, MA, USA) with an excitation wavelength of 250 nm and an emission wavelength of 395 nm. Standard curves for AA quantification were obtained using AA standard solution (18.5 to 300 pmol; Waters Corporation. Cat. N°WAT088122, Milford, MA, USA).

Fatty acid profiles

The analysis of fatty acids in the diets and muscle tissue was performed according to the method described by Folch et al. (1957), with some modifications. The diets and muscle tissues were individually homogenized. Lipids were extracted overnight at 4°C, using a mixture of dichloromethane-methanol (2:1, v/v) and 1% butylated hydroxytoluene (BHT) as antioxidants. The lipids were subjected to saponification using a 0.3 N solution of 90% methanolic KOH, and the unsaponifiable lipids were separated. Fatty acid methyl esters (FAME) from lipid extracts from diet pellets and muscle tissues were prepared according to Christie (1993). FAMES were separated in a gas chromatograph

(Agilent Technologies 6880) equipped with a flame ionization detector (260°C) and a capillary column (Agilent DB-23; 60 m × 0.25 mm, film thickness 0.25 μm), using hydrogen as carrier gas. The initial oven temperature was 140°C. One microliter of the solution containing the FAMES was injected; 5 min later, the temperature was increased (at a rate of 4°C min⁻¹) to 250°C and kept at that temperature for an additional 10 min. Fatty acids were identified by comparison with the retention times of FAME standards (37 Component FAME Mix and PUFA1, Supelco/Sigma-Aldrich). The concentration of each fatty acid was calculated from the corresponding area in the chromatogram using C19 fatty acid as the internal standard and the Agilent ChemStation software package (version E.02.00.493).

Statistical analysis

Results are reported as means and standard deviations. After checking the data's normality and homoscedasticity (Levene's test), a one-way ANOVA was performed to determine if there were significant differences in the biological indices between dietary treatments. A posteriori analysis was performed using Tukey's multiple comparison test when significant differences were found. Differences were considered statistically significant if $P < 0.05$. All statistical tests were performed using Statistica 10.0 (Stat Soft, Inc. Tulsa, OK, USA).

RESULTS

Growth and survival

No significant differences in weight gain (0.65 ± 0.15 g d⁻¹) were observed, and height, SGR, and survival were observed between treatments at the end of the 60-day experiment. However, a trend was observed in which the larger the percentage of HFM included in the diet, the lower the increase in size (0.53 ± 0.01) of the individuals under study. The 100HFM diet was associated with the lowest increase in weight (692%) compared to the 0HFM and 33HFM diets (1672 and 1465%, respectively). The 0HFM, 33HFM, and 67HFM diets had a survival rate ranging from $56.0 \pm 0.9\%$, while the 100HFM diet was associated with the lowest survival rate, $35.0 \pm 13.0\%$ (Table 5).

Biochemical composition of prawn tissue

There were no significant differences between treatments in the muscle tissue of the prawns in terms of protein, CF, ash, and NFE content. The content of these nutrients was similar (Table 6).

Significant variations were found between treatments regarding the fatty acid profile of prawn muscle

Table 3. Ingredients (g kg⁻¹), proximate composition (% dry weight basis), and amino acid composition (% dry weight basis) of the four experimental diets containing different levels of substitution of fishmeal with hydrolyzed feather meal (HFM; 0, 33, 67 and 100% substitution). *Sardine Fishmeal, fish oil, hydrolyzed feather meal, and Poultry oil were obtained from Proteínas Marinas y Agropecuarias SA de CV. Guadalajara. México. **Free nitrogen extract (NFE, %) = 100 - (% crude protein + % total lipid + % ash).

Ingredients (g kg ⁻¹)	Experimental treatments			
	0 HFM	33 HFM	67 HFM	100 HFM
Fishmeal (FM)*	432.0	265.0	94.0	0.0
Hydrolyzed feather meal (HFM)*	0.0	143.0	289.0	369.0
Corn meal	55.0	55.0	55.0	55.0
Fish oil*	47.0	37.0	21.0	0.0
Poultry oil*	0.0	18.0	42.0	68.0
Cornstarch	369.0	388.0	405.0	411.0
Gelatin	60.0	60.0	60.0	60.0
Rovimix	30.0	30.0	30.0	30.0
Stay C	4.0	4.0	4.0	4.0
Sodium benzoate	2.0	2.0	2.0	2.0
Choline chloride	1.0	1.0	1.0	1.0
Tocopherol	0.1	0.1	0.1	0.1
Total	1000	1000	1000	1000
Proximate composition (% of dry matter)				
Moisture	3.2	3.5	3.0	3.2
Crude protein	30.0	30.5	30.6	30.0
Crude fat	8.5	8.4	8.0	8.3
Ash	8.5	8.9	9.2	9.6
NFE**	53.0	52.2	52.2	52.1
Essential amino acids (% of dry diet)				
LYS	2.1	1.7	1.1	1.1
MET	1.9	1.1	1.0	0.8
THR	1.6	1.6	1.7	1.6
ARG	2.0	2.0	2.2	2.0
PHE	1.3	1.3	1.5	1.4
HIS	0.7	0.6	0.5	0.4
ILE	1.3	1.4	1.6	1.5
LEU	2.2	2.2	2.2	2.5
VAL	1.5	1.8	1.9	1.8
Non-essential amino acids (% of dry diet)				
ALA	2.9	3.1	2.7	3.0
PRO	1.5	2.1	2.5	2.7
TYR	0.8	0.9	0.9	1.0
ASP	2.6	2.3	2.0	1.9
SER	1.3	1.9	2.5	2.7
GLU	3.7	3.5	3.2	3.1
GLY	2.5	2.5	2.4	2.4
TAU	0.15	0.10	0.05	0.00

at the end of the experiment (Table 6). Saturated fatty acids (SFA) showed significant differences in C14:0 and C16:0, decreasing their concentration as the proportion of HFM in the diet increased. Concerning monounsaturated fatty acids (MUFA), significant differences were found only in C18:1n9, increasing as the proportion of HFM increased. Polyunsaturated fatty acids (PUFAs) showed the same behavior, with α -linolenic acid (C18:2n6) increasing as the proportion of

HFM increased. Fat deposition ranged from 2.6 to 5.7%. The content of eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) decreased as the proportion of HFM increased, showing significant differences between treatments, as shown by the broken-line model (Fig. 1). The proportion found in n3/n6 tended to decrease as the proportion of HFM increased.

Table 4. Fatty acid (FA) composition (% of total fatty acids) of the four experimental diets containing different levels of substitution of fishmeal with hydrolyzed feather meal (HFM; 0, 33, 67 and 100% substitution) used to feed juvenile *Macrobrachium tenellum* prawns. ND: no determinate.

FA	Experimental treatments			
	0 HFM	33 HFM	67 HFM	100 HFM
C15:0	0.87	0.91	0.87	1.01
C16:0	30.16	29.03	29.89	36.56
C17:0	1.35	1.08	0.84	0.47
C18:0	2.31	1.54	0.92	0.19
C20:0	0.35	0.47	0.35	0.52
C23:0	0.32	0.29	0.27	ND
Σ SFA	45.3	42.4	40.1	46
C14:1	0.17	0.2	0.19	0.21
C16:1	12.11	9.94	8.98	8.87
C17:1	0.15	0.19	0.19	0.14
C18:1n7	3.86	5.12	5.51	7.98
C18:1n9	13.77	16.36	18.97	21.4
Σ MUFA	30	31.8	33.8	38.6
C16:2n4	1.36	0.92	0.71	0.37
C16:3n4	0.54	0.68	0.61	1.00
C18:2n6	3.65	5.46	8.11	10.63
C18:3n4	0.41	ND	0.26	0.25
C18:3n3	0.68	0.77	0.98	0.63
C18:4n3	1.04	0.91	0.84	0.36
C20:2	0.36	0.47	0.43	0.56
C20:4n6	0.72	0.78	0.81	0.32
C20:4n3	0.32	0.3	0.2	ND
C20:5n3	9.25	8.13	5.76	ND
C22:5n3	0.87	0.79	0.34	ND
C22:6n3	6.13	5.18	4.1	ND
Σ PUFA	19.9	17.5	13.9	5.7
EPA+DHA	15.3	13.3	9.8	2.8
Σn3/n6	4.1	2.5	1.3	0.3

Table 5. Biological indices for juvenile *Macrobrachium tenellum* prawns fed with four experimental diets containing different levels of substitution of fishmeal with hydrolyzed feather meal (HFM; 0, 33, 67 and 100% substitution). Values are given as mean and \pm standard deviation (SD), n = 3. The biological indices were calculated based on 60 days of treatment. SGR: specific growth rate. *Values in the same row with different superscripts are statistically different $P < 0.05$, a>b.

Biological indices	Experimental treatments			
	0 HFM	33 HFM	67 HFM	100 HFM
Initial weight (g)	0.05 \pm 0.02	0.05 \pm 0.02	0.05 \pm 0.02	0.05 \pm 0.02
Final weight (g)	0.88 \pm 0.41	0.78 \pm 0.1	0.63 \pm 0.09	0.53 \pm 0.01
Initial length (mm)	11.9 \pm 2.7	11.9 \pm 2.7	11.9 \pm 2.7	11.9 \pm 2.7
Final length (mm)	39.06 \pm 7.2	45.49 \pm 3.7	41.29 \pm 2.3	40.6 \pm 3.5
Increase in weight (%)	1,672 \pm 836	1,465 \pm 218	1,161 \pm 186	962 \pm 259
Increase in size (ind mm ⁻¹ d ⁻¹)	0.42 \pm 0.1	0.53 \pm 0.1	0.46 \pm 0.0	0.45 \pm 0.1
Increase in weight (ind g ⁻¹ d ⁻¹)	0.014 \pm 0.007	0.012 \pm 0.002	0.010 \pm 0.002	0.008 \pm 0.002
SGR	4.7 \pm 0.7	4.6 \pm 0.2	4.2 \pm 0.3	3.9 \pm 0.4
Survival (%)	53.0 \pm 18.0 ^{ab}	65.0 \pm 13.0 ^a	50.0 \pm 8.0 ^{ab}	35.0 \pm 13.0 ^b

Table 6. Proximate (% dry matter) and main fatty acid (FA) composition (% of total fatty acids) of muscle tissue of juvenile *Macrobrachium tenellum* fed with four experimental diets containing different levels of substitution of fishmeal with hydrolyzed feather meal (HFM; 0, 33, 67 and 100% substitution). *Values in the same row with different superscripts are statistically different $P < 0.05$. a>b>c, NFE: free nitrogen extract. ND: no determinate.

	Experimental treatments			
	0 HFM	33 HFM	67 HFM	100 HFM
Crude protein (%)	34.1 ± 1.9	33.6 ± 0.9	33.3 ± 2.2	33.7 ± 3.0
Crude fat (%)	3.1 ± 0.8	3.7 ± 0.4	3.9 ± 0.4	3.9 ± 1.7
Ash (%)	18.6 ± 1.9	16.9 ± 2.6	18.3 ± 2.0	20.8 ± 1.8
NFE	44.1	45.8	45.5	41.6
FA (% of total)				
FA	0 HFM	33 HFM	67 HFM	100 HFM
C10:0	1.54 ± 0.9	0.77 ± 0.3	0.80 ± 0.5	0.37 ± 0.1
C12:0	0.21 ± 0.01	0.17 ± 0.05	0.26 ± 0.1	0.12 ± 0.02
C13:0	1.15 ± 0.4	2.05 ± 0.6	1.01 ± 0.3	0.40 ± 0.17
C14:0	8.22 ± 2.0 ^a	7.10 ± 0.8 ^a	7.931 ± 1.57 ^a	3.90 ± 1.4 ^b
C15:0	1.24 ± 0.03	1.10 ± 0.1	ND	0.88 ± 0.13
C16:0	32.4 ± 0.4 ^a	28.90 ± 1.3 ^b	24.54 ± 2.0 ^c	26.6 ± 2.7 ^{bc}
C17:0	0.57 ± 0.2	0.62 ± 0.3	0.5 ± 0.04	0.60 ± 0.06
C20:0	0.30 ± 0.0	0.30 ± 0.03	0.29 ± 0.01	0.24 ± 0.01
Σ Saturated	45.1 ± 2.8 ^a	41.0 ± 2.6 ^a	39.7 ± 1.3 ^{ab}	33.0 ± 4.7 ^b
C14:1	0.60 ± 0.1	0.64 ± 0.1	1.17 ± 0.2	0.5 ± 0.22
C16:1	14.31 ± 1.3	13.0 ± 0.6	11.77 ± 3.0	12.13 ± 4.8
C17:1	1.6 ± 0.1	1.45 ± 0.1	1.55 ± 0.2	1.83 ± 0.94
C18:1n7	7.0 ± 1.7	6.57 ± 0.4	6.2 ± 0.7	6.50 ± 0.9
C18:1n9	15.37 ± 0.6 ^d	19.03 ± 1.4 ^c	20.4 ± 1.1 ^b	24.57 ± 1.2 ^a
Σ MUFA	34.1 ± 6.0	40.7 ± 1.9	40.1 ± 2.8	41.0 ± 2.4
C16:2n4	0.34 ± 0.01	0.94 ± 0.1	0.38 ± 0.1	0.13 ± 0.02
C16:3n4	0.97 ± 0.1	ND	1.13 ± 0.2	0.84 ± 0.14
C18:2n6	2.64 ± 0.1 ^c	3.83 ± 0.3 ^b	5.17 ± 0.5 ^a	5.73 ± 0.22 ^a
C18:3n4	0.24 ± 0.01 ^a	0.25 ± 0.03 ^a	0.31 ± 0.14 ^a	0.17 ± 0.0 ^b
C18:3n3	0.34 ± 0.1	0.41 ± 0.01	0.33 ± 0.1	0.25 ± 0.025
C18:4n3	0.26 ± 0.0	0.22 ± 0.02	0.24 ± 0.05	0.22 ± 0.02
C20:2	0.78 ± 0.3	0.58 ± 0.1	0.46 ± 0.2	0.52 ± 0.32
C20:4n6	1.5 ± 0.03	1.70 ± 0.3	1.75 ± 0.6	3.0 ± 1.72
C20:4n3	0.8 ± 0.1	ND	ND	ND
C20:5n3	3.9 ± 0.26 ^a	4.66 ± 0.6 ^a	4.07 ± 1.23 ^a	1.21 ± 0.2 ^b
C22:5n3	0.22 ± 0.05	0.16 ± 0.04	0.12 ± 0.0	0.13 ± 0.0
C22:6n3	2.9 ± 0.2 ^a	2.18 ± 0.4 ^b	2.01 ± 0.4 ^b	0.61 ± 0.4 ^c
Σ PUFA	13.8 ± 0.3	14.1 ± 1.2	15.4 ± 1.7	16.0 ± 5.0
EPA+DHA	6.7 ± 0.1	6.2 ± 0.8	6.5 ± 1.7	1.82 ± 0.4
Σn3/n6	2.3 ± 0.7 ^a	1.3 ± 0.2 ^a	0.9 ± 0.1 ^{ab}	0.7 ± 0.04 ^b

No significant differences were observed between treatments regarding the content of most of the essential and non-essential AA in the prawn muscle tissue. Only the histidine (His) content showed a significant difference in the analyzed tissue, its concentration increasing as the proportion of HFM increased. In the 100% HFM treatment, the concentration of histidine was greater than 1.1 ± 0.1 , different than the concentrations obtained with the 0 and 33% HFM treatments (Table 7).

DISCUSSION

The balanced feed industry for aquaculture has carried out the partial replacement of the FM with terrestrial animal by-products (Glencross et al. 2020); this is thanks to its low carbohydrate content compared to vegetable sources and its similar AA profile VS FM (Stone 2003, Li et al. 2021). In particular, HFM has been described as an ingredient with a high content of keratin and as a low digestibility protein source (Poppi

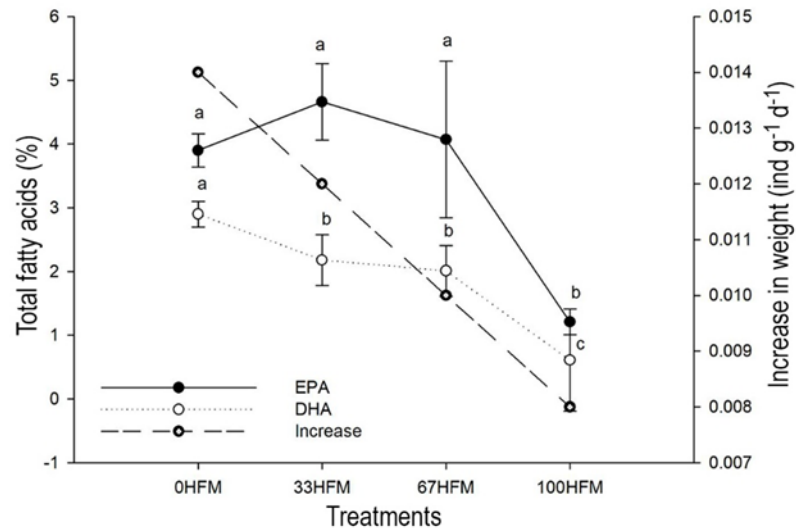


Figure 1. Effect of the substitution of hydrolyzed feather meal (HFM) in EPA and DHA levels in the muscle tissue of juvenile *Macrobrachium tenellum* fed with four experimental diets for 60 days (HFM; 0, 33, 67, and 100% substitution). Values are given as mean and \pm standard deviation (SD), $n = 3$. Values with different superscripts are statistically different $\alpha < 0.05$.

et al. 2011), showing different levels of inclusion or substitution of FM in different aquatic organisms (Yu 2008), such as golden pompano *Trachinotus ovatus*, gilthead seabream *Sparus aurata*, prawns *Macrobrachium nipponense*, European sea bass *Dicentrarchus labrax*, of 12 to 25% of replacement (Campos et al. 2017, Psafakis et al. 2020, Zohu et al. 2022). In this way, our results of the biological indices did not show significant differences in any of the parameters. However, the productive indices tended to decrease as the proportion of HFM increased to substitute FM (Table 5); this could be due to the keratin content, as well as its low content of essential fatty acids. Generally, it has been reported that 85-90% of the protein content of feather meal comes from keratin, which in its natural state has little nutritional value (since it is generally considered to have relatively low digestibility). Keratin cannot be fully utilized as a nutrient if it is deficiently hydrolyzed (Mendoza et al. 2000). However, other authors (Bureau 2000, Mendoza et al. 2000, Montoya-Martínez et al. 2016, 2018b, Campos et al. 2017) have proposed the use of HFM as a possible replacement for proteins of marine origin. The advantages of feather hydrolysate are its low cost and its relatively high digestibility in fish (NRC 1993) such as rockfish, *S. schlegelii* (Lee 2002); Nile tilapia, *O. niloticus* (Suloma et al. 2014); European sea bass, *D. labrax* (Campos et al. 2017); as well as crustaceans such as white shrimp, *Penaeus vannamei* (Mendoza et al. 2000); and shrimp, *M. tenellum* (Montoya-Martínez

et al. 2018a). In the same vein, it is known that the hydrolysis process used by the industry to produce HFM affects the product's amino acid quality, digestibility, and palatability (Florida 2019). In particular, Fakhfakh et al. (2011) have reported an increase in digestibility of up to 98% of the protein.

Regarding the nutritional value of HFM, it has been reported to be rich in cysteine, threonine, and arginine but deficient in lysine and methionine (Campos et al. 2017). However, the AA profile of the formulations studied here did not show these nutritional properties (Table 3). The results of the biological indices and the proximate analysis of the experimental formulations containing HFM indicate that it is a good ingredient in diets for *M. tenellum*. In the same way, mortalities increased as HFM was included, which could infer that n-3 chain essential fatty acids may decrease biological indices and, most likely, with more study days, could lead to total treatment mortality. A similar response was obtained by D'Abramo & Sheen (1993), who observed that enrichment of PUFAs increases growth by up to 93% in juveniles of the freshwater prawn *Macrobrachium rosenbergii*.

Therefore, it is suggested to conduct specific research to assess the minimum levels of essential fatty acids in substituting FM with HFM for *M. tenellum*.

Based on our broken-line model, Figure 1 shows that the increase in weight of the prawns tended to be smaller as the proportion of HFM increased. It is also

Table 7. Amino acid profile (g 100 g⁻¹) of the muscle tissue of juvenile *Macrobrachium tenellum* fed with four experimental diets containing different levels of substitution of fishmeal with hydrolyzed feather meal (HFM; 0, 33, 67 and 100% replacement). *Values in the same row with different superscripts are statistically different $P < 0.05$.

Amino acids	Experimental treatments			
	0 HFM	33 HFM	67 HFM	100 HFM
LYS	2.2 ± 0.2	2.1 ± 0.5	2.3 ± 0.3	2.3 ± 0.1
MET	1.0 ± 0.2	1.5 ± 1.0	0.7 ± 0.2	1.0 ± 0.3
THR	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.0	1.7 ± 0.1
ARG	3.5 ± 0.4	3.9 ± 0.5	4.2 ± 0.5	4.0 ± 0.6
PHE	1.7 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
HIS	0.8 ± 0.1 ^b	0.8 ± 0.2 ^b	0.9 ± 0.0 ^{ab}	1.1 ± 0.1 ^a
ILE	1.5 ± 0.1	1.8 ± 0.5	1.5 ± 0.1	1.4 ± 0.0
LEU	2.5 ± 0.2	2.0 ± 0.4	2.2 ± 0.0	2.1 ± 0.0
VAL	1.7 ± 0.1	1.4 ± 0.4	1.5 ± 0.2	1.5 ± 0.0
Subtotal	16.5 ± 0.2	16.6 ± 0.3	16.5 ± 0.2	16.6 ± 0.1
Non-essential amino acids				
ALA	3.2 ± 0.2	3.2 ± 0.2	3.2 ± 0.1	3.1 ± 0.1
PRO	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.0
TYR	1.3 ± 0.1	1.5 ± 0.2	1.4 ± 0.0	1.4 ± 0.1
ASP	3.2 ± 0.3	3.1 ± 0.0	3.0 ± 0.1	3.0 ± 0.1
SER	1.5 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.4 ± 0.1
GLU	4.4 ± 0.2	4.1 ± 0.3	4.2 ± 0.1	4.3 ± 0.1
GLY	2.2 ± 0.1	2.0 ± 0.2	2.0 ± 0.2	2.3 ± 0.2
Subtotal	17.1 ± 0.1	16.7 ± 0.2	16.5 ± 0.1	16.8 ± 0.1
Others				
TAU	0.4 ± 0.1	0.3 ± 0.3	0.1 ± 0.2	0.2 ± 0.2
Total	34.0	33.6	33.2	33.6

observed that the total percentage of certain fatty acids in tissue (Table 6) decreases as the proportion of HFM increases due to the proportion established in the treatments (Tables 2-4), with a higher concentration of C18:1n9 and C18:2n6 as the HFM content increases, and a decrease in C20:5n3 and C22:6n3 (EPA and DHA). With a higher proportion of HFM, there is a lower incorporation of ARA, EPA, and DHA in the diet and the muscle tissue. Furthermore, it can be observed that river prawns mobilize and utilize these essential fatty acids for their physiological functioning, as they are not maintained in the same proportion.

The concentration of some fatty acids showed an inversely proportional relationship with the percentage of HFM in the diet. The content of ARA (C20:4n6), EPA (C20:5n3), and DHA (C20:6n3) decreased significantly in muscle tissue as the proportion of HFM increased (Table 6), which could have contributed to the decrease in the values of the biological indices associated with the experimental treatments. The role played by these fatty acids in developing prawn juveniles still must be elucidated.

A decrease in the content of EPA and DHA was observed in the diets and the muscle tissue of *M.*

tenellum as the amount of HFM increased as a substitution for FM. However, the decrease in the content of these PUFAs did not significantly affect the biological indices of the four treatments, even when it is known that EPA and DHA are considered essential fatty acids with high nutritional value (Cavalli et al. 1999), suggests that *M. tenellum* does not require high incorporation of fish oil in the diet. A further study could assess the specific requirement of these fatty acids in this prawn species. The most relevant PUFAs, such as arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), are generally recognized as important nutritional components (Crawford et al. 1976, Harrison 1990, Henderson et al. 1996). Their importance lies in their primary function as precursors of biologically active eicosanoids, vital components of cell membranes, and regulators of cell functions. EPA and DHA have also been found to improve the hatching process and larval quality in *M. rosenbergii* (Cavalli et al. 1999, 2001). Assessing the minimum requirements of EPA and DHA for the ontogenic development, fattening growth, and reproduction of prawn species would be very useful. It is known that n-3 and n-6 PUFAs (particularly EPA,

DHA, and ARA) cannot be synthesized *de novo* by most animals. So they or their precursors, such as α -linolenic acid (ALA, C18:3n-3, precursor to EPA and DHA) and linoleic acid (LIN, 18:2n-6, precursor to ARA) (Bell et al. 1986, Sargent et al. 1995) must be included in the diet. González-Baró & Pollero (1998) found that *M. borellii* is not capable of synthesizing ARA or EPA from shorter and more saturated fatty acids, which require elongation and desaturation of the chain. The authors did not detect the activity of the enzymatic system that converts dihomo- γ -linolenic acid (DHGLA, 20:3n-6) to ARA. In the present study, linoleic acid showed a significant increase in the 33, 67, and 100% substitution treatments and the muscle tissue of the organisms under study. However, the content of ARA in muscle tissue did not show significant differences between the four treatments at 60 days, while the content of EPA and DHA decreased as the proportion of HFM increased; this could indicate that *M. tenellum* cannot synthesize these fatty acids. However, a further study on enzymatic expressions should be carried out to examine this hypothesis.

A balance between the absolute concentrations of the two types of PUFAs (omega-3 and omega-6: n-3 and n-6, respectively) is important (Sargent et al. 1995). The results of the present study show that the substitution of 33%, and up to 67%, of FM for HFM in the diet of juvenile river prawns is optimal since the content of EPA and DHA did not decrease drastically in these treatments. It can be inferred that the 100% FM diet exceeds the physiological capacity of the species since the content of EPA and DHA in the organisms fed with that diet remained at the same levels as in those fed with the 33 and 67% HFM diet. The proportions of HFM used in the present experiment differ from those proposed by Tacon et al. (2011), who in a compilation of data on the use of poultry by-products indicated that the maximum proportion intervals for carnivorous fish and crustacean species should be between 10 and 15%, depending on the by-product. The maximum proportion of HFM for omnivorous/herbivorous species is indicated to be between 10 and 20%. From the results of the biological indices and the AA profiles, no significant differences are observed among the treatments, and this indicates that the physiological performance of prawn river was not compromised; however, there is a tendency to reduce the variables when FM was 100% substituted by HFM. These responses have been observed in fish, pigs, and livestock, where the use of HFM has a beneficial impact on animal nutrition (Madrid 2014, Godínez-Siordia et al. 2021).

The interaction between HFM and chicken oil (PO) cannot be described in this study as an experimental design was not set up to make inferences between each protein or lipid source. However, PO has been described as a lipid source that enhances the palatability of dietary ingredients, does not impair growth, and has shown good digestibility, as has been observed in carnivorous fish, such as yellowtail kingfish, salmon, and trout (Bowyer et al. 2012, Emery et al. 2013, Hatlen et al. 2013, Turchini et al. 2013). Similarly, the ideal inclusion level for this lipid source in *M. tenellum* has yet to be reported. However, Boateng et al. (2023) replaced fishmeal with poultry by-product meal in *M. rosenbergii*. They found that a 75% replacement of both protein and lipid did not result in significant differences in growth rates and proximate composition of the organisms. Based on these premises, it can be inferred that *M. tenellum* has plasticity in incorporating different protein and lipid sources, as it did not show significant differences in growth.

The organisms under study might have the necessary enzymatic capacity to take advantage of the new protein source without drastically reducing growth. This result agrees with that reported by Montoya-Martínez et al. (2018a,b), who evaluated *in vitro* the digestibility, attractability, and palatability of this nutrient (HFM) in the diet of *M. tenellum* and reported high digestibility values for the HFM. Further studies with different levels of PUFAs and lipids sources are needed to assess their effect on the physiological performance of cultivated organisms. It would help develop balanced feed formulations for exploiting *M. tenellum* in the central coastal region of Mexico.

Conflict of Interest Statement

The authors declare no conflicts of interest regarding this research article.

Data Availability Statement

The authors declare that all the data reported here were generated during the bioassay in the Laboratory of Water Quality and Experimental Aquaculture, Department of Biological Sciences, University Center of the Coast, University of Guadalajara. All additional information can be requested from the corresponding research.

Ethics Statement

The Secretariat of Agriculture and Rural Development approved all procedures of organism management. Mexican Official Standard (NORMA Oficial Mexicana NOM-646062-ZOO 1999).

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