Turk. J. Fish.& Aquat. Sci. 23(10), TRJFAS21999
https://doi.org/10.4194/TRJFAS21999

RESEARCH PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

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

Mary Cruz Sánchez-Alcade[1], Manuel García-Ulloa[1], Emmanuel Martínez Montaño[2,3], Claudia Castro-Martínez[1], Píndaro Álvarez-Ruíz[1], Hervey Rodríguez González[1*]

[1]Instituto Politécnico Nacional, Centro Interdisciplinario de Investigaciones para el Desarrollo Regional Unidad Sinaloa, Guasave, Sinaloa, 81100, Mexico.
[2]Maestría en Ciencias Aplicadas, Unidad Académica de Ingeniería en Biotecnología, Universidad Politécnica de Sinaloa (UPSIN), Carretera Municipal Libre Mazatlán Higueras Km 3, Mazatlán, Sinaloa, 82199, Mexico.
[3]Investigadores por México CONACyT, Consejo Nacional de Ciencia y Tecnología, Mexico City, Mexico.

How to Cite

Sánchez-Alcade, M.C., García-Ulloa, M., Montaño, E.M., Castro-Martínez, C., Álvarez-Ruíz, P., Rodríguez González, H.(2023). Use of Enzyme Mixtures in Diets Based on Animal and Plant Ingredients for *Litopenaeus vannamei*: Effect on Digestibility, Growth, and Enzyme Activity. *Turkish Journal of Fisheries and Aquatic Sciences*, *23(10)*, *TRJFAS21999.* https://doi.org/10.4194/TRJFAS21999

Article History
Received 02 June 2022
Accepted 07 June 2023
First Online 23 June 2023

Corresponding Author
Tel.: +525552098800
E-mail: hrodriguezg@ipn.mx

Keywords
Digestive capacity
Exogenous enzymes
Fishmeal
Growth
Soybean paste.

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 isoproteic 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 still 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 enzymes 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., 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,

Table 1. Ingredients of experimental diets (g).

Ingredients	Control diets		Diets + enzymes	
	FM	SP	FM	SP
Fish meal	500	0	500	0
SP Soybean paste	0	650	0	650
Wheat flour	397	227	396.9	226.9
Grenetin	40	40	40	40
Enzymes mixture*	-----	------	0.05	0.05
Fish oil**	30	40	30	40
Soy lecithin	30	40	30	40
Vitamin Premix**	1	1	1	1
Mineral Premix**	2	2	2	2

Two groups of diets based on fish meal (FM) and soybean paste (SP) were developed. FM and SP = control diets. The enzyme mixtures were: Protease/Xylanase (P/X), Protease/Cellulase (P/C), and Xylanase/Cellulase (X/C) added in equal parts at 0.0025% each one (0.05 g of enzyme mix per kg of feed). **Fish oil, Vitamin Premix and Mineral Premix were provided by the company VIMIFOS SA DE CV. (Cd Obregon, Sonora, Mexico).

Bakersfield, CA, USA) that supplied water to 24 plastic tanks (Length 50 × Base 30 × Heigth 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 ºC) 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/m^2, 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 weighed in an Eppendorf tube at a ratio of 1:5 (weight/volume) and mixed with a buffer solution (50 mM Tris-HCl, pH 8.0), to, finally, be stored at -80°C until use.

The concentration of the soluble protein was determined following the methodology of Bradford (1976) using bovine serum albumin (BSA) as the protein concentration standard (20–2000 µg mL^{-1}). The enzymatic activity of proteases, α-amylases, and lipases was obtained according to standard methods (Walter, 1984; Gjellesvik *et al*., 1992; Vega-Villasante *et al*., 1993; Navarrete del Toro *et al*., 2011), using 1% casein with 0.05 M Tris-HCl buffer (pH 8, 25°C) as the substrate for the determination of proteases, and 1% potato starch with 50 mM sodium acetate buffer ($C_2H_3NaO_2$) as the substrate for amylase determination. For lipase determination, 4-nitrophenyl octanoate (4-NPO) was dissolved in 0.5 M Tris-HCl, pH 7.4, at 25°C plus 6 mM sodium taurocholate and 0.1 M NaCl, as substrate (Arriaga-Hernandez et al., 2021).

In vivo Protein Digestibility

After the experiment, protein digestibility was determined by adding 1% chromium oxide (Cr_2O_3) 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- to 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).

$$x = [(Y - O.OO32)(0.2089)]$$
$$\%\ chromium\ oxide = X/A$$

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

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

Determination of the N-NH_4 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.

Origin (O)	Enzymes mixture (EM)	Moisture	Crude protein	Lipids	Crude fiber	Ash	NFE*
FM	-	3.86	36.27	11.46	4.16	9.96	30.52
	P/C	3.84	36.77	15.13	3.56	13.05	19.63
	P/X	3.96	36.09	11.30	3.36	10.28	30.72
	X/C	3.46	36.04	14.22	3.94	10.80	24.94
SP	-	3.9	34.15	6.45	3.50	4.46	48.3
	P/C	2.29	33.76	9.48	2.69	5.03	42.24
	P/X	3.63	33.64	8.22	4.47	4.43	45.03
	X/C	4.75	35	10.08	4.93	5.07	39.96

*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-NH_4 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-NH_4 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-NH_4 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-NH_4 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-NH_4 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-Emerenciano & 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 3. Productive parameters of white shrimp (*L. vannamei*) fed for 90 days with different enzyme mixtures.

Origin (O)	Enzyme Mixture(EM)	Final weight (g)	Weight gain (g)	Biomass Total (g)	Survival (%)	AGR (g)	FCR (g)	SGR (%)
FM	-	6.91±0.99	5.95±1.00	69.06±0.00ab	80.56±12.73	0.100±0.016	1.87±0.04ab	12.11±1.8
	P/C	7.83±0.34	6.87±0.33	83.57±4.52a	97.22±4.81	0.116±0.005	1.71±0.01b	13.59±0.4
	P/X	7.01±0.41	6.05±0.41	72.44±4.05ab	94.44±4.81	0.102±0.006	1.84±0.01ab	12.20±0.6
	X/C	6.91±0.21	5.96±0.20	64.45±15.9b	94.44±4.81	0.100±0.003	1.72±0.03ab	12.12±0.2
SP	-	5.97±0.51	5.02±0.52	63.73±3.4b	97.22±4.81	0.085±0.008	2.26±0.03a	10.41±1.0
	P/C	6.15±1.01	5.19±1.01	59.44±19.7b	97.22±4.81	0.088±0.017	2.29±0.02a	10.73±1.7
	P/X	5.80±0.11	4.85±0.11	67.62±3.3ab	100±0.00	0.082±0.002	2.23±0.02a	10.18±0.2
	X/C	6.45±0.13	5.50±0.13	75.24±3.7ab	88.89±19.25	0.093±0.002	1.98±0.01ab	11.34±0.2
2-way ANOVA								
O		$P < 0.05$	$P < 0.05$	N.S.	N.S.	$P < 0.05$	N.S.	$P < 0.05$
EM		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
O*EM		N.S.	N.S.	$P < 0.05$	N.S.	N.S.	$P < 0.05$	N.S.

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 decreased. 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-Palafox *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 different enzyme mixtures for 90 days.

Origin (O)	Enzyme mixture (EM)	Proteolytic activity (U/mg protein)	Lipolytic activity (U/mg protein)	Amylolytic activity (U/mg protein)	Digestibility (%)	N-NH_4 (mg/L)
FM	-	102.32±38.4	$0.0063±0.0006_c$	1.47±0.07b	87.41±0.08e	6.44±5.2
	P/C	178.81±24.4	$0.0147±0.0006_b$	1.61±0.27b	92.46±0.07b	4.26±2.6
	P/X	149.83±24.0	$0.0074±0.0012_c$	1.46±0.05b	82.69±0.09e	5.88±1.2
	X/C	220.28±60.0	$0.0052±0.0011_c$	3.47±0.19a	76.96±0.09g	9.17±0.2
SP	-	47.98±20.44	$0.0084±0.0006_c$	1.50±0.06b	90.23±0.09c	9.81±2.0
	P/C	120.10±23.66	$0.0177±0.0006_b$	1.55±0.02b	89.83±0.03c	4.60±1.9
	P/X	122.13±13.94	$0.0075±0.0004_c$	1.77±0.02b	78.68±0.06f	7.01±2.7
	X/C	173.39±23.51	$0.0235±0.0028_a$	1.66±0.03a	94.12±0.09a	7.37±0.4
2-way ANOVA						
O	N.S.	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	N.S.
EB	N.S.	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
O*EB	N.S.	N.S.	$P < 0.05$	$P < 0.05$	$P < 0.05$	N.S.

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.

Y	X	Regression equation	r	P
PROT	PF	y = -98.69+35.92x	0.50	0.0138
PROT	AMI	y = 59.83+42.48x	0.62	0.0013
AMI	DG	y = 8.57-0.08x	0.56	0.0041
LIP	DG	y = -0.056+0.0008x	0.75	<0.0001
LIP	N-NH_4	y = 0.0004+0.004x	0.50	0.0131
DG	N-NH_4	y = 75.71+3.94x	0.52	0.0099

Values of enzymatic activity of Proteases U/mg protein (PROT), values of enzymatic activity of amylases 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-NH_4), 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 carbohydrase) in the diet of white shrimp. It is accepted that proteases break peptide bonds, achieving a smaller size and free amino acids, while carbohydrases 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 of 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-NH_4 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 (Secretaría 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 -EDI), and the Institutional Scholarship for Researcher Development (Beca de Estímulo Institucional de Formación de Investigadores -BEIFI) provided by the IPN; the National Researcher System (SNI-CONACyT).

References

AOAC. (2016). *Métodos oficiales de análisis del Catálogo Internacional AOAC en línea* (20th ed., Vol. 2). Gaithersburg. https://catalogo.latu.org.uy/opac_css/index.php?lvl=notice_display&id=31273

Arriaga-Hernández, D., Hernández, C., Martínez-Montaño, E., Ibarra-Castro, L., Lizárraga-Velázquez, E., Leyva-López, N., & Chávez-Sánchez, M. C. (2021). Fish meal replacement by soybean products in aquaculture feeds for white snook, *Centropomus viridis*: Effect on growth, diet digestibility, and digestive capacity. *Aquaculture*, *530*, 735823. https://doi.org/10.1016/J.AQUACULTURE.2020.735823

Ashraf, G., Saad, A., Mohamed, H., Zaki, S., & Ehab, E.-H. (2018). Dietary effects of Azolla pinnata combined with exogenous digestive enzyme (Digestin™) on growth and nutrients utilization of freshwater prawn, *Macrobrachium rosenbergii* (de Man 1879). *Journal of Oceanology and Limnology*, *36*(4), 1434–1441. https://doi.org/10.1007/s00343-018-7019-7

Bhunia, B., Basak, B., Bhattacharya, P., & Dey, A. (2013). Process engineering studies to investigate the effect of temperature and pH on kinetic parameters of alkaline protease production. *Journal of Bioscience and Bioengineering*, *115*(1), 86–89. https://doi.org/10.1016/J.JBIOSC.2012.08.003

Boyd, C. E. (2017). General relationship between water quality and aquaculture performance in ponds. In *Fish Diseases:*

Prevention and Control Strategies (pp. 147–166). Elsevier Inc. https://doi.org/10.1016/B978-0-12-804564-0.00006-5

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Cabrera, S. M. J. (2018). Efecto de alimentos funcionales para camarón blanco (*Penaeus vannamei*) sobre la expresión de genes asociados a la resistencia al virus de la mancha blanca (*WSSV*) y a una cepa toxigénica de *Vibrio parahaemolyticus (ahpnd*). In Centro de Investigaciones Biologicas del Noroeste, S.C. http://dspace.cibnor.mx:8080/bitstream/handle/123456789/2832/cabrera_m TESIS.pdf?sequence=1&isAllowed=y

Carrillo, O., & Vega-Villasante, F. (2000). Aditivos alimentarios como estimuladores del crecimiento de camarón. *Memorias Del V Simposium Internacional de Nutrición Acuícola.*, *19–22*, 90–101. https://www.uanl.mx/utilerias/nutricion_acuicola/V/archivos/nolasco.pdf

Cobo Abrantes, R., & Pérez Jar, L. (2018). Aspectos generales del cultivo y la genética del camarón blanco del Pacífico *Litopenaeus vannamei* (Boone, 1931). Item Type Journal Contribution. *Revista Cubana de Investigaciones Pesqueras*, *35*(1), 18–23. http://hdl.handle.net/1834/15129

Coelho-Emerenciano, M. G., & Massamitu-Furuya, W. (2006). Ensilado de maíz en dietas para postlarva de camarón de agua dulce *Macrobrachium rosenbergii*. *Investigaciones Marinas*, *34*(2), 57–61. https://doi.org/10.4067/S0717-71782006000200006

Costavalo Muñoz, J. A. (2021). Composición química y enzimas digestivas en juveniles de tilapia roja *(oreochormis mossambicus x oreochromis niloticus)* alimentados con β-glucano en dietas. Universidad Técnica Estatal de Quevedo.

Dalsgaard, J., Bach Knudsen, K. E., Verlhac, V., Ekmann, K. S., & Pedersen, P. B. (2016). Supplementing enzymes to extruded, soybean-based diet improves breakdown of non-starch polysaccharides in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*, *22*(2), 419–426. https://doi.org/10.1111/anu.12258

Davis, D.A., Samocha, T.M., Bullis, R.A., Patnaik, S., Browdy, C.L., Stokes, A.D. & Atwood, H.L. (2004) Practical diets for *Litopenaeus vannamei* (Boone, 1931): working towards organics and/or all plant production diets. In: Avances en Nutrición Acuícola VII: Memorias del Séptimo Simposium Internacional de Nutrición Acuícola. 16–19 noviembre (ed. by L.E. Cruz Suárez, D. RicqueMarie, M.G. Nieto López, D. Villarreal, U. Scholtz & M. González), pp. 202–214. Noviembre, Hermosillo, Sonora, Mexico.

Escobar Gil, C., Pachamoro Leytón, M., & Reyes Avalos, W. (2017). Supervivencia y crecimiento de machos adultos del camarón de río *Cryphiops caementarius molina*, 1782 (*Crustacea, Palaemonidae*) expuestos a salinidades. *Ecología Aplicada*, *16*(2), 75–82. https://www.redalyc.org/journal/341/34153892001/html/

Faillace, J., Vergara, R., & Suarez, A. (2016). Evaluación de una fórmula alimenticia para camarón de cultivo (*L. vannamei*) con inclusión de proteína vegetal a base de harina de soya. *Revista AquaTIC*, *44*, 12–29. http://www.revistaaquatic.com/ojs/index.php/aquatic

FAO. (1989). *Nutricion y alimentacion de peces y camarones cultivados manual de capacitacion*. https://www.fao.org/3/ab492s/AB492S01.htm#ch2.

Furukawa, A., & Tsukahara, H. (1966). On the acid digestion of chromic oxide as an index substance in the study of digestibility of fish feed. *Bulletin of the Japanese Society for the Science of Fish*, *32*, 502–506.

García-Ulloa, M., Hernandez-Llamas, A., Armenta-Soto, S. de J., & Rodríguez-González, H. (2017). Substituting fishmeal with mixtures of wheat, corn and soya bean meals in diets for the white leg shrimp, *Litopenaeus vannamei* (Boone): effect on production parameters and preliminary economic assessment. *Aquaculture Research*, *48*(9), 4864–4873. https://doi.org/10.1111/ARE.13306Gjellesvik, D. R., Lombardo, D., & Walther, B.T. (1992). Pancreatic bile salt dependent lipase from cod (*Gadus morhua*): Purification and properties. *Biochimica et Biophysica Acta (BBA)/Lipids and Lipid Metabolism*, *1124*(2), 123–134. https://doi.org/10.1016/0005-2760(92)90088-d

Hamidoghli, A., Won, S., Farris, N. W., Bae, J., Choi, W., Yun, H., & Bai, S. C. (2020). Solid state fermented plant protein sources as fish meal replacers in whiteleg shrimp *Litopaeneus vannamei*. *Animal Feed Science and Technology*, *264*, 114474. https://doi.org/10.1016/j.anifeedsci.2020.114474

Juárez-Morales, P., Cortes Cuevas, A., Arce Menocal, J., Del Río García, J. C., Gómez Verduzco, G., & Avila González, E. (2020). Efecto de un complejo multienzimático y un probiótico en gallinas de postura alimentadas con dietas sorgo-soya-canola. *Revista Mexicana De Ciencias Pecuarias*, *11*(2), 369–379. https://doi.org/10.22319/RMCP.V11I2.4843

León-Balderrama, J. I., Carrazco-Escalante, J. C., Leyva-León, E., León-Balderrama, J. I., Carrazco-Escalante, J. C., & Leyva-León, E. (2020). La topología y algunas características clave de la Red de Conocimiento de la acuicultura de camarón desarrollada en Ahome, Sinaloa. *Estudios Sociales. Revista de Alimentación Contemporánea y Desarrollo Regional*, *30*(56). https://doi.org/10.24836/ES.V30I56.1032

López-Flores, A. R., Luna-Urban, C., Buenrostro-Figueroa, J. J., Hernández-Martínez, R., Huerta-Ochoa, S., Escalona-Buendía, H., Aguilar-González, C. N., Prado-Barragán, L. A., López-Flores, A. R., Luna-Urban, C., Buenrostro-Figueroa, J. J., Hernández-Martínez, R., Huerta

Ochoa, S., Escalona-Buendía, H., Aguilar-González, C. N., & Prado-Barragán, L. A. (2016). Effect of pH, temperature and protein and carbohydrate source on the production of proteases by *Yarrowia lipolytica* in solid culture. *Revista Mexicana de Ingeniería Química*, *15*(1), 57–67. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S166527382016000100057&lng=es&nrm=iso&tlng=es

Martínez-Aispuro, J. A., Figueroa-Velazco, J. L., Cordero-Mora, J. L., Sánchez Torres-Esqueda, M. T., & Martínez-Aispuro, M. (2016). Dietas para cerdos en iniciación incluyendo salvado de trigo y adicionadas con xilanasas. *Ecosistemas y Recursos Agropecuarios*, *4*(10), 80. https://doi.org/10.19136/era.a4n10.996

Martínez Córdova, L. R., Martínez Porchas, M., & Robles Porchas, G. R. (2022). Alternativas de Acuacultura Sostenible: Aspectos Nutricionales. In L. E. Cruz Suárez,

M. Tapia Salazar, M. G. Nieto López, D. A. Villarreal Cavazos, J. Gamboa-Delgado, & C. A. Martínez Palacios (Eds.), *Investigación e Innovación en Nutrición Acuícola* (Universida, p. 473).

Maynard, L. A., & Loosli, J. K. (1969). Animal nutrition. In *Animal nutrition.* (6ta ed., Issue 6th ed). McGraw-Hill Book Co. Inc.

Mendoza, R., De Dios, A., Vazquez, C., Cruz, E., Ricque, D., Aguilera, C., & Montemayor, J. (2001). Fishmeal replacement with feather-enzymatic hydrolyzates co-extruded with soya-bean meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture Nutrition*, *7*(3), 143–151. https://doi.org/10.1046/J.1365-2095.2001.00164.X

Morales-Campos, B. K., González-Melo, M., Sánchez-Santillán, P., Torres-Salado, N., Rojas-García, A. R., & Maldonado-Peralta, M. de los A. (2019). Producción de celulasas y lacasas de *Pleurotus ostreatus* por fermentación sólida usando subproductos de maíz como sustratos. *Revista Mexicana de Agroecosistemas*, *6*(2), 16–18.

Moreno Garrido, L. (2022). uso de extractos de macroalgas como aditivos en la dieta de la dorada *(sparus aurata*) sobre el crecimiento y bienestar animal [Universidad de Cádiz]. https://rodin.uca.es/bitstream/handle/10498/27648/TFG_Lucía Moreno Garrido.pdf?sequence=1&isAllowed=y

Navarrete del Toro, M. de los A., García-Carreño, F. L., & Córdova-Murueta, J. H. (2011). Comparison of digestive proteinases in three penaeids. *Aquaculture*, *317*(1–4), 99–106. https://doi.org/10.1016/j.aquaculture.2011.03.035

Núñez-Torres, O. P. (2017). Journal of the Selva Andina animal science. In *Journal of the Selva Andina Animal Science* 4(2), 93-94. http://www.scielo.org.bo/scielo.php?script=sci_arttext&pid=S2311-25812017000200001&lng=es&nrm=iso&tlng=es

Ordoñez Rumiche, E. M., Del Carpio Ramos, P. A., & Cayo Colca, I. S. (2018). Suplementación alimenticia con orégano (*Origanum vulgare*) y complejo enzimático en pollos de carne: I. Indicadores Productivos. *UCV - HACER: Revista de Investigación y Cultura, Vol. 7(1)*, 31–44. https://dialnet.unirioja.es/servlet/articulo?codigo=6317321&info=resumen&idioma=SPA

Patil, D. W., & Singh, H. (2014). Effect of papain supplemented diet on growth and survival of post-larvae of *Macrobrachium rosenbergii*. *International Journal of Fisheries and Aquatic Studies*, *1*(6), 176–179. https://www.fisheriesjournal.com/archives/2014/vol1issue6/PartC/162.pdf

Piñeros-Roldan, A. J., Gutiérrez-Espinosa, M. C., & Castro-Guerrero, S. R. (2014). Sustitución total de la harina de pescado por subproductos avícolas suplementados con aminoácidos en dietas para juveniles de *Piaractus brachypomus*, Cuvier 1818. *ORINOQUIA*, *18*(2), 13–24. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0121-37092014000200002&lng=en&nrm=iso&tlng=

Ponce-Palafox, J. T., Esparza-Leal, H., Arredondo-Figueroa, J. L., Martinez-Palacios, C. A., & Ross, L. G. (2017). The effect of protein and lipid level on the specific dynamic action and post-prandial nitrogen excretion in subadult of white shrimp *Litopenaeus vannamei*. *Revista de Biología Marina y Oceanografía*, *52*(1), 131–141. https://www.redalyc.org/articulo.oa?id=47950143011

Rabello, C. B. V., Costa, M. J., Nogueira, W. C. L., Barbosa, J. G., Rios-Alva, J. C., Wyatt, C. L., York, T. W., Serrano, M. P., & Oviedo-Rondón, E. O. (2021). Effects of graded levels of exogenous xylanase in corn-soy diets with two amino acid density and fat levels postpellet in broiler chickens: live performance, energy utilization, digestibility, and carcass characteristics. *Poultry Science*, *100*(2), 820–834. https://doi.org/10.1016/j.psj.2020.10.046

Rachmawati, D., & Samidjan, I. (2016). Effect of phytase enzyme on growth boost in the artificial feed made of plant protein to shorten production time of giant tiger prawn [*Penaeus monodon*, (Fabricus 1798)]. *Aquatic Procedia*, *7*, 46–53. https://doi.org/10.1016/J.AQPRO.2016.07.006

SENASICA. (2003). *Manual de Buenas Prácticas de Producción Acuícola de Camarón para la Inocuidad Alimentaria*. https://www.gob.mx/cms/uploads/attachment/file/311356/ManualBPCam.pdf

Shahzad, M. M., Bashir, S., Hussain, S. M., Javid, A., Hussain, M., Ahmed, N., Khan, M. K. A., Furqan, M., Liaqat, I., Rafique, T., & Khalid, F. (2021). Effectiveness of phytase pre-treatment on growth performance, nutrient digestibility and mineral status of common carp (*Cyprinus carpio*) juveniles fed moringa by-product based diet. *Saudi Journal of Biological Sciences*, *28*(3), 1944–1953. https://doi.org/10.1016/J.SJBS.2020.12.046

Siccardi III, A., Lawrence, A., Gatlin III, D., Fox, J., Castille, F., Perez-Velazquez, M., & González-Félix, M. (2006). *Requerimientos de energía y proteína digerible para crecimiento y mantenimiento de subadultos de Litopenaeus vannamei | Avances en Nutrición Acuicola*. https://nutricionacuicola.uanl.mx/index.php/acu/article/view/171

Song, H. L., Tan, B. P., Chi, S. Y., Liu, Y., Chowdhury, M. A. K., & Dong, X. H. (2017). The effects of a dietary protease-complex on performance, digestive and immune enzyme activity, and disease resistance of *Litopenaeus vannamei* fed high plant protein diets. *Aquaculture Research*, *48*(5), 2550–2560. https://doi.org/10.1111/are.13091

Stefanello, C., Vieira, S. L., Carvalho, P. S., Sorbara, J. O. B., & Cowieson, A. J. (2016). Energy and nutrient utilization of broiler chickens fed corn-soybean meal and corn-based diets supplemented with xylanase. *Poultry Science*, *95*(8), 1881–1887. https://doi.org/10.3382/ps/pew070

Strickland, J. D. H., & Parsons, T. R. (1972). *A practical handbook of seawater analysis* (2nd ed.). Supply and Services Canada. https://epic.awi.de/id/eprint/39262/1/Strickland-Parsons_1972.pdf

Tacon, A. G. (1997). Reemplazos de harina de pescado: ¿Revisión de los antinutrientes en las semillas oleaginosas y las legumbres? ¿Un factor limitante para la Revolución Verde de los alimentos acuáticos? *Cahiers Options Mediterraneennes (CIHEAM).*

Tidwell, J. H., Coyle, S. D., Bright, L. A., & Yasharian, D. (2005). Evaluation of plant and animal source proteins for replacement of fish meal in practical diets for the largemouth bass *Micropterus salmoides*. *Journal of the World Aquaculture Society*, *36*(4), 454–463. https://doi.org/10.1111/j.1749-7345.2005.tb00393.x

Valdez-González, F. J., Gutiérrez-Dorado, R., García-Ulloa, M., Cuevas-Rodríguez, B. L., & Rodríguez-González, H. (2018). Effect of fermented, hardened, and dehulled of chickpea (*Cicer arietinum*) meals in digestibility and antinutrients in diets for tilapia (*Oreochromis niloticus*).

Spanish Journal of Agricultural Research, *16*(1), e0605–e0605. https://doi.org/10.5424/SJAR/2018161-11830

Valdivia, A. L., Matos, M. M., Rodríguez, Z., Pérez, Y., Rubio, Y., & Vega, J. (2019). Enzymatic additives and their use on animal rearing Los aditivos enzimáticos, su aplicación en la crianza animal. *Cuban Journal of Agricultural Science*, *53*(4).

Vega-Villasante, F., Nolasco, H., & Civera, R. (1993). The digestive enzymes of the pacific brown shrimp *Penaeus californiensis*. I-Properties of amylase activity in the digestive tract. *Comparative Biochemistry and Physiology -- Part B: Biochemistry And*, *106*(3), 547–550. https://doi.org/10.1016/0305-0491(93)90130-W

Walter, H. E. (1984). Proteinases: methods with haemoglobin, casein and azocoll as substrates. In *Methods of enzymatic analysis.* (H.U Bergme, pp. 270–277)

Wang, J., Zhang, H., Yang, Q., Tan, B., Dong, X., Chi, S., Liu, H., & Zhang, S. (2020). Effects of replacing soybean meal with cottonseed meal on growth, feed utilization and non-specific immune enzyme activities for juvenile white shrimp, *Litopenaeus vannamei*. *Aquaculture Reports*, *16*, 100255. https://doi.org/10.1016/j.aqrep.2019.100255

Wong, M. H., Tang, L. Y., & Kwok, F. S. (1996). The use of enzyme-digested soybean residue for feeding common carp. *Biomed Environ Sci.*, 9(4), 418–423.

Xie, S. wei, Liu, Y. jian, Zeng, S., Niu, J., & Tian, L. xia. (2016). Partial replacement of fish-meal by soy protein concentrate and soybean meal based protein blend for juvenile Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, *464*, 296–302. https://doi.org/10.1016/J.AQUACULTURE.2016.07.002

Yao, W., Li, X., Kabir Chowdhury, M. A., Wang, J., & Leng, X. (2019). Dietary protease, carbohydrase and micro-encapsulated organic acid salts individually or in combination improved growth, feed utilization and intestinal histology of Pacific white shrimp. *Aquaculture*, *503*, 88–95. https://doi.org/10.1016/J.AQUACULTURE.2018.12.06

Yun, H., Shahkar, E., Hamidoghli, A., Lee, S., Won, S., & Bai, S. C. (2017). Evaluation of dietary soybean meal as fish meal replacer for juvenile whiteleg shrimp, *Litopenaeus vannamei* reared in biofloc system. *International Aquatic Research*, *9*(1), 11–24. https://doi.org/10.1007/S40071-017-0152-7/FIGURES/1