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Nutritional Value of *Macrobrachium rosenbergii* Prawns Fed on Extruded Feeds Enriched with Linseed and Whey Proteins

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Abstract

Three extruded diets were formulated and used to cultivate *Macrobrachium rosenbergii* prawns. For ninety days, the prawns were fed on the similarly balanced isonitrogeneous and isoenergetic diets, based on albumin and linseed (Linseed diet), on whey proteins concentrate (WPC) and soybean oil (Whey proteins diet), and on albumin and soybean oil (Control diet). Every thirty days, the shrimps were weighed and submitted to analyses. The results showed that the average body weights for the shrimps fed on the Linseed and Whey proteins diets were approximately sixty percent higher than for those fed on the Control diet. No significant differences were found for the protein content and the total lipid content in shrimps fed on the diets. However, the Linseed diet led to the lowest cholesterol level and improved the ratio between the sum of n-6 and the sum of n-3 fatty acids in the whole body of shrimps. WPC was used for the first time as a protein source for shrimp feeds, and may be considered as a component to be used in extruded diets for aquaculture. The results suggested that the experimental diets succeeded to provide shrimps with greater weight gain and nutritional value.

Keywords: Cholesterol; Fatty acids; Linseed; Shrimp feed; Whey proteins

Introduction

Population growth, combined with the demand for good-quality seafood, has contributed to the development of shrimp aquaculture. The total annual aquaculture production of all species of freshwater prawns had risen to almost 444000t in 2009 [1]. As an economic activity, the cultivation of the freshwater prawn *Macrobrachium rosenbergii* constitutes 52% of the global total [1]. Its culture has expanded continuously, and reveals that this species has a high potential for aquaculture [2].

Fish meal and fish oil have been the main sources of proteins and lipids in the diets for several cultured specimens [3,4]. In spite of their importance, a coniderable reduction in the use of these ingredients is expected in coming years. Limited availability and variable supply are primary concerns [3]. The availability of fish meal and oil is largely dependent upon weather patterns, and excessive exploitation has caused reduction in supply and increasing prices [3,4]. On the other hand, feed manufacture requires nutritional and cost-effective formulations based on the content and availability of indispensable nutrients, with a reduction in feed cost per unit production [5].

United Nations (UN) Food and Agriculture Organization's (FAO) estimates that 75% of the world's fisheries are fully or overexploited [6]. Replacement of fish meal with alternative sources of proteins from terrestrial animals or plants has been encouraged [6]. Additionally, the use of wild fish in the form of fish meal and fish oil as inputs for aquaculture feeds, relies on marine species that are renewable, but often overexploited for human use [7]. Many studies raise questions about the sustainability of the various alternatives for aquatic feed ingredients. Fish meal and fish oil are limited. Fish oil may in the future be a scarcer commodity than fish meal for use in aqua feeds [7,8].

Supplementation with other ingredients was shown necessary to improve the nutritional quality of such diets [3]. The soluble proteins of milk, also known as whey proteins, are by products of the dairy industry. They have been used in infant and sportsmen formulas because of their high nutritional value, with high levels of essential amino acids, especially branched-chain amino acids. They also have a high level of calcium and bioactive peptides [9,10]. Many positive observations pertaining to the application of whey proteins in wearing pig diets, chickens diets and in calf milk replacers can be found in the literature [11]. Whey proteins are not yet, a competitively priced source of dietary protein for the animal feed industry, with values 7-8 times more expensive than protein from regular soybean meal [11].

Accordingly, the substitution of fish oil in feed compositions has deserved attention. While terrestrial animal fats are rich in saturated fatty acids, vegetal oils are characterized by a high C18-fatty acids content. Recently, experiments were conducted to determine apparent digestibility coefficients of lipid and fatty acids by juvenile halibut. The apparent digestibility of poultry fat (saturated acids) was lower than that of vegetal oils (unsaturated fatty acids). The highest apparent digestibility coefficient was found in groups fed on linseed oil [12]. Linseed oil is considerably good alternative lipid source for salmonids, freshwater fish and prawns. The prices of vegetable oils are more stable and even less expensive than fish oil [13].

Nutritionally, linseed (*Linum usitatissimum*) offers excellent sources of nutrients and energy because it is one of the richest sources of alpha-

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linolenic acid [14,15]. Results from an increasing number of studies clearly indicate that oilseed lipids can be valuable ingredients for fish feed formulations. The replacement of dietary fish oil by vegetable oils had better survival and growth of the freshwater prawn *Mcrobrachium rosenbergii* [16]. Linseed can be included into livestock feeds to modify the fatty acid composition of meat, milk and eggs, providing additional health benefits to consumers [17,18]. Lipids and the associated C-18 polyunsaturated fatty acids (PUFA), linoleic (18:2 n-6) and linolenic (18:3 n-3) acids, as well as n-3 and n-6 highly-unsaturated fatty acids (HUFA) (eicosapentanoic acid, EPA; docosahexanoic acid, DHA; and arachidonic acid, AA), are required in shrimp and other crustaceans feeds [19].

Addition of whey proteins and sources of unsaturated fatty acids, together with other technological resources, may contribute to improve the quality of aquaculture feeds. One of these resources consists of extrusion, which is a process that submits the feed formulation to mixing, shearing and heating under high pressure before the extrudate is forced through a die. Extrusion is the most important process used in the production of fish feeds and pet foods. During the process, feeds undergo reactions that could be beneficial if their nutritional value is improved or detrimental if nutrients are destroyed or become resistant to digestion [20,21]. Studies with blue shrimp (*Litopenaeus stylirostris)* have shown that prior heat treatment was necessary to improve digestibility and possibly inactivate anti-nutritional factors [22]. Denatured proteins are more easily digested compared to the native globular structure. Shear forces play an important role in changing the nutritive value of proteins [21]. This technology also improves the physical characteristics of the pellet, mainly water stability, which is very important for crustacean feeding [22].

Although several studies were reported on partial or complete replacement of fish meal and fish oil in diets for other shrimp species, only a few studies were performed for *Macrobrachium rosenbergii* juveniles with the objective of evaluating their nutritional values. In this work, new extruded feeds enriched with whey proteins and linseed was obtained and the nutritional value of shrimps fed for three months with such diets were evaluated monthly.

Materials and Methods

Defatted egg albumin was purchased from Galgrin Group Ltda. (Caitité, BA, Brazil). Whey protein concentrate (WPC), Alacen™ 450, was supplied by Probiótica Produtos Naturais (São Paulo, SP, Brazil). According to the manufacturer, the concentrate contains 82.3 wt% total proteins. Regular corn starch (CS) with 0.5% gluten, and 12 wt% moisture content was supplied by Corn Products Brazil (São Paulo, SP, Brazil). Food grade butylated hydroxy toluene was supplied by Vetec Química Fina Ltda. (Duque de Caxias, RJ, Brazil). A mixture of minerals and vitamins was purchased from Nestlé Purina (São Paulo, Brazil). Linseed brown was purchased from Erva Viva. Vitamin C was supplied by Hoechst do Brasil Química e Farmacêutica S.A. (Novo Hamburgo, RS, Brazil). A commercial kit CAT. Nº 01400 from Laborlab Produtos para Laboratórios Ltda. (Guarulhos, SP) was used for the quantification of cholesterol in shrimps. All other reagents and solvents were purchased from Vetec Química Fina Ltda. (Rio de Janeiro, Brazil).

Experimental animals and installation

The experiment took place in Itaipuaçu, Maricá, in Rio de Janeiro State (RJ). One thousand juveniles freshwater prawns (*Macrobrachium rosenbergii*), 45-days old, with average initial weight of 130–190 mg, were purchased from Santa Helena Farm, Silva Jardim, RJ. 250 juveniles were separated at the time initial (0 day) to carry out chemical analyses and weighing. Juveniles are very small, requiring a larger number of prawns for chemical analyses. Seven hundred fifty were divided into three groups: 1st, a test group fed on a diet based on egg albumin complemented with linseed (Linseed diet); 2nd, a test group fed on a diet based on whey proteins concentrate and soybean oil (Whey proteins diet); 3rd, a control group fed on a diet based on egg albumin and soybean oil (Control diet). The experiments were performed in polyethylene tanks of 1000 L, covered by a screen. Shelters were provided to serve as refuges. The water in the tanks was aerated with electric pumps (Sarlo Better, model S300–110 V) with a capacity of 280 L/h, and partially changed daily, after being siphoned. In the afternoon, dirt accumulated at the water surface was removed. For 90 days, the prawns were fed in the morning and in the evening with a diet corresponding to 5 wt% of their body weight [23].

Experimental diets and feeding

The diets were formulated according to SEBRAE [23], with the bromathological characteristics recommended for shrimp feed, which consist of protein (25-30 wt%), carbohydrate (30-40 wt%), fat (6-8 wt%), fiber (6-8 wt%), ash (8-10 wt%), moisture (up to 10 wt%), Ca/P ratio (2.5/1). Raw ingredients and formulations compositions are shown in Table 1.

The diet based on linseed was formulated taking into account its cellulose and oil content. In the formulation of the diet based on whey proteins, the lipid content in whey was considered. Linseeds were milled in a conventional blender shortly before processing, and the resulting flour was used.

Preparation of diets

The experimental formulations, with approximately 25 wt% moisture, were prepared by mixing dry ingredients for 30 min in a domestic blender. Diets were prepared by extrusion in a Haake Rheocord 9000 system (Karlsruhe, Germany), equipped with a single-screw extruder, and four heating zones. The barrel length-to-diameter ratio was 25:1. The screw speed was maintained at 20-40 rpm, and

Ingredients (wt. %)	Linseed diet	Whey protein diet	Control diet
Albumin	33.03	-	38.18
Linseed	20.07	-	-
Whey proteins concentrate	-	45.65	-
Corn starch	39.03	36.28	41.15
Soybean oil	-	3.95	6.55
Cellulose	-	6.25	6.25
BHT[a]	0.02	0.02	0.02
Calcium carbonate	2.10	2.10	2.10
Dicalcium phosphate	5.30	5.30	5.30
Minerals and vitamins mixture[b]	0.4	0.4	0.4
Vitamin C[c]	0.05	0.05	0.05
Total	100	100	100

[a] Buthyl hydroxyl toluene (antioxidant); [b] Minerals and vitamins mixture consisting of Mg (0.4 g/kg), Mn (10 mg/kg), Cu (50 mg/kg, Zn (100 mg/kg), I (0.3 mg/kg), Se (0.15 mg/kg), vit. A (3800 IU/kg), vit. D3 (1900 IU/kg), vit. E (140 IU/kg), vit. K (20 mg/kg), folic acid (7 mg/kg), choline (1400 mg/kg), biotin (0.20 mg/kg), niacin (130 mg/kg), calcium pantothenate (40 mg/kg), thiamin (15 mg/kg), riboflavin (20 mg/kg), pyridoxine (20 mg/kg), vit B12 (20 mcg/kg); [c]vit. C (L-ascorbic acid-2-monophosphate (150 mg/kg)

Table 1: Ingredients and composition of experimental diets.

the temperatures of the heating zones were 110, 105, 105, and 90°C from feed zone to die end. The samples were extruded via an attached circular die. After extrusion, the diets were dried to a moisture content around 6 wt% using a forced air oven at temperatures below 55°C.

Chemical analyses

Thirty shrimps (whole body) from each group were individually weighed before the experiments and every 30 days. Although individual weights were recorded, only the average weight values within each tank were used in the data analysis.

The chemical analyses of the shrimp whole body, at different stages of growth (0, 30, 60 and 90 days), were performed in triplicate. On the first day of the experiment, and every 30 days, 70 shrimps were collected from each group to be submitted to analyses. Lipids were extracted from crushed shrimps with chloroform/methyl alcohol/water mixture (1:2:0.8), and quantified according to the Bligh-Dyer method [24]. The protein content was determined by the micro-Kjeldahl method [25].

The enzymatic method (cholesterol oxidase, COD) was used to determine the cholesterol content in shrimps [26,27]. The kit from Laborlab is composed of ready-to-use reagents: a standard reagent (cholesterol solution at 200 mg/dL), enzymatic reagent (lipase ≥ 300 U/mL, COD ≥ 20 U/mL), color reagent 1 (4-aminophenazone at 0.025 mol/L) and color reagent 2 (phenol at 0.055 mol/L). The working reagent was prepared by mixing 0.5 mL of color reagent 1, 0.5 mL of color reagent 2, 19 mL of water, and 0.2 mL of the enzymatic reagent. After lipid extraction and saponification, 2 mL of the working reagent were added to the unsaponifiable fraction. The reaction was carried out in a water bath at 37°C for 10 min. Then, the absorbance of the resulting products was read at 505 nm, in a UV-vis Spectrophotometer Shimadzu model 1601 (Tokyo, Japan), against a control containing only isopropyl alcohol and the working reagent. The cholesterol concentration was determined with a standard curve obtained by plotting absorbance values for standard cholesterol solutions (0.01-0.05 mg/mL).

Fatty acids analyses of diets and whole body of shrimps were carried out on fatty acid methyl esters (FAMEs) diluted in hexane. FAMEs were obtained by base-catalyzed methylation [28]. Separation and analysis of FAMEs were conducted by injecting 1 µL of sample into a Shimadzu gas chromatograph (GC) model QP 2010 (Tokyo, Japan), equipped with a mass spectrometer detector, and a fused-silica capillary column (DB-5ms) from Agilent Technologies (J & W Scientific, CA, USA) with 20 m length, 0.18 µm thickness and 0.25 µm diameter. The GC injector and detector temperatures were set at 250°C; the column oven temperature was set at 110 °C, the column flow was 0.76 mL/min, and the linear velocity was 40 cm/s. Helium was used as the carrier gas at a total flow rate of 42 mL/min. The injector pressure was held constant at 144.0 kPa. The different fatty acids were identified by comparing their mass spectra with the spectra patterns belonging to the library`s equipment. Individual FAME peaks on the gas chromatograms were identified by comparison of retention times.

Fatty acids concentrations were estimated by using the ratio of relative peak area of each fatty acid and the relative peak area of palmitic acid (C16:0), which was the major saturated fatty acid in shrimp samples.

Statistical analysis

The results are presented as mean values ± standard error. The data of different treatments, obtained on every thirty days, were submitted to one-way analysis of variance (ANOVA) with Tukey-Kramer Multiple Comparisons Test for all groups and Student's *t* test unpaired for comparison between two groups. Differences were considered significant at 5%. Statistical analyses were performed by GraphPad InStat 3.01.

Results and Conclusion

The complete substitution of fish meal and fish oil did not affect the survival of *Macrobrachium rosenbergii* juveniles, which was higher than 95%. Also, no negative effect on the palatability of the diets was observed, but an increased feed intake was observed for the diet based on linseed, probably because of its stronger odor.

Although no significant differences in growth rate were observed by Gupta et al. [29] for *Macrobrachium rosenbergii* juveniles fed different experimental isonitrogeneous and isocaloric diets, which varied in terms of percent contribution of major protein sources (fish meal, soybean meal, groundnut oil cake and mustard oil cake), some differences were observed in the present work. Figure 1 shows the variation in weight for the three groups of shrimps, fed for 90 days (Figure 1).

On the 30th day of the experiment, the group fed on the diet based on linseed (0.39 ± 0.06 g) showed a significant improvement in weight ($P<0.05$) in relation to the control group (0.27 ± 0.12 g). This difference disappeared on the 60th day; however, a significant improvement in weight ($P<0.05$) was observed for shrimps fed on the Whey proteins diet (0.58 ± 0.26 g) in relation to the group fed on the Control diet (0.34 ± 0.14 g). On the 90th day, the average weights for the groups fed on the Linseed (0.83 ± 0.28 g) and Whey proteins (0.79 ± 0.19 g) were statistically different ($P<0.05$) in relation to Control diets (0.49 ± 0.12 g). The quality of the lipid profile of linseed might have contributed to the weight gain of shrimps fed on the Linseed diet. Also, the high biological value of proteins of whey (94.6) might have influenced the weight gain of shrimps, compared to the control group, in which the diet was based on egg albumin, with a biological value of 83 [30]. For *Macrobrachium rosenbergii* prawns cultured for 63 days in new earthen ponds to which artificial feed was offered during the last 42 days of culture, the weight gain was higher [31] than those observed in this work. However, for prawns of the same species, fed for 40 days with a diet rich in casein and supplemented with oleic acid and 3.9% lipids, a mean weight similar to those observed for the groups fed on the diets based on linseed and on whey proteins was reported [14]. Anh

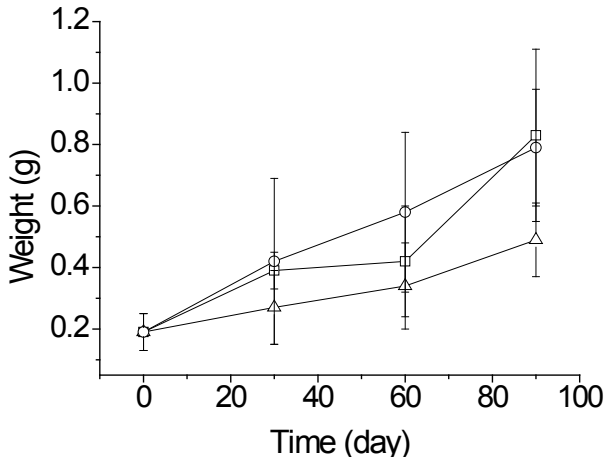

Figure 1: Average weight (g) of *Macrobrachium rosenbergii* prawns along the 90-days experiments; Linseed diet (□), Whey proteins diet (○), Control diet (Δ).

et al. [32] in their studies with diets based on artemia meal, soybean meal, soybean oil, squid oil, gelatin and wheat flour obtained weight values between 0.08- 0.21g, lower to this work, for thirty days. Despite the initial weight being 0.12 g and this work of 0.19 g. Variations in average weight might be attributed to the species, age, availability of food offered to animals (tank size and number of shrimps in the race for food), and type of diet and to the extension of the experiment. In the case of *Macrobrachium rosenbergii* species, males normally occur as three morphotypes, with weights varying in the range 5-250 g [33].

Table 2 shows results from chemical analyses of shrimp whole body in total protein and lipid along the 90-days period.

After 60 days, the group fed on the Whey proteins diet showed a higher protein value (16.37 ± 0.24%) than the other groups fed on the Linseed (11.54 ± 0.49%) and Control (14.98 ± 0.29%) diets (P<0.05). A significant decrease in protein concentration (P<0.05) was observed from the first day up to the 60th day for the group fed on the Linseed diet. However, after 90 days, the protein content for this group was improved and reached a typical value. At the end of the experiments, no statistical difference in protein content was found for the three groups fed on the three different diets, although egg albumin had a lower biological value than whey proteins. For *Litopenaeus vannamei* fed for 8 weeks on a diet based on fish meal, Zhong et al. [34] found a protein content of 16.34 wt%. Protein source was cited as a major factor influencing survival and growth of shrimp [35]. In the present case, the difference in protein source (egg and whey) did not affect survival, and crude protein content in adult *Macrobrachium rosenbergii*. In a study on the growth performance of Pacific white shrimp (*Litopenaeus vannamei*) fed different diets formulated with high levels of soybean meal in combination with 10 wt% either poultry by-product, fish meal, distiller's dried grains with solubles, or pea meal, the diet containing fish meal showed no benefit on growth performance, survival, or feed conversion rate compared with the other formulations [36]. In general, juvenile and sub adult shrimp presented a greater weight gain, percent weight gain, feed efficiency and protein conversion efficiency on a protein-fed basis when fed on a 32% protein diet [37]. Experiment with diets based sardine fishmeal, corn oil, corn flour, wheat bran and soybean meal was found protein content between 13.07–13.95% [38].

As for total lipid, the control group presented the lowest lipid content (1.21 ± 0.11%) (P<0.05) after 30 days. On the 60th day, the groups fed on the Linseed diet (1.63 ± 0.31%) and the Control diet (1.45 ± 0.02%) presented a lower lipid content (P<0.05) than the group fed on the Whey proteins diet (2.21 ± 0.17). However, by incorporating linseed in the diet as the sole source of lipid and fiber, the lipid level was maintained constant until the end of the experiment. After 90 days, the lipid content for whole body of shrimps fed on the Linseed diet was maintained constant along the experimental period. However, no significant differences (P > 0.05) were found for total lipid content in shrimps fed on the different diets. Similarly, for *Litopenaeus vannamei* shrimps fed for 60 days on diets, in which fish meal was replaced by increasing contents of rice protein concentrate [39], no significant difference in tail-muscle composition was found. For several shrimp species, when muscle tissues were investigated [40], the lipid content was lower than those obtained in the present work. Maliwat et al. [38] found lipid content between 0.28–0.76%.

The supposedly high cholesterol content in shrimps has contributed to this crustacean rejection. Consequently, it is important to quantify cholesterol. Figure 2 shows the results found for cholesterol content in whole body, evaluated along the 90-days experimental period.

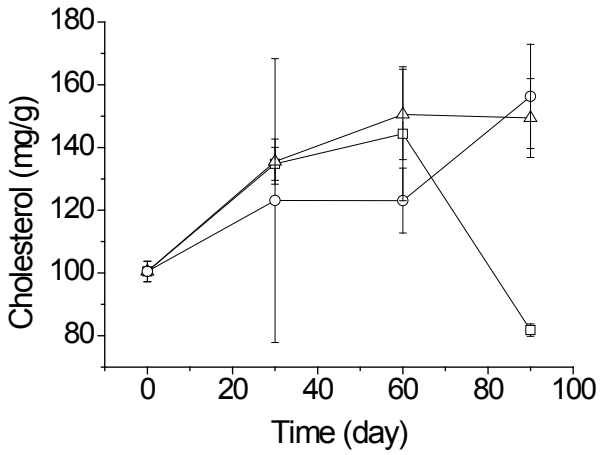

Figure 2: Cholesterol content for whole body of shrimps fed on the different diets along the 90-days experiments; Linseed diet (□), Whey proteins diet (○), Control diet (Δ).

Day	Protein (wt. %)	Lipid (wt. %)
0	15.20 ± 0.14	1.45 ± 0.05
Linseed diet		
30	14.39 ± 1.03	1.63 ± 0.18
60	11.54 ± 0.49	1.63 ± 0.31
90	14.75 ± 0.58	1.68 ± 0.03
Whey proteins diet		
30	15.45 ± 0.94	1.83 ± 0.06
60	16.37 ± 0.24*	2.21 ± 0.17*
90	14.90 ± 0.33	1.56 ± 0.01
Control diet		
30	14.16 ± 1.65	1.21 ± 0.11*
60	14.98 ± 0.29	1.45 ± 0.02
90	14.10 ± 0.42	1.06 ± 0.10

*Superscripts on the same column are significantly different. Significant level (P<0.05) (one-way analysis of variance (ANOVA) with posttest Tukey-Kramer Multiple Comparisons Test)

Table 2: Whole body composition in total protein and lipid from *Macrobrachium rosenbergii* prawns submitted to different treatments along the experimental period.

On the 30th day, a significant increase (P<0.05) in cholesterol content was observed for the shrimps fed on the three diets. Although lower values were determined for shrimps fed on the Whey proteins diet after 30 and 60 days, on the 90th day the highest cholesterol content (156.3 ± 16.6 mg/g) was determined for this group (P<0.05). Contrarily, for shrimps fed on the Linseed diet, after increasing until the 60th day, a significant decrease (P<0.05) in cholesterol content was detected on the 90th day (81.8 ± 1.9 mg/g). This result showed the role of linseed in reducing cholesterol in shrimp tissues. The decrease in cholesterol level found for the group fed on the Linseed diet, compared to the control group and the group fed on the Whey proteins diet may not be attributed to the presence of insoluble fiber in the diet; all the experimental diets had the same amount of fiber. Addition of insoluble fiber does not alter cholesterol level, partly because of hepatic cholesterol synthesis can compensate for poor absorption of cholesterol. Instead, the presence of soluble fibers in diets seems to be responsible for the decrease in cholesterol [41]. Linseed has 10, 22% soluble fiber and 30, 41% insoluble fiber [15]. Soluble fiber may have contributed to the reduction of cholesterol. Viscous fiber could increase intraluminal viscosity and decrease cholesterol content [41]. Also, the hypocholesterolemic effect may be related to the high content

of alpha-linolenic acid (ALA, 18:3n–3), a polyunsaturated fatty acid (PUFA), abundant in linseed [42].

ALA and linoleic acid (LNA, 18:2n-6) and their long-chain derivatives are important components of animal and cell membranes. ALA and LNA are essential polyunsaturated fatty acids (PUFA) that compete for the same enzymes (desaturatases and elongases) to produce their long-chain (20- to 22-carbon atoms) highly unsaturated fatty acids (HUFA) [43]. HUFAs serve as substrates to synthesize eicosanoids (ie, prostaglandins, leukotrienes, prostacyclins, thromboxanes, and lipoxins). The anti-inflammatory properties of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) from ALA are known to exert a protective action against atherosclerosis and to have other beneficial effects on human health, such as insulin resistance and blood pressure. On the other hand, a high intake of LNA was appointed to increase inflammatory eicosanoids, and possibly the risk of chronic diseases, and a value of 4.1 or less was recommended for the ratio between Σ n-6 and n-3 [44,45]. An appropriated balance of fatty acids in food was recommended by several authors. Also, because of the limited ability of crustaceans and fishes for de novo synthesis of PUFAs and HUFAs, they need to acquire these EFAs through their diet.

Table 3 summarizes fatty acids ratios determined for prawns at different stages of maturation.

Before the experimental period (on the denoted day 0), palmitic acid (16:0) was the most abundant in whole body of shrimps, followed by elaidic acid (18:1n-9*trans*), stearic acid (18:0), eicosapentaenoic acid (20:5n-3) and linoleic acid (18:2n-6). A small amount of docosahexaenoic acid (DHA, 22:6n-3*cis*) was also detected as FAME by gas chromatography. This result reflected the fatty acid composition of diets offered to early days prawns, conventionally based on fish meal and fish oil.

The content in fatty acids was also quantified periodically along the experimental period. A significant decrease (P<0.05) in the ratio of the sum of n-3 fatty acids (Σ n-3) to palmitic acid was observed for shrimps fed on the soybean oil-rich Control diet. However, a significant increase in the (Σ n-3) to palmitic acid content could be detected along the feeding trial on the Linseed diet. As expected, shrimps fed on the diets supplemented with soybean oil (Whey proteins and Control diets) presented a higher ratio between the sum of n-6 fatty acids (Σ n-6) and palmitic acid than those fed on the Linseed diet. It is worth noting that the amount of soybean oil added to the Whey proteins diet corresponded to about half the amount added to the Control diet. Particularly on the 60th day, the (Σ n-6)/palmitic acid ratio increased significantly (P<0.05) for the shrimps fed on the Control diet (1.3 ± 0.19) in comparison to the result found for the group fed on the Whey proteins diet (0.77 ± 0.05) and the Linseed diet (0.77 ± 0.12).

Days	Σ n-3/16:0	Σ n-6/16:0	Σ n-9/16:0	Σ n-6/Σ n-3	PUFA/SFA
0	1.73 ± 0.28	0.67 ± 0.04	1.67 ± 0.98	0.39 ± 0.09	1.29 ± 0.05
Linseed diet					
30	0.87 ± 0.37	0.64 ± 0.01	1.52 ± 0.07	0.73 ± 0.24	0.87 ± 0.20
60	1.43* ± 0.04	0.77 ± 0.12	1.61 ± 0.06	0.53 ± 0.06	1.23 ± 0.19
90	1.48^{x} ± 0.12	0.73 ± 0.03	2.02* ± 0.02	0.49^{x} ± 0.01	1.15 ± 0.04^{x}
Whey proteins diet					
30	0.42 ± 0.29	1.11 ± 0.38	1.17 ± 0.01	3.84 ± 3.55	0.94 ± 0.12
60	0.17 ± 0.02	0.77 ± 0.05	1,14 ± 0.03	4,76 ± 0,98	0.83 ± 0.39
90	[a]	–[a]	1.20 ± 0.02	–[a]	–[a]
Control diet					
30	0.48 ± 0.16	1.16 ± 0.36	1.18 ± 0.00	2.70 ± 1.66	1.10 ± 0.14
60	0.17 ± 0.12	1.30* ± 0.19	1.03 ± 0.13	9.95 ± 6.12	1.00 ± 0.14
90	0.15 ± 0.00	1.05^{x} ± 0.07	1.00 ± 0.00	7.02 ± 0.57	0.88 ± 0.07

*Indicates statistical differences among means on the same column. Significant level (P<0.05) (one-way analysis of variance (ANOVA) with post-test Tukey-Kramer Multiple Comparisons Test). ^{x}Indicates statistical differences among means. Significant level (P<0.05) (Unpaired t test). [a]Results were not expressed by chromatography. Σ n-3 corresponds to the sum of alpha-linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), eicosatrienoic acid (20:3n-3), docosapentatrienoic acid (22:5n-3) and docosahexaenoic acid (22:6n-3). Σ n-6 corresponds to the sum of linoleic acid (18:2n-6) and arachidonic acid (20:4n-6). Σ n-9 corresponds to oleic acid (18:1n-9)

Table 3: Ratio of Σ n-3, Σ n-6 and Σ n-9 to palmitic acid (16:0), Σ n-6/Σ n-3 and polyunsaurated fatty acids (PUFA) to saturated fatty acids (SFA) determined for the whole body of *Macrobrachium rosenbergii* prawns fed on the different diets.

For whole body of shrimps fed on the Control and the Whey protein diets, the ratio between the sums of n-9 fatty acids (Σ n-9) to palmitic acid was maintained constant along the experimental period. On the other hand, at the end of the trial, this ratio was significantly higher for the group fed on the Linseed diet. For this group, oleic acid (18:1n-9*cis*) was found to be the major fatty acid in whole body of shrimps. As for the ratio between the sum of n-6 fatty acids to the sum of n-3 fatty acids (Σ n-6/Σ n-3), a significantly lower value (P<0.05) was observed at the end of the experiment for the shrimps fed on the Linseed diet in relation to the Control group. On the 90th day, while a (Σ n-6/Σ n-3) of 0.49 ± 0.01 was determined for the group fed on the Linseed diet; this ratio was 7.02 ± 0.57 for the Control group. This result corroborates previous findings related to the nutritional value of linseed oil in reducing Σ n-6//Σ n-3 [42]. Moreover, the high content in linolenic acid present in linseed oil should have contributed to the lowest cholesterol content and the lowest Σ n-6/ n-3 found at the end of the experiment for whole body of shrimps fed on the Linseed diet. Although similar ratios between the contents in PUFAs and saturated fat acids (SFA) were found for the three groups of shrimps in the initial experimental period, this relation was significantly higher (P<0.05) at the end of the experiment for shrimps fed on the Linseed diet (1.15 ± 0.04) than for the group fed on the Control diet (0.88 ± 0.07). According to the British Department of Health [46], to be considered healthy, meat should have a PUFA/SFA value higher than 0.45. In this work, the PUFA/SFA ratios found for shrimps fed on the three diets were characterized as healthy. The PUFA/SFA ratios were higher than 0.45 in shrimp meat.

Despite differences in experimental conditions, commercial freshwater *Macrobrachium rosenbergii* fed on a diet based on hydrolyzed chicken feather and internal organs, fish flour, fish oil and wheat four, the major fatty acids, analyzed by similar methodology, were palmitic acid and eicosapentaenoic acid (EPA). This shrimp was also significantly higher in stearic and elaidic acids than other wild marine species, and presented a PUFA/SFA of 1.19 [40], similarly to the result found in the present work for shrimps fed on the Linseed diet for 90 days. In a 60-day experiment, the growth, survival and nutritional quality in relation to the increased amount of EPA and docosahexaenoic acid (DHA) of freshwater *Macrobrachium rosenbergii* were improved by feeding post larvae with *Moina micrura* enriched with emulsions containing sunflower oil, cod liver oil and commercially available MaxEPA capsules. A maximum EPA of 14.94 ± 0.17% and a maximum DHA of 7.63 ± 0.19% were found [47].

Fish meal and fish oil were successfully substituted by terrestrial proteins and lipids in isonitrogeneous and isocaloric extruded diets for freshwater *Macrobrachium rosenbergii* prawns. Egg albumin was used together with linseed, which was the source of lipids and fiber. A sample of whey proteins concentrate and soybean oil constituted

the base of the second diet. A Control diet was also prepared based on egg albumin and soybean oil. Juveniles fed on the different diets were compared as for weight gain and whole body composition. A high survival was observed, independently of the type of diet. Outdoors conditions, as well as water maintenance and the quality of ingredients could have contributed to this result. The results from this study provided information regarding the application of whey proteins as an alternative source of protein for shrimp diets. Overall, the results suggested that the experimental diets succeeded to provide shrimps with satisfactory growth and healthy nutritional properties.

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