Use of Enzyme Mixtures in Diets Based on Animal and Plant Ingredients for *Litopenaeus vannamei*: Effect on Digestibility, Growth, and Enzyme Activity

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How to Cite


Abstract

The objective of this research was to determine the effect of adding a mixture of different exogenous enzymes at 0.005% (0.0025% for each enzyme; protease+xylanase [P/X], protease+cellulase [P/C], and xylanase+cellulase [X/C]) to diets, based on fishmeal (FM) or soybean paste (SP), on shrimp’s growth performance, survival, feed conversion, digestibility, and digestive capacity. Eight isoprotilic experimental diets (FM, FM+P/X, FM+P/C, FM+X/C, SP, SP+P/X, SP+P/C, and SP+X/C) were evaluated with three replicates. Shrimps (1 ± 0.2 g) were maintained in a recirculating system at 12 organisms/tank for 90 days. Significant differences (p < 0.05) were found in all the productive parameters of *L. vannamei*. Compared with the control diets (FM and SP), shrimps fed with FM+P/C showed the highest final body weight (7.83 ± 0.34 g) and proteolytic activity (178.81 ± 24.49 U/mg protein), as well as the lowest feed conversion ratio (1.71 ± 0.01). In contrast, the SP+X/C group presented the highest lipolytic activity (0.0235 ± 0.0028 U/mg) and apparent digestibility (94.12 ± 0.09%). In accord with its omnivorous trophic habits, enzyme mixtures included in animal and plant based-diets improved different productive variables of *L. vannamei*, representing a nutritional alternative to optimize feed use in shrimp cultivation.

Introduction

Aquaculture has been the fastest-growing primary activity in the last decade, contributing significantly to future food security on the planet (León-Balderrama et al., 2020). The white legs shrimp *Litopenaeus vannamei* is one of the most important aquatic species due to its high yields and attractive prices in the international market (Cobo Abrantes & Pérez Jar, 2018). However, shrimp farming faces crucial challenges in its consolidation process as an economic and environmentally-sustainable activity. One of the main problems is the increasing demand for a dietary protein source and its high cost, this situation has led food producers to seek alternatives that can contribute to reducing feed costs to make more efficient the use of balanced feed nutrients (García-Ulloa et al., 2017). It is well-accepted that more than 50% of the operating costs of a shrimp farm are related to feeding costs (Núñez-Torres, 2017); therefore, shrimp farmers are continuously seeking cheaper, more digestible, and easily assimilated ingredients (Shahzad et al., 2021).
New feeding techniques are essential for the development of more sustainable aquaculture (Moreno Garrido, 2022), in which the dietary inclusion of enzymes represents an option to increase the bioaccessibility and bioavailability of nutrients (Dalsgaard et al., 2016; Cabrera, 2018); however, these compounds must remain stable and active during digestion so that the food molecules be absorbed effectively (Carrillo & Vega-Villasante, 2000). The use of diverse enzymes becomes important since fishmeal demand is a substantial challenge for shrimp production (Xie et al., 2016).

Investigations related to aquaculture nutrition indicate that the replacement of fishmeal by vegetable protein has been achieved up to 30% without affecting the productive variables (Yun et al., 2017); however with the addition of enzymes in the feed, the replacement can be total. One of the most paramount aspects of evaluating the effect of enzyme mixtures in diets is the relationship between the type of dietary inputs and the feeding habit of the target species (Piñeros-Roldan et al., 2014). In this way, xylanases, carbohydrases, amylases, and cellulases are included in the diets of herbivorous species to fragment the fiber and carbohydrates when plant ingredients are included (Martínez-Aispuro et al., 2016; Juárez-Morales et al., 2020). Incorporating proteases in the diet of organisms that consume proteins of animal origin helps break them up and assimilate them (Ordoñez Rumiche, 2018). It has been reported that the mixture of both groups of enzymes, preferably in diets for omnivorous species such as crustaceans, increases energy and amino acids availability (Rachmawati & Samidjan, 2016).

The addition of proteases and carbohydrases in diets for Litopenaeus vannamei juveniles improves their growth, survival, feed conversion, and enzymatic activity (Song et al., 2017; Yao et al., 2019); consequently, the assimilation of nutrients also increases. However, information on the dietary inclusion of enzyme mixtures to break down animal and plant source ingredients to cultivate white shrimp is scarce. This work aimed to evaluate the effect of enzyme mixtures using protease, cellulase, and xylanase-incorporated in animal and plant-based diets on juvenile shrimp’s productive and physiological parameters.

### Material and Methods

#### Digestive Enzymes

The enzymes (protease, $P = 2,080$ U/g; cellulase, $C = 116,277$ CMCase/g; and xylanase $X = 90,000-110,000$ XU/g) used in this study were provided by *Enzimas Mexicanas* (EnMEx®, S.A. de C.V., Tlalnepantla, Edo de México, Mexico).

#### Ingredients and Experimental Diets

Two groups of diets -based on fishmeal (FM) and soybean paste (SP)- were elaborated as main sources of animal and vegetable protein, respectively (García-Ulloa et al., 2017). Except for control diets (FM and SP), the enzyme mixtures (protease+xylanase [P/X], protease+cellulase [P/C], and xylanase+cellulase [X/C]) were included in equal parts at 0.0025% each (0.05 g of enzyme mixture/kg, Wong et al., 1996; Tacon, 1997).

Eight diets were elaborated (FM, FM+P/X, FM+P/C, FM+X/C, SP, SP+P/X, SP+P/C, and SP+X/C) and evaluated with three replicates each. The ingredients (Table 1) were ground (Grindmaster, Model 505, Louisville, KY, USA) and sieved to <250 µm (No. 60, FIIC, S.A. de C.V., México, Mexico); subsequently, they were mixed in a blender for 30 min (KitchenAid, Model 600, Benson Harbor, MI, USA). The enzymes were added as the last ingredient. Pellets were obtained with a meat mill (TorRey®, M-22R) and dried for 12 h at room temperature to avoid the inactivation of the enzymes (Bhunia et al., 2013; López-Flores et al., 2016). Finally, the diets were stored in plastic bags at 5 °C until use.

#### Proximal Composition of the Diets

The proximal composition of the diets (g/kg, dry weight, Table 2) was determined according to standard methods (AOAC, 2016).

#### Experimental Design

The bioassay was carried out in a recirculation system consisting of a 350-L water container with a submersible pump (Mody Sump Pump, SR, HP 1.3,
Bakersfield, CA, USA) that supplied water to 24 plastic tanks (Length 50 × Base 30 × Height 28 cm) filled at 42-L capacity. Each tank was provided with a diffuser stone connected to a blower (5 HP) to keep the dissolved oxygen concentration above 6 mg/L. Water temperature (25 ± 2 °C) and salinity (35 ± 1 psu) were measured daily; the photoperiod was adjusted to 14:10 h (light: dark). The initial density of _L. vannamei_ was 12 shrimps/tank (80 org/m², 1.0 ± 0.2 g initial weight) obtained from a commercial farm, "Los Ahumada", located in Guasave, Sinaloa. Shrimps were acclimated during one week to the experimental system conditions with a commercial diet before being fed the experimental diets. Shrimp body weight was obtained weekly to adjust the daily food ration (SENASICA, 2003), which was dosed thrice daily (10:00, 13:00, and 17:00 h). Besides, shrimp body weight (g), the total biomass (g), survival (%), absolute growth rate (AGR; g) = initial weight – final weight/time in days (Escobar Gil, 2017), specific growth rate SGR (%) = [(log final body weight - log initial body weight)/time in days] × 100, and feed conversion factor (FCA, g) = food supplied/weight gained (FAO, 1989) were evaluated weekly. The duration of the bioassay was 90 days.

### Enzymatic Activity of the Hepatopancreas

At the end of the bioassay, the organisms were euthanized by thermal shock; the hepatopancreas of each shrimp was extracted and frozen at -70°C until use. Enzyme extracts were prepared as follows: a cold buffer (50 mM Tris-HCl, pH 8.0) was added to the hepatopancreas (<4°C) at a 1:5 ratio (weight/volume). After that, the hepatopancreas was rapidly homogenized at low speed with a Yeto® tissue homogenizer. The homogenized material was placed in Eppendorf tubes and centrifuged at 10,000 g at 4°C for 30 min. The supernatant (crude enzymatic extract) was collected in aliquots and stored at -80°C until used. The enzyme extract's dilution level for quantifying the enzyme activity was determined. The enzyme was gained (FAO, 1989) were evaluated weekly. The conversion factor (FCA, g) = food supplied/weight

### In vivo Protein Digestibility

After the experiment, protein digestibility was determined by adding 1% chromium oxide (CrO₃) to the experimental diets as inert marker for one week. Shrimp feces were collected daily by siphoning 1 hour after feeding (10:00, 13:00, and 17:00 h), placed in Falcon® tubes, and frozen at -70°C until obtaining enough feces to determine protein digestibility. Approximately, 25-30 mL samples were lyophilized and stored in the refrigerator until use (Furukawa & Tsukahara, 1966); the apparent digestibility coefficient (ADC) was obtained with the formula described by Maynard & Loosli (1969).

\[
x = \frac{[(Y - 0.0032)(0.2089)]}{\text{% chromium oxide} \times X/A}
\]

\[
\text{ADC} = 100 \times \left( \frac{\text{% in food}}{\text{% in feces}} \right) \left( \frac{\text{% protein in feces}}{\text{% protein in food}} \right) (100),
\]

where X = weight of chromium oxide, Y = absorbance, A = weight of sample, ADC = apparent digestibility coefficient.

### Determination of the N-NH₃ Concentration in Water

After the digestibility bioassay, six shrimps of each treatment were placed in fiberglass tanks supplied with

### Table 2. Proximal composition (g/kg⁻¹) of the diets used in the experiment.

<table>
<thead>
<tr>
<th>Origin (O)</th>
<th>Enzymes mixture (EM)</th>
<th>Moisture</th>
<th>Crude protein</th>
<th>Lipids</th>
<th>Crude fiber</th>
<th>Ash</th>
<th>NFE*</th>
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</thead>
<tbody>
<tr>
<td>FM</td>
<td>-</td>
<td>3.86</td>
<td>36.27</td>
<td>11.46</td>
<td>4.16</td>
<td>9.96</td>
<td>30.52</td>
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<tr>
<td></td>
<td>P/C</td>
<td>3.84</td>
<td>36.77</td>
<td>15.13</td>
<td>3.56</td>
<td>13.05</td>
<td>19.63</td>
</tr>
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<td></td>
<td>P/X</td>
<td>3.96</td>
<td>36.09</td>
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<td>3.36</td>
<td>10.28</td>
<td>30.72</td>
</tr>
<tr>
<td></td>
<td>X/C</td>
<td>3.46</td>
<td>36.04</td>
<td>14.22</td>
<td>3.94</td>
<td>10.80</td>
<td>24.94</td>
</tr>
<tr>
<td>SP</td>
<td>-</td>
<td>3.9</td>
<td>34.15</td>
<td>6.45</td>
<td>3.50</td>
<td>4.46</td>
<td>48.3</td>
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<tr>
<td></td>
<td>P/C</td>
<td>2.29</td>
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<td>2.69</td>
<td>5.03</td>
<td>42.24</td>
</tr>
<tr>
<td></td>
<td>P/X</td>
<td>3.63</td>
<td>33.64</td>
<td>8.22</td>
<td>4.47</td>
<td>4.43</td>
<td>45.03</td>
</tr>
<tr>
<td></td>
<td>X/C</td>
<td>4.75</td>
<td>35</td>
<td>10.08</td>
<td>4.93</td>
<td>5.07</td>
<td>39.96</td>
</tr>
</tbody>
</table>

*NFE = Nitrogen free extract, Fish meal diets (FM), soybean paste diets (SP). The enzyme mixtures were: Protease/Xylanase (P/X), Protease/Cellulase (P/C), and Xylanase/Cellulase (X/C).*
clear water and constant aeration to determine the effect of the experimental diets on the N-NH₄ water concentration (Strickland and Parsons, 1972). Shrimps were fed with the experimental diets (same last ration and dosage) for three days without water exchange. N-NH₄ was measured at the beginning and end of the bioassay and calculated by the difference in the ammonia concentration.

Statistics

The results are shown as mean ± standard deviation. Data were tested for normality and homoscedasticity using the Kolmogorov-Smirnov and Bartlett tests, respectively. A simple analysis of variance (ANOVA) was used to determine the significant differences among the mean weights between treatments, and a two-way ANOVA and Tukey test were applied to detect the differences among means. Correlations were also performed for productive and metabolic parameters. Significance was established at $P < 0.05$ (Software Statistica 7.0, Statsoft, Tulsa, Oklahoma).

Results

Growth Performance and Feed Efficiency

The interaction of control diets + enzyme mixtures affected the total biomass and FCR ($P < 0.05$). The highest biomass (83.57 ± 4.52 g) and the lowest FCR (1.71 ± 0.01 g) were obtained in shrimp fed FM+P/C. Final weight (7.16 ± 0.63 g), weight gained (6.20 ± 0.63 g), AGR (0.10 ± 0.01 g), and SGR (12.50 ± 1.07 %/d) were higher for the FM diet ($P < 0.05$; Table 3).

Physiological Variables

The mixture of enzymes was associated with the differences in the activity of the enzymes in the hepatopancreas, digestibility, and N-NH₄ in the water ($P < 0.05$). Proteolytic activity in the hepatopancreas was higher with the FM diet supplemented with the three enzyme mixtures (FM+P/C = 178.81 ± 24.49 U/mg; FM+P/X = 149.83 ± 24.05 U/mg; FM+X/C = 220.28 ± 60.03 U/mg). However, the activity of lipases and amylases did not show a similar trend (Table 4). The highest activity of amylases in the hepatopancreas (3.47 ± 0.19 U/mg) was obtained for FM+X/C treatment, whereas for lipases (0.0235 ± 0.0028 U/mg) and digestibility (94.12 ± 0.09%), the highest values were recorded for the SP+X/C diet ($P < 0.05$; Table 4). The lowest concentration of N-NH₄ in the water was recorded in the P/C mixture of both diets (FM+P/C = 4.26 ± 2.68 mg/L and SP+P/C = 4.60 ± 1.93 mg/L) ($P < 0.05$; Table 4).

Correlations

Moderate correlations ($P < 0.05$) were obtained between the productive and metabolic parameters. The highest correlation was found between lipase activity and digestibility ($r = 0.75$), whereas the lowest ($r = 0.50$) was obtained for protease activity with final weight and between lipase activity and N-NH₄ in the water ($P<0.05$; Table 5).

Discussion

Due to the high cost of balanced feeds and the growing lack of essential inputs for their formulation and preparation, aquaculture nutritionists continue to search and test different sources of protein, both of animal and vegetable origin, generating different results (Coelho-Emerecianco & Massamitu-Furuya, 2006; Valdez-González et al., 2018; Wang et al., 2020). In this study, the shrimp fed with an FM diet supplemented with the different enzyme mixtures recorded the highest values in most of the productive and metabolic parameters of L. vannamei. We assumed that it was

<table>
<thead>
<tr>
<th>Origin (O)</th>
<th>Enzyme (EM)</th>
<th>Final weight (g)</th>
<th>Weight gain (g)</th>
<th>Biomass Total (g)</th>
<th>Survival (%)</th>
<th>AGR (g)</th>
<th>FCR (%)</th>
<th>SGR (%/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM</td>
<td>-</td>
<td>6.91±0.09</td>
<td>5.95±1.00</td>
<td>69.06±0.00ab</td>
<td>80.56±12.73</td>
<td>0.100±0.016</td>
<td>1.87±0.04ab</td>
<td>12.11±1.8</td>
</tr>
<tr>
<td></td>
<td>P/C</td>
<td>7.83±0.34</td>
<td>6.87±0.33</td>
<td>83.57±4.52a</td>
<td>97.22±4.81</td>
<td>0.116±0.005</td>
<td>1.71±0.01b</td>
<td>13.59±0.4</td>
</tr>
<tr>
<td></td>
<td>P/X</td>
<td>7.01±0.41</td>
<td>6.05±0.41</td>
<td>72.44±4.05ab</td>
<td>94.44±4.81</td>
<td>0.102±0.006</td>
<td>1.84±0.01ab</td>
<td>12.20±0.6</td>
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<tr>
<td></td>
<td>X/C</td>
<td>6.91±0.21</td>
<td>5.96±0.20</td>
<td>64.45±15.9b</td>
<td>94.44±4.81</td>
<td>0.100±0.003</td>
<td>1.72±0.03ab</td>
<td>12.12±0.2</td>
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<tr>
<td>SP</td>
<td>-</td>
<td>5.97±0.51</td>
<td>5.02±0.52</td>
<td>63.73±3.4b</td>
<td>97.22±4.81</td>
<td>0.085±0.008</td>
<td>2.26±0.03a</td>
<td>10.41±1.0</td>
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<td></td>
<td>P/C</td>
<td>6.15±1.01</td>
<td>5.19±1.01</td>
<td>59.44±19.7b</td>
<td>97.22±4.81</td>
<td>0.088±0.017</td>
<td>2.29±0.02a</td>
<td>10.73±1.7</td>
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<tr>
<td></td>
<td>P/X</td>
<td>5.80±0.11</td>
<td>4.85±0.11</td>
<td>67.62±3.3ab</td>
<td>100±0.00</td>
<td>0.082±0.002</td>
<td>2.23±0.02a</td>
<td>10.18±0.2</td>
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<td></td>
<td>X/C</td>
<td>6.45±0.13</td>
<td>5.50±0.13</td>
<td>75.24±3.7ab</td>
<td>88.89±19.25</td>
<td>0.093±0.002</td>
<td>1.98±0.01ab</td>
<td>11.34±0.2</td>
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2-way ANOVA

<table>
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<tr>
<th>O*EM</th>
<th>P &lt; 0.05</th>
<th>P &lt; 0.05</th>
<th>N.S.</th>
<th>N.S.</th>
<th>P &lt; 0.05</th>
<th>N.S.</th>
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<td>O</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>P &lt; 0.05</td>
<td>N.S.</td>
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<tr>
<td>EM</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>P &lt; 0.05</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Absolute growth rate (AGR), food conversion ratio (FCR), specific growth rate (SGR), fish meal diets (FM), soybean paste diets (SP). The enzyme mixtures were: Protease/Xylanase (P/X), Protease/Cellulase (P/C), and Xylanase/Cellulase (X/C). Data expressed as mean ± standard deviation. Means with different letters indicate significant differences ($P < 0.05$) among diets. N.S. = There is no significant difference ($P > 0.05$).
related to two aspects: the enhanced proteolytic and amylolytic activity in the hepatopancreas of shrimp treated with FM diets and the essential amino acid composition and palatability of fishmeal (Davis et al., 2004; Tidwell et al., 2005; Faillace et al., 2016; Hamidoghli et al., 2020).

By themselves, the mixtures of the different enzymes studied did not cause differences in the productive parameters, which could be explained by the amount of crude protein in the diets -regardless of its source of origin- being sufficient to meet the nutritional requirements of the organisms (Faillace, 2016). Ashraf et al. (2018) and Mendoza et al. (2001) found a positive effect on shrimp development due to the addition of enzymes when the protein content in the food is increased. Therefore, to determine in shrimp the beneficial effect of the dietary inclusion of enzymes on their productive parameters, the crude protein content in the feed should be at least optimal. Siccardi et al. (2006) pointed out that the percentage of protein in shrimp feed can vary from 15 to 40%; meanwhile Ponce-Palafoux et al. (2017) recommended an optimal protein content of 30 to 35%, which is similar to that adjusted in the present study.

The interaction between the enzyme mixtures and dietary proteins from different origins affected the physiological parameters of the white shrimp. Consistent with our results, Hamidoghli et al. (2020) documented that the inclusion of enzymes in the diet of L. vannamei increased the proteolytic and amylolytic enzymatic activities, which would stimulate the secretion of digestive enzymes and the appetite of crustaceans. Stefanello et al. (2016) mention that adding xylanase in shrimp plant-based diets improves digestibility. The aforementioned can be explained because xylanase helps in the digestion of xylan, producing xylooligosaccharides, which act as prebiotics that increase the production of fatty acids, delay gastric emptying, and increase the time in the transit of the digestive bolus, allowing better digestion of proteins (Rabello et al., 2021). These findings relate to those found in the present study, in which the organisms fed the FM+X/C and SP+X/C diets presented better digestibility and a higher activity of digestive enzymes. The lowest enzymatic activity was shown in the two control diets. Valdivia et al. (2019) pointed out that shrimp fed with diets without enzymes depicted a

**Table 4.** Enzyme activity (U/mg protein) in hepatopancreas, digestibility (in vivo), and nitrogen compounds released in white shrimp (L. vannamei) fed with enzyme mixtures for 90 days.

<table>
<thead>
<tr>
<th>Origin (O)</th>
<th>Enzyme mixture (EM)</th>
<th>Proteolytic activity (U/mg protein)</th>
<th>Lipolytic activity (U/mg protein)</th>
<th>Amylolytic activity (U/mg protein)</th>
<th>Digestibility (%)</th>
<th>N-NH₄ (mg/L)</th>
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<tbody>
<tr>
<td>FM</td>
<td>-</td>
<td>102.32±38.4</td>
<td>0.0063±0.0006</td>
<td>1.47±0.07b</td>
<td>87.41±0.08e</td>
<td>5.04±2.6</td>
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<tr>
<td></td>
<td>P/C</td>
<td>178.81±24.4</td>
<td>0.0147±0.0006</td>
<td>1.61±0.27b</td>
<td>94.26±0.07b</td>
<td>4.26±2.6</td>
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<tr>
<td></td>
<td>P/X</td>
<td>149.83±24.0</td>
<td>0.0074±0.0012</td>
<td>1.46±0.05b</td>
<td>82.69±0.09e</td>
<td>5.88±1.2</td>
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<tr>
<td></td>
<td>X/C</td>
<td>220.28±60.0</td>
<td>0.0052±0.0011</td>
<td>3.47±0.19a</td>
<td>76.96±0.09g</td>
<td>9.17±0.2</td>
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<tr>
<td>SP</td>
<td>-</td>
<td>47.98±20.4</td>
<td>0.0048±0.0006</td>
<td>1.50±0.06b</td>
<td>90.23±0.09c</td>
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<td>P/C</td>
<td>120.10±23.6</td>
<td>0.0177±0.0006</td>
<td>1.55±0.02b</td>
<td>89.83±0.03c</td>
<td>4.60±1.9</td>
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<td>P/X</td>
<td>127.13±36.9</td>
<td>0.0075±0.0004</td>
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<td>78.68±0.06f</td>
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<td>X/C</td>
<td>173.39±23.5</td>
<td>0.0235±0.0028</td>
<td>1.66±0.03a</td>
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2-way ANOVA

<table>
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<tr>
<th>O</th>
<th>N.S.</th>
<th>P &lt; 0.05</th>
<th>P &lt; 0.05</th>
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<td>O*EB</td>
<td>N.S.</td>
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</tbody>
</table>

Fish meal diets (FM), soybean paste diets (SP). The enzyme mixtures were: Protease/Xylanase (P/X), Protease/Cellulase (P/C), and Xylanase/Cellulase (X/C). Data expressed as mean ± standard deviation. Means with different letters indicate significant differences (P < 0.05) among diets. N.S. = There is no significant difference (P > 0.05).

**Table 5.** Correlation of productive and metabolic parameters of L. vannamei fed with diets supplemented with a mixture of Protease/Xylanase (P/X), Protease/Cellulase (P/C), and Xylanase/Cellulase (X/C) enzymes. Regression equations that relate physiological response (enzymatic activity (U/mg protein) in the hepatopancreas, digestibility in vivo (%), nitrogenous compounds (mg/L), and productive parameters.

<table>
<thead>
<tr>
<th>Y</th>
<th>X</th>
<th>Regression equation</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROT</td>
<td>PF</td>
<td>y = -98.69+35.92x</td>
<td>0.50</td>
<td>0.0138</td>
</tr>
<tr>
<td>PROT</td>
<td>AMI</td>
<td>y = 59.83+42.48x</td>
<td>0.62</td>
<td>0.0013</td>
</tr>
<tr>
<td>AMI</td>
<td>DG</td>
<td>y = 8.57-0.08x</td>
<td>0.56</td>
<td>0.0041</td>
</tr>
<tr>
<td>LIP</td>
<td>DG</td>
<td>y = -0.056+0.0008x</td>
<td>0.75</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LIP</td>
<td>N-NH₄</td>
<td>y = 0.0040+0.004x</td>
<td>0.50</td>
<td>0.0131</td>
</tr>
<tr>
<td>DG</td>
<td>N-NH₄</td>
<td>y = 75.71+3.94x</td>
<td>0.52</td>
<td>0.0099</td>
</tr>
</tbody>
</table>

Values of enzymatic activity of Proteases U/mg protein (PROT), values of enzymatic activity of amyloses U/mg protein (AMI), values of enzymatic activity of lipases U/mg protein (LIP), values of percentage of digestibility (DG), nitrogenous compound values mg/L (N-NH₄), final weight values in grams (PF).
limited digestibility of proteins, revealing that shrimp feed supplemented with enzymes improves the zootechnical parameters of shrimp. Costalvo Muñoz (2021) stated that enzymes stimulate and increase the speed of biological reactions achieving greater availability and digestion of nutrients. The effect of the interaction between diets with different sources of origin and the enzyme mixture is similar to that reported by Song et al. (2017), Cabrera (2018), and Yao et al. (2019), who obtained an increase in biomass and a reduction in FCA by including enzyme mixtures (protease-complex and carboxylase) in the diet of white shrimp. It is accepted that proteases break peptide bonds, achieving a smaller size and free amino acids, while carboxylases degrade cellulose, which allows the release of energy and absorption of nutrients (Martínez Córdova et al., 2022; Morales-Campos et al., 2019), which could partially explained the results.

Boyd (2017) highlighted that water quality plays an essential role in sustainable production systems. The addition of enzymes in the feed aims to improve the productive parameters and contribute to reducing the organic matter, which is discharged into the environment. In this study, the P/C enzyme mixture significantly reduced the ammonium levels of the water. Patil (2014) emphasized that both the use of enzymes and the increase in the digestibility of the nutrients in the diet could reduce the nitrogen released into the environment. The afore stated would confirm the correlation found between digestibility and enzymatic activity with the ammonium concentration in the water, derived from the addition of the enzyme mixture to the diet of L. vannamei juveniles.

Conclusion

The addition of enzymes to the experimental diets optimized growth, weight gain, survival, digestibility, the enzymatic activity of the hepatopancreas in the white shrimp, and N-NH₄ level in the culture water. It was found that, specifically, FM+P/C increased L. vannamei growth and decreased its FCA; however, the SP+X/C diet promoted, in turn, digestibility and amylolytic and lipid activity in the hepatopancreas. Therefore, enzyme mixtures are presented as critical nutritional tools to optimize the use of dietary nutrients in white shrimp.

Ethical Statement

The experiment has been conducted in an ethical and responsible. The animals used not mistreated or misused. was used for academic purposes only.

Funding Information

Funding was provided by Instituto Politécnico Nacional (Secretaria de Investigación y Posgrado IPN; Grant: SIP-IPN No. 20201950; 20210281; 20220645).

Author Contribution

The responsibilities of the authors were as follows: M.S.: Writing, original draft, experiment, M.G.: Writing, revision and editing, E.M.: Methodology, Validation, C.C.: Methodology, Validation, P.A.: Methodology, Validation. H.R.: Writing Review & Edition, critical review of the article. All authors read and approved the final version manuscript.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Acknowledgements

M.C. S.-A. is a recipient of a doctoral fellowship from CONACyT. H. R.-G., C. C.-M., and M. G.-U. thanks to the Commission of Operation and Promotion of Academic Activities (Apoyos económicos de la Comisión de Operación y Fomento de Actividades Académicas - COFAA), Researcher Development Grants (Estímulos al Desempeño de los Investigadores -ED), and the Institutional Scholarship for Researcher Development (Beca de Estímulos Institucional de Formación de Investigadores -BEIFI) provided by the IPN; the National Researcher System (SNI-CONACyT).

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