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# Effect of Replacing Fishmeal with *Plukenetia volubilis* Cake on Growth, Digestive Enzymes, and Body Composition in Whiteleg Shrimp (*Litopenaeus vannamei*)

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**Abstract:** A feeding trial was carried out on a shrimp farm located in the Santa Rosa province of El Oro, Ecuador, with four isonitrogenous and isolipidic experimental diets, designed with increasing levels of substitution of fish meal by *P. voluvilis*, (D-0), 15% (D-15), 25% (D-25), and 50% (D-50). The obtained results indicated that the 50% replacement with *P. volubilis* in practical diets had no noticeable negative effects on the growth performance of *L. vanameii* juveniles. The total weight of shrimps fed with 25% and 50% diets (16.04 g and 16.72 g, respectively) and the abdomen weight (10.32 g) of shrimps fed with the D-50 diet were high, with significant differences regarding those fed with the D-0 diet. No adverse effects on muscle composition were found. Significant differences were observed only in groups D-25 (for chymotrypsin) and D-50 (chymotrypsin and alkaline protease). Trypsin and amylase activity was not affected by the inclusion of *P. volubilis*. The results of this experiment indicated that *P. volubilis* cake is a possible alternative to fish meal in shrimp feeding; however, it should be studied in more depth to establish the maximum replacement percentage and to identify the adequate treatments to eliminate antinutritional factors.

**Keywords:** *Plukenetia volubilis* cake; *Litopenaeus vannamei*; fish meal replacement; digestive enzymes



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## 1. Introduction

Crustacean culture represents 12% of the aquaculture production by species. The shrimp species is one of the most important aquaculture species, with an estimated production in 2016 of 5,180,563 tons, of which, 4,155,827 tons were *Litopenaeus vannamei* [1]. Whiteleg shrimps need a high percentage of protein in their diet. Dietary protein in shrimp feed ranges from 25% to 33% [2], of which, a minimum of 12% is fish meal [3]. Fish meal is an essential ingredient in fish/crustaceans feed, but is an unsustainable source due to overfishing and the increased demand for livestock feed [4]. Hence, a decrease in the fish meal included in shrimp feed and an increase in the employed alternative protein are foreseen [5]. In fact, aquaculture depends increasingly less on fishmeal. The FIFO (fish in–fish out) lowered from 0.63 in 2000 to 0.22 in 2015, with 0.91 for crustaceans in 2000 and 0.46 in 2015 [6].

The alternative proteins used in aquafeeds are mainly derived from plants. Vegetable sources have some advantages over animal-based sources, such as lower prices, more availability, and improved composition [7,8]. Of all plant sources, soy is the most widely used because of its excellent nutritional quality, high digestibility, high protein quality and quantity, and the best amino acid profile of available vegetable protein sources. Despite its good nutritional value, the inclusion percentages do not exceed 40% [9] due to antinutritional factors that reduce feed utilization, absorption, and feed conversion ratio, as these sometimes affect the growth of shrimps. The bioprocessing of soy could eliminate these antinutritional factors. On the other hand, soy meal has a strong environmental impact,

such as its contribution to deforestation, the use of water, and the utilization of GMOs and pesticides [10]. Thus, the search for other sustainable sources that allow higher replacement percentages and diversify aquafeed ingredients remains as an important animal nutrition research goal.

*Plukenetia volubilis* (sacha inchi, SI) is a partly woody and perennial plant belongs to the Euphorbiaceous family, which is native to Peruvian jungles. The flour from the seeds contains approximately 48% oil and 27% proteins, which are rich in cysteine, tyrosine, threonine, and tryptophan [11]. The fatty acids of sacha inchi are mostly unsaturated, with about 85% of polyunsaturation, and with an n6/n3 ratio, reported by Hamaker et al. [12], of approximately 0.81, comprising approximately 33–34% linoleic acid and 50–51% linolenic acid [12,13]. These fatty acids are important for health and nutrition, and for the prevention of cardiovascular disease, rheumatoid arthritis, and cancer [13,14]. These unsaturated fatty acids are also important for the development of aquatic organisms, and are the main source of high amounts of unsaturated fatty acids for humans. Other bioactive compounds have also been observed in SI oil, such as carotenes [15], polyphenolic compounds [15,16], tocopherols [15,17,18], and phytosterols [18]. For all these reasons, SI seeds should be considered as an important dietary source of health-promoting phytochemicals [15]. Currently, SI oil is commercialized for medicinal applications for skin care and as a nutritional supplement for humans. SI oil is obtained by pressing nuts, separating oil, and obtaining a rich protein paste by-product which can be used in animal feed. The post-oil extraction residue from sacha inchi presents a high content of proteins (59%) [19]. This cake represents 62–70% of seed and contains 12–17% of oil [20]. SI cake has been examined as a fish meal substitute for several fish species, such as red tilapia (*Tilapia* spp.) [21], *Colossoma macropomum*, *Brycon amazonicus* [22], and rainbow trout (*Oncorhynchus mykiss*) [23], all with promising results. In rainbow trout, SI digestibility is better than other vegetable sources [23], and, depending on the animal species, SI can be included in the diet at 10–30% without affecting growth rates [21,22].

Given the above considerations, the aim of this work was to study the effect of the replacement of fish meal by *Plukenetia volubilis* on whiteleg shrimp growth, nutrition indices, digestive enzymes, and final body composition.

## 2. Materials and Methods

### 2.1. Sacha Inchi Cake

The sacha inchi (*P. volubilis*) seeds were provided by the agricultural cooperative, "Aso-Inchi Cantón Las Lajas", in the province of El Oro (Ecuador). To obtain sacha inchi cake, seeds were extruded at a low temperature (below 45 °C) to extract the sacha inchi oil. The amino acid profile of the sacha inchi cake was obtained through the Protein Chemistry Service of the Biological Research Centre (CSIC, Madrid, Spain), following the basic principle of operation developed by Spackman et al. [24], by continuous flow chromatography (Biochrom 30 series). Tryptophan was not detected, and asparagine and glutamine were deaminated and detected as aspartic acid and glutamic acid, respectively (Table 1, Figure S1).

**Table 1.** Amino acid composition (g % 100 g dry matter) of sacha inchi cake compared to fish meal as reference.

| | Sacha Inchi Cake | Fish Meal |
|---|---|---|
| *Non-essential amino acids* | | |
| Asp | 3.62 | 3.81 |
| Tyr | 1.34 | 1.33 |
| Ser | 1.98 | 1.69 |
| Glu | 4.34 | 5.19 |
| Pro | 1.29 | 1.79 |
| Gly | 3.01 | 2.74 |
| Ala | 1.19 | 2.98 |
| Cys | 0.66 | 0 |

**Table 1.** *Cont.*

| | Sacha Inchi Cake | Fish Meal |
|---|---|---|
| *Essential amino acids* | | |
| Thr | 1.23 | 1.57 |
| Val | 1.18 | 1.71 |
| Met | 0.33 | 1.39 |
| Ile | 0.91 | 1.38 |
| Leu | 1.97 | 2.94 |
| Phe | 0.73 | 0.72 |
| His | 0.75 | 1.39 |
| Lys | 1.48 | 3.12 |
| Arg | 2.86 | 2.3 |
| ***Total*** | 28.85 | 37.15 |

### 2.2. Experimental Diets

Four isonitrogenous (350 g crude protein $kg^{-1}$) diets were prepared in the Department of Aquaculture at the Technical University of Machala, Ecuador. Experimental diets were formulated, including sacha inchi cake as a partial substitute for fish meal. In order to formulate isonitrogenous diets, the quantity of soy meal and squid meal varied among the diets. A sacha-inchi-free diet was used as the control (CT), and three experimental diets were designed with increasing levels of fish meal replacement by sacha inchi cake: 15% (D-15), 25% (D-25), and 50% (D-50) regarding the fish meal. Briefly, feed ingredients, including vitamins and minerals, were finely ground, weighed, and mixed in a vertical helix ribbon mixer (Bathammex 178716, 10-L capacity, Bathammex, Mexico DF, Mexico). Then fish oil was added to the latter ingredients to be mixed together. Water was slowly added until the diets began to clump and form a homogeneous dough. The dough was then extruded by a mill machine (Torrey M-22R1, Torrey, Mexico DF, Mexico) to obtain pellets that were 4 mm in diameter. Finally, the pellets were dried at room temperature for 24 h. The pellets were broken and sieved with sieves of different mesh sizes, and kept in sealed plastic bags at −20 °C until their use. The formulation and chemical composition of the experimental diets are shown in Tables 2 and 3, respectively.

**Table 2.** Ingredient composition and proximate composition (g $kg^{-1}$ on dry matter basis) of the experimental diets.

| | CT | D-15 | D-25 | D-50 |
|---|---|---|---|---|
| *Ingredients (g kg dry matter$^{-1}$)* | | | | |
| Fish meal | 186.9 | 158.9 | 140.2 | 93.5 |
| Sacha inchi | 0.00 | 28.0 | 46.7 | 93.5 |
| Soy meal | 313.1 | 313.1 | 303.7 | 294.4 |
| Wheat meal | 414.6 | 414.6 | 414.6 | 414.6 |
| Squid meal | 18.7 | 18.7 | 28.0 | 37.4 |
| Vitamin | 10.0 | 10.0 | 10.0 | 10.0 |
| Mineral | 10.0 | 10.0 | 10.0 | 10.0 |
| Fish oil | 46.7 | 46.7 | 46.7 | 46.7 |
| *Proximate composition (g kg dry matter$^{-1}$)* | | | | |
| Crude protein | 359.6 | 359.7 | 356.6 | 356.5 |
| Total lipid | 87.8 | 87.9 | 84.7 | 83.8 |
| NDF | 189.3 | 128.1 | 189.7 | 195.8 |
| Ash | 86.7 | 86.7 | 82.1 | 83.6 |
| MELN | 465.9 | 465.7 | 476.6 | 477.1 |

CT: control diet; D-15: 15% sacha inchi cake in respect to fish meal, D-25: 25% sacha inchi cake in respect to fish meal, D-50: 50% sacha inchi cake in respect to fish meal. Fish meal, GALDECUN S.A., Jaramijó, Ecuador. CP, 65.0; EE, 10.8; FB, 4.0; ash, 19.0. Sacha inchi cake, Aso-inchi Cooperativa, Las Lajas-El Oro, Ecuador. CP, 59; EE, 7.8; FB, 12.24; ash, 7.88. Soy meal, Camari (Fondo Ecuatoriano Populorum Progressio), Quevedo-Los Ríos, Ecuador (PB, 48.9; EE, 2.2; FB, 3.79; ash, 7.88. Wheat meal, Camari (Fondo Ecuatoriano Populorum Progressio), Quevedo-Los Ríos, Ecuador (PB, 10; EE, 2.5; FB, 3.25; ash, 1.54. Squid meal, PCO, Chacras-El Oro, Ecuador (PB, 50.6; EE, 6.8; FB, 2.7; ash, 8.54). Vitamins and minerals, Adilisa, Guayaquil, Ecuador. Vitamins (kg): A, 8500 UI; $D_3$, 3000 UI; E, 30,250 mg; $K_3$, 9000; $B_{11}$, 25 mg; $B_2$, 12.$B_6$, 24.5 mg; $B_{12}$, 16.5 mg; niacin, 38.65 mg; calcium pantothenate, 25. Mg; folic acid, 4250 mg; biotin, 350 mg; inositol, 55 mg; C (35%), 75 mg; antioxidant, 500 mg. Minerals: Co, 400 mg; Se, 400 mg; Cu, 10,000 mg; Fe, 120,000 mg; Mg, 85,000 mg; I, 500 mg; Zn, 45,000 mg; Antioxidants, 9047 mg.

**Table 3.** Fatty acid composition (% fatty acids) of sacha inchi cake and the experimental diets used in the feeding trial.

| | Sacha Inchi Cake | CT | D-15 | D-25 | D-50 |
|---|---|---|---|---|---|
| 14:0 | | 4.20 ± 0.03 | 4.46 ± 0.08 | 3.91 ± 0.23 | 4.31 ± 0.02 |
| 16:0 | 3.79 ± 0.22 | 23.21 ± 0.15 | 24.32 ± 0.49 | 23.08 ± 0.07 | 23.17 ± 0.20 |
| 16:1n7 | | 4.71 ± 0.01 | 4.94 ± 0.01 | 4.52 ± 0.18 | 4.80 ± 0.04 |
| 18:0 | 2.42 ± 0.08 | 5.07 ± 0.04 | 5.26 ± 0.30 | 5.33 ± 0.41 | 5.10 ± 0.09 |
| 18:1n9 | 9.28 ± 0.19 | 14.29 ± 0.22 | 14.57 ± 0.04 | 15.00 ± 0.40 | 14.52 ± 0.14 |
| 18:1n7 | 0.33 ± 0.04 | 2.54 ± 0.04 | 2.66 ± 0.03 | 2.63 ± 0.05 | 2.63 ± 0.01 |
| 18:2n6 | 38.83 ± 0.16 | 13.21 ± 0.01 | 14.15 ± 0.08 | 15.18 ± 0.43 | 13.58 ± 0.13 |
| 18:3n3 | 44.42 ± 0.15 | 3.11 ± 0.01 | 1.98 ± 0.05 | 5.04 ± 0.39 | 3.63 ± 0.00 |
| 20:4n6 | | 1.70 ± 0.03 | 1.95 ± 0.01 | 1.79 ± 0.01 | 1.87 ± 0.02 |
| 20:5n3 | | 6.91 ± 0.12 | 8.01 ± 0.40 | 6.81 ± 0.22 | 7.73 ± 0.10 |
| 22:5n3 | | 1.23 ± 0.01 | 1.49 ± 0.03 | 1.30 ± 0.05 | 1.42 ± 0.02 |
| 22:6n3 | | 14.48 ± 0.52 | 16.56 ± 0.86 | 15.36 ± 0.45 | 16.75 ± 0.02 |

CT: control diet; D-15: 15% sacha inchi cake in respect to fish meal; D-25: 25% sacha inchi cake in respect to fish meal; D-50: 50% sacha inchi cake in respect to fish meal. Values are presented as mean ± SD of triplicate determination ($n$ = 3).

### *2.3. Feeding Trial and Sampling*

The feeding trial was carried out in the shrimp farm, "Noblecilla Salas", located in Santa Rosa, El Oro Province, Ecuador (3°23′45″ S; 79°57′21″ W), using 1 $m^3$ cages placed in a 1-hectare pond. Prior to the experiment, the shrimps were acclimatized to the culture environment for 3 weeks and fed a commercial diet (Nicovita Shrimp feed). Once acclimatized, 120 *L. vannamei* juveniles were used, with an average initial weight of 3.41 ± 0.31 g. The shrimps were randomly distributed and stocked in eight cubical 1 $m^3$ cages at the bottom of pond at a density of 15 individuals per cage. Each experimental feed was tested in duplicate (4 diets × 2 cages each) for 10 weeks. All the shrimp groups were fed with the experimental diets 3 times daily (7% of their body weight) at 08:00 h, 12:00 h, and 16:00 h. Every week, the total weight of the shrimps in each cage was registered to adjust the daily amount of feed. The water quality parameters measured during the experimental trial were optimal for the growth and survival of white shrimp and averaged: temperature (26.6 °C), salinity (24.5‰), pH (7.19), and $O_2$ (4–5 mg/mL). A natural light–dark cycle was used during the trial.

Weekly, the shrimps in each cage were individually weighed, and the standard length was measured to determine the growth and the feed utilization parameters. At the end of the feeding trial, the crude protein, total lipid content, and fatty acid profile of the abdomen samples were determined by standard methods, as described in Section 2.6 Finally, for the enzymatic activity analysis, the hepatopancreas from 10 shrimps per treatment were pooled to obtain enzymatic extracts. Briefly, hepatopancreas were manually homogenized in distilled water at 4 °C to a final concentration of 0.5 g $mL^{-1}$, and supernatants were obtained after centrifugation (12,000 rpm, 12 min, 4 °C). The total soluble protein in the enzyme extracts was determined according to Bradford [25], using bovine serum albumin as the standard.

### *2.4. Animals Ethics*

Crustaceans are not regulated by European animal welfare legislation [26]. However, the animals were kept and slaughtered under production conditions; they were immersed in ice and then frozen. During the experimental period, they were not subjected to any procedure, and all analyses were carried out post-mortem.

### *2.5. Growth Performance and Nutrient Utilization*

Final weight and feed intake were used to calculate the different parameters: specific growth rate (SGR, %) = (Ln (Wf) − Ln (Wi)/days) × 100, where Wf and Wi were the final

and the initial weight, respectively, (g); daily gain (DG, g $day^{-1}$) = (Wf − Wi)/days; feed conversion ratio (FCR) = total feed intake as a dry basis (g)/weight gain (g).

Cephalotorax, abdomen, abdomen + exoskeleton, and hepatopancreas were weighted, and the total length of the cephalothorax and abdomen were measured.

### *2.6. Proximate Composition and Fatty Acids Analysis*

Chemical analysis of the sacha inchi diets and the edible part of the abdomen (without the exoskeleton) samples were conducted by AOAC [27] procedures. Dry matter and ash were determined gravimetrically after drying at 105 ± 0.5 °C and after combustion at 500 °C in a muffle furnace, respectively, until a constant weight. Crude protein content was determined by the Kjeldahl method (Nx.6.25). Total lipids were determined by ethyl ether extraction (Soxhlet technique). The fatty acid rofile of the sacha inchi cake, feeds, and abdomen without the exoskeleton were determined by gas chromatography using a modification of the direct transesterification method described by Lepage and Roy [28] that requires no prior separation of the lipid fraction [29].

All the analyses were performed in triplicate.

### *2.7. Digestive Enzymes Activity*

The total alkaline protease activity in the hepatopancreas extracts was determined according to Alarcon et al. [30], using 5 g $L^{-1}$ of casein in 50 mM of Tris HCl (pH 9.0) as the substrate. One unit of total protease activity was defined as the amount of enzyme that released 1 µg of tyrosine per min in the reaction mixture by considering an extinction coefficient for tyrosine of 0.008 $\mu g^{-1}$ $mL^{-1}$ $cm^{-1}$, measured at 280 nm. Trypsin and chymotrypsin activities were measured using 0.5 mM BAPNA (N-a-benzoyl-DL-arginine-4-nitroanilide) as the substrate according to Erlanger et al. [31], and 0.2 mM SAPNA (N-succinyl-(Ala)2-Pro-Phe-P-nitroanilide) according to Del Mar et al. [32], respectively, in 50 mM Tris-HCl buffer, pH 8.5, containing 10 mM $CaCl_2$. For trypsin and chymotrypsin, one unit of enzyme activity (U) was defined as the amount of enzyme that released 1 µmol of p-nitroanilide per min, using 8800 M $cm^{-1}$ as the extinction coefficient, measured at 405 nm. The amylase activity was determined according to the Somogyi–Nelson method using soluble starch (2% *w*/*v*) as the substrate, as described by Robyt and Whelan [33]. One unit of activity (U) was defined as the amount of enzyme capable of producing 1 mg of maltose per minute.

All the assays were performed in triplicate, and the specific enzymatic activity was expressed as U g $tissue^{-1}$.

### *2.8. Statistical Analysis*

All the statistical analyses were performed with the Stagraphics Plus 4.0 (Rockville, MD, USA) software. The results were expressed as the mean ± standard deviation of at least three determinations. The data with parametric distribution were analyzed by a one-way analysis of variance (ANOVA), and the significant differences between treatments ($p < 0.05$) were determined by Tukey's multiple comparison test. The data with nonparametric distribution were analyzed by the Kruskal–Wallis test, and significant differences were determined using box and whisker plot graphs.

## 3. Results

### *3.1. Nutrition and Growth Indices*

Table 4 displays the nutrition and growth indices. Statistical differences in the final weight were found among treatments; the final weight trended to increase with the level of sacha inchi replacement in respect to the fish meal, due to the increased weight of the cephalothorax and abdomen (with or without the exoskeleton). No differences in the total cephalothorax or abdomen length were observed among treatments.

The hepatopancreas weight was similar among treatments; statistical differences only appeared between D-25 and D-15 groups. Regarding the SGR, D-50 was the treatment with

a higher SGR, and was statistically different to CT and D-25. No statistical differences in FCR were observed among the different experimental diets, except between the control and D-50.

**Table 4.** Growth performance and nutrient utilization of *L. vannamei* fed the experimental diets for 70 days.

| | CT | D-15 | D-25 | D-50 | *p* Value |
|---|---|---|---|---|---|
| **Final body weight** | 14.25 ± 1.02 $^{a}$ | 14.57 ± 1.34 $^{ab}$ | 16.04 ± 0.25 $^{ab}$ | 16.72 ± 0.67 $^{b}$ | 0.031 |
| **Cephalothorax weight** | 3.99 ± 0.59 $^{ab}$ | 3.65 ± 0.36 $^{a}$ | 4.48 ± 0.41 $^{b}$ | 4.40 ± 0.82 $^{b}$ | 0.009 |
| **Abdomen weight** | 8.74 ± 1.17 $^{ab}$ | 8.86 ± 1.04 $^{ab}$ | 9.75 ± 0.99 $^{ab}$ | 10.32 ± 1.71 $^{b}$ | 0.024 |
| **Abdomen + exoskeleton** | 10.20 ± 1.77 $^{ab}$ | 10.24 ± 0.95 $^{a}$ | 11.34 ± 1.07 $^{ab}$ | 12.21 ± 1.98 $^{b}$ | 0.013 |
| **Hepatopancreas weight** | 0.42 ± 0.13 $^{ab}$ | 0.45 ± 0.11 $^{a}$ | 0.60 ± 0.09 $^{b}$ | 0.48 ± 0.15 $^{ab}$ | 0.011 |
| **Total length** | 12.76 ± 1.40 | 13.10 ± 0.39 | 13.30 ± 0.59 | 13.65 ± 0.53 | 0.127 |
| **Cephalothorax length** | 5.65 ± 0.30 | 5.65 ± 0.30 | 5.49 ± 0.31 | 5.30 ± 0.42 | 0.079 |
| **Abdomen length** | 8.96 ± 0.61 | 8.96 ± 0.61 | 8.95 ± 0.28 | 9.13 ± 0.52 | 0.843 |
| **DG** | 0.15 ± 0.01 $^{a}$ | 0.16 ± 0.02 $^{ab}$ | 0.17 ± 0.01 $^{ab}$ | 0.19 ± 0.01 $^{b}$ | 0.028 |
| **SGR** | 1.95 ± 0.10 $^{a}$ | 2.13 ± 0.19 $^{ab}$ | 2.03 ± 0.09 $^{a}$ | 2.36 ± 0.06 $^{b}$ | 0.015 |
| **FCR** | 4.72 ± 0.46 $^{b}$ | 4.45 ± 0.54 $^{ab}$ | 4.09 ± 0.14 $^{ab}$ | 3.69 ± 0.15 $^{a}$ | 0.042 |

CT: control diet; D-15: 15% sacha inchi cake; D-25: 25% sacha inchi cake; D-50: 50% sacha inchi cake. DG: daily gain; SGR: specific growth rate; FCR: feed conversion ratio. Values are presented as mean ± SD. Values in the same row with different lowercase letters indicate significant differences among treatments ($p < 0.05$).

### 3.2. Proximal Composition and Fatty Acid Profile

The protein content of the abdomen was higher in the shrimps fed the control diet than for any other treatment (Table 5). Conversely, no differences in crude fat content were found among treatments.

**Table 5.** Body chemical composition (g $kg^{-1}$ dry weight) of *L. vannamei* fed the experimental diets for 70 days.

| Dietary Treatment | Crude Protein | Total Lipids |
|---|---|---|
| CT | 86.14 ± 5.40 $^{b}$ | 3.86 ± 0.55 |
| D-15 | 73.98 ± 2.61 $^{a}$ | 3.65 ± 0.40 |
| D-25 | 74.11 ± 1.29 $^{a}$ | 3.86 ± 0.50 |
| D-50 | 75.05 ± 1.88 $^{a}$ | 3.37 ± 0.28 |
| *p* value | < 0.001 | 0.055 |

CT: control diet; D-15: 15% sacha inchi cake; D-25: 25% sacha inchi cake; D-50: 50% sacha inchi cake. Values are presented as mean ± SD of triplicate determination ($n = 3$). Values in the same column with different lowercase letters indicate significant differences among treatments ($p < 0.05$).

No differences in the fatty acid profile of muscle were found, except for 18:0, which lowered when the sacha inchi cake was included, regardless of the inclusion percentage (Table 6). Nevertheless, SFA, MUFA, and PUFA decreased with sacha inclusion, being statistically different between the control and CT and D-50 for SFA and MUF, and between CT and the other three experimental diets for PUFA. No differences for n-3, n-6, or n-3/n-6 were observed.

**Table 6.** Fatty acid composition (% fatty acids) of *L. vannamei* fed with experimental diets for 70 days.

| | CT | D-15 | D-25 | D-50 | *p* Value |
|---|---|---|---|---|---|
| 14:0 | 0.72 ± 0.06 | 0.86 ± 0.08 | 0.78 ± 0.20 | 0.94 ± 0.10 | 0.430 |
| 15:0 | 0.66 ± 0.06 | 0.76 ± 0.01 | 0.75 ± 0.14 | 0.83 ± 0.16 | 0.536 |
| 16:0 | 18.04 ± 0.39 | 16.77 ± 0.84 | 16.98 ± 1.14 | 16.65 ± 0.42 | 0.376 |
| 16:1n7 | 1.98 ± 0.36 | 2.12 ± 0.09 | 1.99 ± 0.34 | 2.22 ± 0.67 | 0.939 |
| 17:0 | 2.05 ± 0.19 | 2.17 ± 0.16 | 2.04 ± 0.22 | 1.50 ± 0.48 | 0.252 |
| 18:0 | 10.70 ± 0.05 $^{c}$ | 10.11 ± 0.02 $^{b}$ | 9.96 ± 0.10 $^{b}$ | 9.23 ± 0.20 $^{a}$ | 0.001 |
| 18:1n9 | 11.23 ± 0.64 | 9.50 ± 0.07 | 9.96 ± 1.31 | 8.81 ± 0.22 | 0.114 |
| 18:1n7 | 2.69 ± 0.20 | 2.75 ± 0.12 | 2.66 ± 0.21 | 2.56 ± 0.26 | 0.826 |
| 18:2n6 | 8.11 ± 0.17 | 7.63 ± 0.15 | 7.87 ± 0.27 | 8.14 ± 0.16 | 0.158 |

**Table 6.** *Cont.*

| | CT | D-15 | D-25 | D-50 | *p* Value |
|---|---|---|---|---|---|
| 18:3n6 | 0.57 ± 0.05 | 0.55 ± 0.02 | 0.53 ± 0.07 | 0.56 ± 0.02 | 0.799 |
| 18:3n3 | 1.12 ± 0.21 | 1.31 ± 0.13 | 1.25 ± 0.29 | 1.74 ± 0.35 | 0.238 |
| 20:1n9 | 0.63 ± 0.20 | 0.44 ± 0.03 | 0.40 ± 0.00 | 0.37 ± 0.05 | 0.159 |
| 20:4n6 | 4.53 ± 0.51 | 4.20 ± 0.12 | 4.01 ± 0.17 | 4.05 ± 0.01 | 0.344 |
| 20:5n3 | 12.06 ± 0.34 | 12.22 ± 1.38 | 11.35 ± 0.80 | 11.00 ± 0.36 | 0.489 |
| 22:5n3 | 0.68 ± 0.01 | 0.73 ± 0.07 | 0.73 ± 0.01 | 0.71 ± 0.06 | 0.847 |
| 22:6n3 | 14.84 ± 1.41 | 12.97 ± 0.69 | 13.37 ± 0.96 | 12.31 ± 0.09 | 0.175 |
| SFA | 32.17 ± 0.74 $^{b}$ | 30.67 ± 0.62 $^{ab}$ | 30.51 ± 0.47 $^{ab}$ | 29.15 ± 0.39 $^{a}$ | 0.027 |
| MUFA | 16.53 ± 0.12 $^{b}$ | 14.81 ± 0.13 $^{ab}$ | 15.01 ± 0.76 $^{ab}$ | 13.96 ± 1.09 $^{a}$ | 0.044 |
| PUFA | 41.57 ± 0.41 $^{b}$ | 39.60 ± 1.61 $^{a}$ | 39.12 ± 1.50 $^{a}$ | 38.52 ± 0.73 $^{a}$ | 0.019 |
| n-3 | 28.36 ± 0.80 | 27.22 ± 1.86 | 26.70 ± 1.47 | 25.76 ± 0.86 | 0.380 |
| n-6 | 13.21 ± 0.39 | 12.39 ± 0.25 | 12.41 ± 0.03 | 12.75 ± 0.13 | 0.071 |
| n-6/n-3 | 0.47 ± 0.03 | 0.46 ± 0.04 | 0.47 ± 0.02 | 0.50 ± 0.02 | 0.617 |

CT: control diet; D-15: 15% sacha inchi cake; D-25: 25% sacha inchi cake; D-50: 50% sacha inchi cake. Values are presented as mean ± SD. Values in the same row with different lowercase letters indicate significant differences among treatments ($p < 0.05$).

### 3.3. Digestive Enzymes

Enzyme activities measured in the hepatopancreas extracts of *L. vannamei* fed with the experimental diets are shown in Figure 1. Significantly lower ($p < 0.05$) total alkaline protease activities were found in shrimps fed with the highest percentage of replacement by sacha inchi cake diets (D-25 and D-50), whereas chymotrypsin only showed significant differences between the control diet and D-25. The total protease activity decreased at higher percentages of substitution, D- 25, and D-50. Trypsin and amylase activities did not differ among the experimental groups.

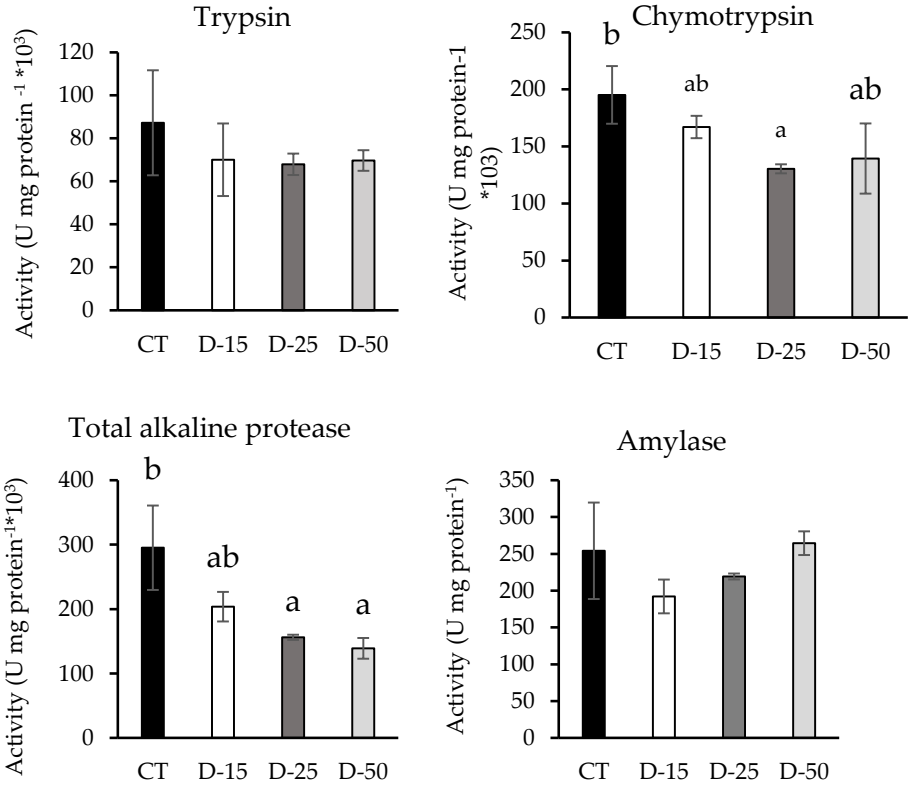


**Figure 1.** Enzyme activities measured in hepatopancreas extracts of *L. vannamei* fed with experimental diets for 70 days. Values are presented as mean ± SD ($n = 6$). Values with different lowercase letters indicate significant differences among treatments ($p < 0.05$).

## 4. Discussion

### *4.1. Growth Performance*

In this experiment, the effect of inclusion was studied at three levels of fish meal replacement by sacha inchi cake (15%, 25%, and 50%) in the diet for *Litopenaeus vannamei*. The results showed that replacing fish meal by sacha inchi cake tended to increase the final weight by increasing the cephalothorax and abdomen weight (Table 5); shrimps fed with D-50 showed higher values of growth indices and better FCR. In an experiment carried out by Miranda-Gelvez et al. [21], tilapia was fed with a diet of 10% fish meal replacement level by sacha inchi; no differences in weight gain, SGR, PER, or hepatosomatic indices were found compared to the control group. Nevertheless, in tilapia, the nutrition and growth rates worsened with a 25% of sacha inchi replacement level. Similar results were found for blackfin pacu, *Colossoma macropomum*, whereas a higher final weight was found in amazon brycon, *Brycon amazonicus*, fed with a diet that included 30% *P. volubilis* [22].

The lowest SGR was found in the shrimps fed with the D-25 diet. This could be related to the heavier hepatopancreas weight observed in this group, and could be provoked by hepatotoxicity, leading to lower enzyme secretion and lower digestive efficiency, as described in poultry fed with sacha inchi cake [34,35]. However, in shrimps fed with 50% fish meal replacement level (D-50), the SGR was higher than that observed for the CT and D-25 groups. As such, it is plausible to question if sacha inchi was responsible for the observed lower SGR and/or the heavier hepatopancreas weight obtained in the D-25 group. A decrease in chymotrypsin activity was observed in the D-25 group, which could be related with the lower SGR observed in this group.

### *4.2. Proximal Composition and Fatty Acid Profile*

Publications on this subject are currently scarce, so no studies about the effect of sacha inchi on body composition or muscle quality in shrimps were found. The present results showed a lower protein content in the edible part of the abdomen of the shrimps fed with the experimental diets at all the fish meal replacement levels, with no changes observed in crude fat (Table 5). In production terms, the reduction of protein could be balanced with the higher final weight obtained in shrimps fed with experimental diets. On the other hand, sacha inchi seeds have been considered a new lipid source for humans for their high n-3 polyunsaturated fatty acid (PUFAs n-3) content. Lipid extraction waste is a cake rich in protein (about 59% CP) [19], and has an adequate amino acid profile that can be used in animal feeding. The proximal composition of sacha inchi cake showed 51.12% $\pm$ 1.08 of crude protein and 10.51% $\pm$ 0.06 of crude fat, which are lower protein values than those described for fish meal. A whole meal made with a fatty fish, such as herring, might contain about 71% protein, 9% fat, 8% water, and 12% minerals, whereas a meal made mainly with white fish and white fish offal, and dried to the same extent, might contain about 66% protein, 5% fat, 8% water, and 21% minerals [36].

The fatty acid profile of the abdomen showed no differences among treatments, except for 18:0, which was higher for the control shrimps (Table 6). Nevertheless, this difference could not be related to sacha inchi because the higher percentage of fatty acids of sacha inchi cake corresponded to 18:2n6 and 18:3n3, with only 2.4% corresponding to 18:0. Furthermore, the fatty acid profile of the abdomen from the shrimps fed various lipid sources generally resembles the lipids from the feed [37]. As such, by taking into account the low fat level in sacha inchi cake (10.5% approx.) and the sacha inchi inclusion in diets, it is foreseeable that it will have barely any effect on diet (Table 3) and, therefore, no effect on muscle composition (Table 6).

The ability to synthesize PUFAs n-3 in marine shrimps appears to be limited [38], and eicosapentaenoic acid (20:5n3, EPA) and docosahexaenoic acid (22:6n3, DHA) are essential for these animals, including *L. vannamei* [39]. Many plant-based feed ingredients display a high n-6/n-3 ratio [40], but this did not apply to sacha inchi cake. This means it is possible to maintain a low n-6/n-3 ratio in the shrimps fed with sacha inchi. However, the n-6/n-3 ratio increased proportionally as the inclusion of sacha inchi increased (Table 6).

Nevertheless, this did not seem to be connected to the sacha inchi itself, but to some other ingredients, since adrenic acid (22:4n6) increased in the experimental diets, and sacha inchi does not contain this fatty acid.

### *4.3. Digestive Enzymes*

Córdova-Murueta and García-Carreño [41] pointed out *P. vannamei* is able to modulate enzymatic secretions according to the type of protein eaten. As described in previous research, chymotrypsin displays greater activity than trypsin when specific synthetic substrates are used [42,43]. Nevertheless, the inclusion of *P. volubilis* affected digestive enzyme activities as herein determined, except for trypsin and amylase (Figure 1). In sacha inchi seeds, diverse antinutritional factors have been detected, including trypsin inhibitors [44]. Lázaro-Aguilar [45] observed greater activity for trypsin inhibitors in cake (46.19 UTI $mg^{-1}$ urinary trypsin inhibitor) when compared to seeds (31.9 UTI $mg^{-1}$). However, the present results showed only a slight decrease in trypsin activity when sacha inchi cake was included, with no statistical differences when comparing to the control diet (Figure 1). Conversely, a reduction in chymotrypsin and alkaline protease activities was found when the inclusion of sacha inchi increased in the experimental diets. Bueno-Borges et al. [44] reported a reduction in the trypsin inhibitor according to the roasting temperature. Ortiz-Chura et al. [23] described good digestibility for sacha inchi in rainbow trout compared to other studied vegetable sources, which the authors associated with the fact that it had been pre-processed (extrusion and extraction of oil). Probably, the increase in temperature during the lipid extraction process of sacha inchi seeds brought about some loss in the effect of the trypsin inhibitor, as well as other antinutritional factors.

In relation to other antinutritional components, Ruiz et al. [46] discovered that *P. volubilis* cake led to a tannin concentration of 6.35 mg 100 $g^{-1}$, a much lower value than that reported in soybeans (34.9 mg 100 $g^{-1}$).

Despite this reduction in the chymotrypsin activity of alkaline protease, growth was not stunted, and the shrimps fed with sacha inchi presented similar growth rates to the control diet. Rojo-Arreola et al. [47] described a phenotypic plasticity of digestive peptidases to dietary inhibitors in *L. vannamei*, which allowed digestive unbalance to be overcome. This has been described in Crustacea [48] and for other Arthropod taxa, including Chelicerata [49] and Insecta [50].

On the other hand, Le Moullac et al. [51] found that the protein source was important for carbohydrate digestibility, and that it could affect amylase expression. Nevertheless, in the present study, the inclusion of sacha inchi did not affect amylase activity.

## 5. Conclusions

Previous research showed the inclusion of plant source proteins in the diet of *L. vannamei* must not exceed 40% [9]; however, in the present study, 50% of the fish meal was replaced by sacha inchi cake without compromising the nutritional and growth indices of the shrimps. Protein decrease in the abdomen was observed for those groups fed with sacha cake; nevertheless, a higher final weight regarding control was also observed. In conclusion, the results of this study showed that sacha inchi cake can be a promising alternative protein source to fish meal for feeding *L. vannamei*, although more studies are needed in order to establish the maximum replacement percentages, as well as the adequate treatments to eliminate antinutritional factors.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes7050244/s1, Figure S1: g, amino acids/100 g, DM.

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**Institutional Review Board Statement:** Crustaceans are not regulated by European animal welfare legislation (EU Directive 2010/63). However, the animals were kept and slaughtered under production conditions; they were immersed in ice and then frozen. During the experimental period, they were not subjected to any procedure, and all analyses were carried out post-mortem.

**Data Availability Statement:** http://hdl.handle.net/10835/13974.

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