

Effect of hydrolysable tannins on growth performance, hepatopancreatic morphology, and immune-related gene expression of white shrimp (*Penaeus vannamei*) fed low fishmeal diets

Efecto de los taninos hidrolizables sobre el crecimiento, morfología hepatopancreática y expresión de genes relacionados con la inmunidad del camarón blanco (*Penaeus vannamei*) alimentado con dietas bajas en harina de pescado

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ABSTRACT

A 90-days feeding trial evaluated hydrolysable tannins (HT) on growth performance, hepatopancreatic morphology, and immune-related gene expression of Pacific white shrimp. Four diets were formulated: control diet (CD) and three diets with HT inclusion (D1: 0.1 %, D2: 0.2 % and D3: 0.3 %). Four hundred shrimps (0.31 g ± 0.04 g) were randomly distributed into 16 tanks (150 L, 4 replicates/each diet, 25 shrimp/tank). After feeding trial, shrimp fed D1 had significantly higher ($P < 0.05$) final weight (2.24 g), weekly weight gain (681.15 %), and specific growth performance (2.37 % d⁻¹) compared with shrimp fed CD. Histologically, shrimp fed D1 and D2 had better hepatopancreatic morphology condition with elevated lipid reserves and atrophy absence, higher B cell height (24.5 – 26.5 µm) and epithelial area (8566 – 8594 µm²) compared with shrimp fed CD, suggesting beneficial effects on shrimp intracellular digestion. The transcriptional response suggested that shrimp fed D1 significantly ($P < 0.05$) upregulated genes related with antimicrobial activity (9.9-fold change) and antioxidant activity (3.9 fold-change) compared with shrimp fed CD, D2 and D3. Therefore, 0.1 % HT had beneficial effects on growth performance, hepatopancreatic morphology, and immune-related gene expression, suggesting their potential as a natural feed additive in shrimp formulations.

Key words: Shrimp, aquaculture, shrimp nutrition, natural feed additives, hydrolysable tannins.

RESUMEN

Un ensayo de alimentación de 90 días evaluó los efectos de taninos hidrolizables (TH) sobre el rendimiento productivo, la morfología hepatopancreática y la expresión de genes relacionados con la inmunidad del camarón blanco. Se formularon cuatro dietas: un control (CD) y tres con inclusión de TH

(D1: 0.1 %, D2: 0.2 % y D3: 0.3 %). Cuatrocientos camarones (0.31 g ± 0.04 g) fueron distribuidos en 16 tanques (150 L, 4 réplicas por dieta, 25 camarones por tanque). Los camarones alimentados con D1 tuvieron significativamente mayor ($P < 0.05$) peso final (2.24 g), ganancia de peso semanal (681.15 %) y tasa de crecimiento específico (2.37 % d⁻¹) comparado con camarones alimentados con CD. Histológicamente, los camarones de D1 y D2 presentaron mejor condición del hepatopáncreas, con mayores reservas lipídicas y ausencia de atrofia, así como mayor altura de células B (24.5–26.5 µm) y área epitelial (8566–8594 µm²), sugiriendo beneficios en digestión intracelular. Además, D1 promovió la expresión de genes relacionados con actividad antimicrobiana (9.9 veces) y estrés oxidativo (3.9 veces) frente a CD, D2 y D3. Por tanto, la inclusión de 0.1 % de HT mejora el crecimiento, la salud y la inmunidad del camarón, sugiriendo su potencial como aditivo natural en su alimentación.

Palabras clave: Camarón, acuicultura, nutrición del camarón, aditivos naturales, taninos hidrolizables.

INTRODUCTION

Pacific white shrimp (*Penaeus vannamei*) represents 62 % of world shrimp aquaculture production with increasing growth (FAO, 2024). As in many other animal production systems, the growth and production of farmed shrimp depends on the external supply of nutritional inputs and feeds (Tacon, 2002; Iber and Kasan, 2021; Eggink *et al.*, 2025); consequently, it is essential to optimize formulated diets to meet the nutritional requirements of shrimp, adapting them to different development stages, culture system and farm production levels (Nunes *et al.*, 2014). Therefore, feed formulation requires improvement to ensure shrimp aquaculture production. In recent years, the development of shrimp formulated feeds

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has faced several challenges; for example, the need to reduce the use of fishmeal and incorporate alternative ingredients, animal or plant-based, into the formulations (Olsen and Hasan, 2012; Gasco *et al.*, 2018; Nunes *et al.*, 2019; Hodar *et al.*, 2020; Casillas-Hernández *et al.*, 2022).

The fishmeal reduction in aquafeed includes additives and functional ingredients to reduce nutritional deficiencies and stress condition of shrimp (Novriadi *et al.*, 2023). However, it is essential to select alternative ingredients that meet certain nutritional characteristics, including a low level of fiber and antinutrients, as well as high nutrient digestibility and palatability (Jannathulla *et al.*, 2019). Nevertheless, the incorporation of ingredients that replace fishmeal has generated the need to include additives that counteract the nutritional deficiencies of the alternative ingredients (Ogunkalu, 2019; Hussain *et al.*, 2024).

In aquafeeds, additives are nutritive or non-nutritive ingredients that are supplemented in small quantities for specific purposes, such as improving the quality of cultivated organisms, preserving the physical and chemical properties of diets, or maintaining a healthy aquatic environment (Bai *et al.*, 2015). Numerous studies have evaluated the effects of feed supplementation with phytogenic natural additives, such as hydrolysable tannins (HT) or condensed tannins (CT), to evaluate their effects on growth, nutrition, and disease resistance in aquatic organisms (Buyukcapar *et al.*, 2011; Encarnação, 2016; Kesselring *et al.*, 2021). Due to its structural composition, HT could be enzymatic or non-enzymatic hydrolyzed more quickly than CT (Buyukcapar *et al.*, 2011); but HT has a stronger antibacterial and antioxidant effect compared to CT due to its biological activities (Zhu *et al.*, 2021). Moreover, stronger astringency has been reported in CT, which reduces palatability, feed intake, nutrient digestibility, and consequently inhibits shrimp growth (Peng *et al.*, 20021). Therefore, tannins have been considered anti-nutritional factors for decreasing feed palatability (Novriadi *et al.*, 2021). Nevertheless, low tannin inclusion could improve nutrient digestion and absorption in aquatic organisms (Novriadi *et al.*, 2023).

The dietary use of HT has been evaluated in some fishes as rohu (*Labeo rohita*), Atlantic striped bass (*Morone saxatilis*) and Tilapia (*Oreochromis niloticus*) to improve growth and antibacterial activity against pathogens (Prusty *et al.*, 2007; Ashraf *et al.*, 2009; Abdel-Tawwab *et al.*, 2023). Previous studies have suggested that HT supplementation of 0.15 g/Kg - 4 g/Kg in diets with high fishmeal inclusion (22 % - 23 %) improved growth performance, immunity, digestive enzyme activity, antioxidant activity, stress tolerance, pathogen resistance, transcriptomic and metabolic responses, and microbial composition of shrimp (Zhu *et al.*, 2021; Gong *et al.*, 2022; Huang *et al.*, 2024; Zhang *et al.*, 2024; Eissa *et al.*, 2023). Also, previous research has been indicated that HT supplementation of 0.025g/Kg - 0.3 g/Kg in diets with low fishmeal inclusion (10 % - 15 %) had positive functional effects on hemocytes count, lysozyme activity, pathogen resistance and hepatopancreatic histology of shrimp (Novriadi *et al.*, 2021;

Bolivar-Ramirez *et al.*, 2022; Novriadi *et al.*, 2023). Therefore, the aim of this study was to confirm the HT effects on growth performance, hepatopancreatic morphology, and immune-related gene expression of *P. vannamei* fed low fishmeal diets.

MATERIALS AND METHODS

Experimental diet preparation

P. vannamei requirements were used to develop four experimental shrimp diets that contain around 36 % of protein. The control diet (CD) was formulated with a mixture of 10 % fishmeal and 43 % soybean meal without HT supplementation, and the other three diets were supplemented with different HT (Tanin Sevnica Slovenia; purity > 60 %) levels (D1: 0.1 %, D2: 0.2 %, and D3: 0.3 %) obtained from the sweet chestnut (*Castanea sativa*). The formulation and proximate composition of experimental diets are shown in Table 1.

Shrimp feeding trial and sample collection

P. vannamei were obtained from a shrimp farm from Sonora, Mexico. The qPCR validation of pathogen-free shrimp was according to the Manual of Diagnostic Tests for Aquatic Animals of the World Organization for Animal Health (WOAH, 2024). Shrimp were acclimated for a week in 1500-L tanks with seawater under controlled conditions (temperature 30 ± 0.5 °C, dissolved oxygen ≥ 4 mg/L, salinity 37 g/L, pH ≥ 7 and photoperiod of 12 light hours) in the aquaculture laboratory prior to the feeding trial and fed with a commercial feed.

Shrimp were fasted for 24 h after the acclimation period. Then, 400 healthy shrimp (0.31 ± 0.04 g) were randomly distributed in 16 circular tanks (150 L) at a density of 25 shrimp per tank (equivalent to a density of 167 shrimp/m³). Four replicate tanks were randomly assigned to each dietary treatment. Shrimp were fed an initial ration of 12 % their biomass to satiation divided into three rations during the day (8:00, 13:00 and 16:00 h) for 90 days, the ration was adjusted daily based on residual feed presence or absence. Temperature (27.84 to 28.36 °C), salinity (36.98 to 37.07 g/L), dissolved oxygen (4.00 to 5.17 mg/L), and pH (7.41 to 7.84) were recorded. The seawater was replaced at a rate of 30% per day. The dead shrimp, feces, uneaten feed, and molts were removed daily. After feeding trial and 24-h fasting period, three shrimp were randomly collected from each replicate to obtain 400 µL of hemolymph for transcriptional response analysis (Martinez-Porchas *et al.*, 2020). Then, shrimps were anesthetized on ice for histological analysis, twelve whole shrimp (three shrimp per replicate) were sampled from each treatment and fixed them in AFA Davidson solution.

Growth performance

Shrimp were counted and weighed to estimate the growth performance according to the equations reported by previous studies (Piérri *et al.*, 2015; Peña-Rodriguez *et al.*, 2017; Gonzalez-Galaviz *et al.*, 2020).

Final weight (FW, g) = (Σ Final individual weight)/Final number of shrimps

Weight gain (WG, %) = 100 × (Final weight - Initial weight)/

Table 1. Ingredients and proximate composition of experimental diets (g/kg dry weight).**Tabla 1.** Ingredientes y composición proximal de dietas experimentales (g/kg peso seco).

Ingredients (g/kg dry weight)	CD	D1	D2	D3
Fishmeal ¹	100.0	100.0	100.0	100.0
Soybean meal ²	431.4	431.4	431.4	431.4
Wheat flour ³	150.0	150.0	150.0	150.0
Soy protein isolate ⁴	93.6	93.6	93.6	93.6
Corn starch ⁵	103.4	102.4	101.4	100.4
Fish oil ⁶	56.6	56.6	56.6	56.6
Soy lecithin ⁷	10.0	10.0	10.0	10.0
Vitamin premix ⁸	20.0	20.0	20.0	20.0
Mineral premix ⁹	20.0	20.0	20.0	20.0
Pellet binder ¹⁰	15.0	15.0	15.0	15.0
Hydrolysable tannin ¹¹	0.0	1.0	2.0	3.0
Proximate analysis (%)				
Dry matter	93.26	92.22	92.89	90.82
Crude protein	36.40	36.27	36.50	35.91
Crude lipid	6.50	7.50	6.90	7.10
Crude fiber	3.40	3.40	3.30	3.10
Ash	7.70	7.40	7.50	7.80
Starch	23.60	22.50	22.90	22.60
Gross energy (Kcal)	4218	4283	4247	4238
Free amino acids mg/100g				
Glycine	11.40	8.50	9.34	14.94
Alanine	49.50	36.95	39.61	47.68
Serine	<1.60	<1.06	<1.06	<1.05
Proline	1.30	<1.15	<1.15	<1.15
Valine	32.85	24.27	27.43	30.50
Threonine	6.55	4.03	3.66	3.93
Cysteine	<1.22	<1.22	<1.22	<1.22
Isoleucine	21.30	15.48	17.39	16.95
Leucine	51.57	29.29	33.91	30.62
Asparagine	6.67	<1.32	<1.32	<1.32
Aspartate	45.67	39.69	41.46	35.59
Lysine	112.45	64.77	82.03	<1.46
Glutamine	<1.46	<1.46	<1.46	<1.46
Glutamate	82.73	62.14	73.00	70.45
Methionine	10.93	5.94	6.79	1.50
Histidine	160.41	152.65	159.50	153.09
Phenylalanine	34.69	20.77	23.24	16.05
Arginine	189.74	146.70	145.08	142.85
Tyrosine	33.80	26.08	25.89	25.58
Tryptophane	<2.04	16.13	20.36	<2.05

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Initial weight

Specific growth rate (SGR, % day⁻¹) = $100 \times (\ln \text{ final weight} - \ln \text{ initial weight}) / \text{days}$

Survival rate (SR, %) = $100 \times (\text{Final number of shrimps} / \text{Initial number of shrimps})$

Feed intake = Feed Input (dry weight) - Feed collected (dry weight)

Feed conversion rate = Feed intake / Final biomass

Hepatopancreatic histology

The shrimp hepatopancreas previously fixed in Davidson's solution were processed according to the method described by Bell and Lightner (1998). The rotary microtome (LEICA RM2115RT, Leica Biosystems, Nussloch, Germany) was used to cut 4 µm-thick histological sections, which were fixed and stained with Hematoxylin and eosin. The slides were observed and digitized according to the method described by Casillas-Hernández *et al.* (2021a). The hepatopancreas' cell height was measured using the digital image system Image-Pro Premier software v9.0 (Media Cybernetics Inc., Rockville, MD, USA) based on tissue images.

Transcriptional response of immune-related genes

To separate the hemocytes from the plasma, the hemolymph was centrifuged at 3500 rpm for 10 min at 4 °C. The total RNA from hemocytes was extracted with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and treated with RNA-free DNase (Promega[®], Madison, WI, USA). cDNA was synthesized with Total RNA (500 ng) using the ImProm-II[™] Reverse Transcription System (Promega[®]) and oligo d(T)20 (T4OLIGO). The cDNA synthesis was carried out by reverse transcription at 42 °C for 60 min; then reverse transcriptase was inactivated at 70 °C for 15 min to stop the reaction. The cDNA was diluted with 80 µL of ultrapure water, and 5 µL were used as template for real time quantitative PCR (qPCR) reaction. Transcriptional response was analyzed from five immune-related genes (Hc: hemocyanin; pPO: prophenoloxidase; LGBP: lipopolysaccharide- and β-glucan-binding protein; MnSOD: cytosolic manganese superoxide dismutase, and HSP60: heat shock protein 60) and β-actin as reference gene (Table 2) (Zhang *et al.*, 2013). The qPCR was conducted on a CFX Connect[™] Real-Time PCR detection system (BioRad, Hercules, CA, USA) using SensiFAST[™] SYBR[®] Hi-ROX Kit (Bioline[™], London, UK). The qPCR conditions were initial denaturation at 95 °C for 10 min, followed by 40 denaturation cycles at 95 °C for 15 s, and annealing/extension at 60 °C for 1 min. An analysis of the dissociation curve (60–95 °C) at a temperature transition rate of 0.5 °C/s was performed for each pair of primers. The relative quantification method was used for gene expression analysis according to Rodríguez-Anaya *et al.* (2020) and Casillas-Hernández *et al.* (2021b).

Statistical analysis

The results of growth performance, hepatopancreas' cell height, and transcriptional response of immune-related genes are presented as mean ± SE and were evaluated by

Table 2. Specific primers used for transcriptional response of immune related genes of *P. vannamei*.**Tabla 2.** Cebadores específicos para análisis de respuesta transcripcional de genes relacionados con la inmunidad de *P. vannamei*.

Gene	Forward/Reverse Sequence	Amplicon Length (bp)	Efficiency (%)	Correlation coefficient (R ²)	GenBank accession number
<i>β-actin</i>	5'-CCACGAGACCACCTACAAC-3' 5'-AGCGAGGGCAGTGATTTC-3'	142	91	0.99	AF300705
<i>hemocyanin (Hc)</i>	5'-GTCTTAGTGGTTCTTGGGCTTGTC-3' 5'-GGTCTCCGTCTGAATGTCTCC-3'	124	98	0.98	X82502
<i>prophenoloxidase (pPO)</i>	5'-CGGTGACAAAGTTCCTCTTC-3' 5'-GCAGGTGCGCGTAGTAAG-3'	122	99	0.99	AY723296
<i>lipopolysaccharide- and β-glucan-binding protein (LGBP)</i>	5'-CCATGTCCGGCGGTGGAA-3' 5'-GTCATCGCCCTTCCAGTTG-3'	122	110	0.99	EU102286
<i>cytosolic manganese superoxide dismutase (MnSOD)</i>	5'-TGTTGCACAAGCATTGACGA-3' 5'-CCAGCCAGAGCCTTCACTCC-3'	94	90	0.98	DQ005531
<i>heat shock protein 60 (HSP60)</i>	5'-ATTGTCCGCAAGGCTATC-3' 5'-ATCTCCAGACGCTTCCAT-3'	102	103	0.99	FJ710169

one-way ANOVA (after homoscedasticity and normality tests). Tukey's test was performed for means comparison if any significance was observed. Significance was set at 95 % probability levels. Statistical analysis was performed with Statgraphics Centurion XVI.

RESULTS AND DISCUSSION

Growth performance

The growth performance of shrimp fed diets supplemented with HT was determined and compared with shrimp fed CD (Table 3). Shrimp fed D1 and D2 showed higher FW and SGR values, but D1 significantly influenced all indicator of shrimp growth performance (FW, WG %, SGR). Growth performance values were not different between shrimp fed CD and shrimp fed D3. HT supplementation (D1-D3) significantly improved FCR. No differences were observed in the survival rate of all dietary treatments. It has been reported that HT supplementation of 0.15 g/Kg – 4 g/Kg in diets with high fishmeal inclusion (Zhu *et al.*, 2021; Gong *et al.*, 2022; Huang *et al.*, 2024; Zhang *et al.*, 2024) and 0.025g/Kg - 0.3 g/Kg in diets with low fishmeal inclusion improved shrimp growth performance (Novriadi *et al.*, 2021; Bolivar-Ramirez *et al.*, 2022; Novriadi *et al.*, 2023). In this study, growth performance and feed utilization were improved in shrimp fed D1 and D2 compared with

shrimp fed CD and D3. Previous research with *P. vannamei* under similar density (153 shrimp/m³), low fishmeal diets (10 % fishmeal) and same HT supplementation of 0.1 g/Kg - 0.3 g/Kg reported that 0.2 g/Kg - 0.3 g/Kg of HT supplementation could significantly improve the shrimp growth, but the feeding trial was conducted for 60 days with shrimp of 4.24 g as initial weight; in addition, the experimental diets were enriched with lysine and methionine (Novriadi *et al.*, 2021). Therefore, shrimp size, feeding trial duration and essential amino acid supplementation could contribute to the differences found in the present study.

Hepatopancreatic histology

The histological structure of hepatopancreas was compared with shrimp fed CD (Table 4). The number of B cells were significantly lower ($P < 0.05$) in shrimp fed D3 while no significant differences were observed in shrimp fed D1 and D2, B cell height was significantly higher ($P < 0.05$) in shrimp fed D1 and D2 but significantly lower ($P < 0.05$) in shrimp fed D3, and epithelial area was significantly lower ($P < 0.05$) in shrimp fed D3 without no significant differences in shrimp fed D1 and D2. In shrimp fed CD, the tissue appears well-preserved, with a normal tubular structure and organization. Varying degrees of vacuolar development in B cells are observed, along with

Table 3. Effect of experimental diets on growth performance of *P. vannamei*.**Tabla 3.** Efectos de dietas experimentales sobre el crecimiento de *P. vannamei*.

Treatment	FW (g)	WG (%)	SGR (% day ⁻¹)	FCR	SR (%)
CD	1.84 ± 0.50 ^a	530.40 ± 251.07 ^a	2.10 ± 0.47 ^a	2.43 ± 1.10 ^b	82.7 ± 0.38
D1	2.24 ± 0.64 ^b	681.54 ± 294.70 ^b	2.37 ± 0.44 ^c	2.06 ± 0.68 ^a	80.2 ± 0.40
D2	2.17 ± 0.56 ^b	632.74 ± 281.77 ^{ab}	2.29 ± 0.45 ^{bc}	1.91 ± 0.66 ^a	87.0 ± 0.34
D3	1.92 ± 0.43 ^a	554.63 ± 212.72 ^a	2.18 ± 0.37 ^{ab}	1.77 ± 0.56 ^a	96.7 ± 0.18
<i>p-value</i>	0.000	0.001	0.001	0.000	0.056

Values are mean ± SEM of three replicates, and values in the same row with different letters are significantly different ($P < 0.05$).

Table 4. Histological structure of the hepatopancreas from *Penaeus vannamei* fed with the experimental diets for 90 days.**Tabla 4.** Estructura histológica del hepatopáncreas de *P. vannamei* alimentados con dietas experimentales por 90 días.

Parameters	Treatments				p-value
	CD	D1	D2	D3	
Number of B cells	7 ± 1.3 ^a	7 ± 1.0 ^a	8 ± 2.5 ^a	5 ± 0.9 ^b	0.000
B cell height (µm)	22.6 ± 5.7 ^b	24.5 ± 4.8 ^c	26.3 ± 4.4 ^d	20.6 ± 4.2 ^a	0.000
Epithelial area (µm ²)	7395 ± 2347 ^b	8566 ± 1230 ^b	8594 ± 1631 ^b	5494 ± 1562 ^a	0.000

Values are mean ± SEM of three replicates, and values in the same row with different letters are significantly different ($P < 0.05$).

regions of mild tubular atrophy. No lesions consistent with pathological conditions were identified in this section (Figure 1A). In the histological analysis of treatment D1, a higher prevalence of vacuoles in B cells is observed, along with improved tubular conditions. There is no evidence of atrophy, and an increase in epithelial tissue thickness is noted (Figure 1B). For treatment D2, notable characteristics include the presence of empty intertubular spaces, without hemocyte infiltration suggestive of an inflammatory response, potentially indicative of edema. A loss of turgor in B cells is also evident (Figure 1C). In treatment D3, widespread atrophy is present, with no indication of an inflammatory response. A marked loss of turgor and a reduction in epithelial thickness (atrophy, at) are observed, with some sections retaining only the basal membrane (Figure 1D).

The hepatopancreas is the main digestive gland related to synthesis and secretion of digestive enzymes, absorption and assimilation of nutrients, and is also an essential indicator of shrimp metabolism (Vogt *et al.*, 1985; Novriadi *et al.*, 2022). In this study, we found that after 90 days the histomorphology condition of the hepatopancreas showed significant changes in shrimp treated with HT (Fig 2). In shrimp fed CD, well-preserved tissue and normal tubular arrangement were observed; however, slightly tubular atrophy was observed that could be due to the absence of HT in the diet. Shrimp fed D1 presented the highest vacuoles amount in B cells, higher epithelial tissue thickness, some R cells, low hemocytes presence in intertubular regions, higher lipid reserves and no cell atrophy was observed. This condition is associated with the fundamental role of B and R cells on digestion, absorption,

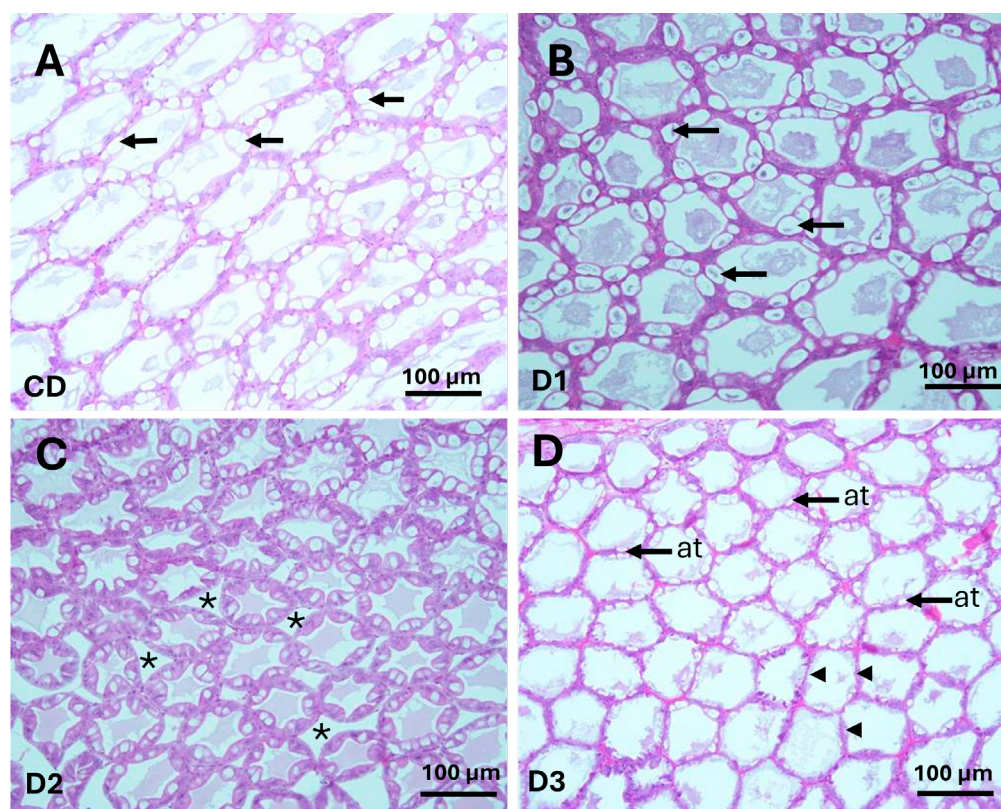


Figure 1. Cross sections of hepatopancreatic tubules, at basal-medial height: (a) Control group (CD); (b) Shrimp fed with inclusion of 0.1 % (D1); (c) 0.2 % (D2) and (d) 0.3 % (D3). B cells (arrows); empty intertubular spaces (asterisks); b cells atrophy (at); basal membrane (arrowhead). 20X magnification. Hematoxylin-eosin staining.

Figura 1. Cortes transversales de los túbulos del hepatopáncreas, a altura basal-medial: (a) Grupo control (CD); (b) Grupo de camarones alimentados con inclusión de 0,1 % (D1); (c) 0,2 % (D2); y (d) 0,3 % (D3). Células B (flechas); espacios intertubulares vacíos (asteriscos); atrofia de células B (at); membrana basal (punta de flecha). Aumento 20X. Tinción con hematoxilina-eosina.

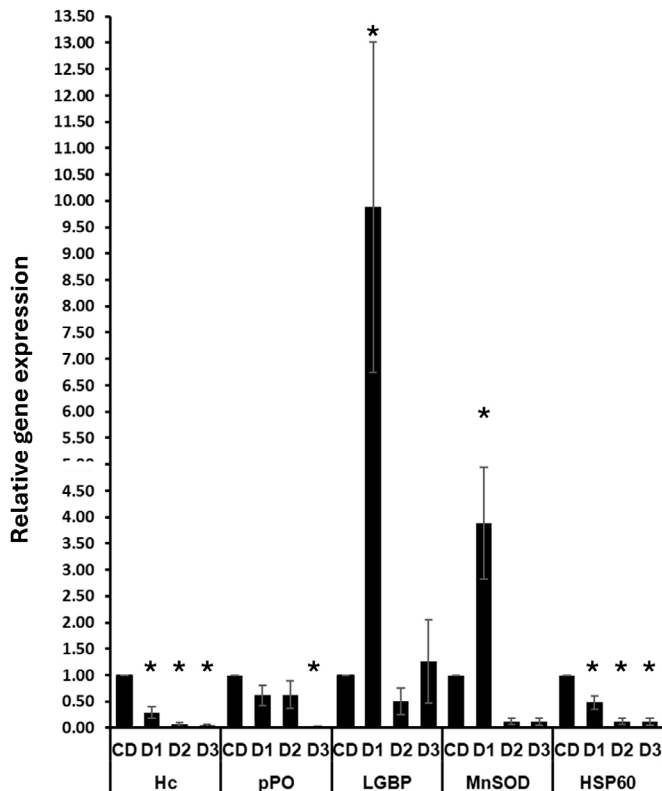


Figure 2. Transcriptional response of immunity-related genes from shrimp fed control diet and diets supplemented with hydrolysable tannins (HT). Hc: hemocyanin, pPO: prophenoloxidase, LGBP: lipopolysaccharide and β -glucan-binding protein, MnSOD: cytosolic manganese superoxide dismutase, HSP60: heat shock protein. Data are presented as means $\pm$ SE, values with * are significantly different ($P < 0.05$). CD: Control diet, D1: 0.1 % HT, D2: 0.2 % HT, and D3: 0.3 % HT.

Figura 2. Respuesta transcripcional de genes relacionados con la inmunidad en camarones alimentados con dieta control y dietas suplementadas con taninos hidrolizables (TH). Hc: hemocianina, pPO: profenoloxidasas, LGBP: proteína de unión a lipopolisacáridos y β -glucanos, MnSOD: superóxido dismutasa de manganeso citosólica, HSP60: proteína de choque térmico. Los datos se presentan como media $\pm$ error estándar; los valores con * son significativamente diferentes ($P < 0,05$). CD: dieta control, D1: 0,1 % TH, D2: 0,2 % TH y D3: 0,3 % TH.

storage, and probably, excretion and detoxification (Barker *et al.*, 1977; Ruiz *et al.*, 2020).

The highest tannin doses used during the feeding trial (0.2 % and 0.3 %) generated a notable presence of intertubular vacant spaces in the hepatopancreas, inflammatory response, turgor loss in B cells, generalized atrophy and lower lipid reserves (Fig. 2). Unlike previous research with similar diet formulation and the same HT inclusion indicated 0.2 % to 0.3 % of HT in shrimp feed containing 10 % of fishmeal for improving growth, haemato-immunological parameters, and to prevent hepatopancreas histological alterations (Novriadi *et al.*, 2021; 2023), but it is important to highlight that the size of shrimp was larger than the current study and the experimental diets used in this study were not enriched with methionine and lysine. It has been reported that amino acid deficiency could contribute to hepatopancreas affection and consequently weakened the shrimp's immune response

(Casillas-Hernández *et al.*, 2022). Moreover, elevated HT inclusion decreased the activity of aminotransferases in shrimp hepatopancreas impairing the protein biosynthesis and causing tissue damage (Yao *et al.*, 2019). Therefore, the 0.1 % HT inclusion could be viable in low fishmeal diets with optimal essential amino acid levels for early culture stage with postlarvas or small juveniles of shrimp.

Transcriptional response of immune-related genes

The transcriptional response of immune-related genes of white shrimp fed diets supplemented with HT was determined and compared with the shrimp fed CD (Figure 2). Hc was significantly downregulated ($p < 0.05$) in shrimp fed D1 (0.29-fold change), D2 (0.07-fold change), and D3 (0.04-fold change). pPO was significantly downregulated ($P < 0.05$) in shrimp fed D3 (0.02-fold change), without significant differences in shrimp fed D1 (0.62-fold change) and D2 (0.63-fold change). D1 diet significantly upregulated ($P < 0.05$) LGBP (9.9-fold change) and MnSOD (3.9-fold change) genes of shrimp, no significant differences were observed in shrimp fed D2 and D3. HSP60 was significantly downregulated ($P < 0.05$) in shrimp fed D1 (0.48-fold change), D2 (0.13-fold change), and D3 (0.10-fold change). However, shrimp fed D1 had a higher gene expression ($P < 0.05$) than shrimp fed D2 and D3. The transcriptional response of immune-related genes is very important to obtain data concerning the shrimp health status (Rodríguez-Anaya *et al.*, 2020).

The tannin supplementation effects on the relative expression of genes related to immunity (Hc, pPO, LGBP), antioxidant capacity (MnSOD), and stress tolerance (HSP60) were analyzed for molecular evaluation of the shrimp defense mechanisms. The main function of Hc and pPO genes is pathogen recognition and the phenoloxidase (PO) system activation, their expression could be affected by different factors such as environmental stressors (Rodríguez-Anaya *et al.*, 2020). Previous research has shown that PO activity could be inhibited by HT in a dose-dependent manner (Huang *et al.*, 2024) comparable to our results. Nevertheless, antimicrobial peptides (AMP) related with pathogen recognition (Huang *et al.*, 2024), superoxide dismutase (SOD) related with antioxidant defense (Gong *et al.*, 2022) and heat shock proteins (HSP) related with stress tolerance (Zhang *et al.*, 2024) improved in shrimp fed diet supplemented with HT. The previous results are important because the prophenoloxidase (pPO) system could be activated by HSP (Zhang *et al.*, 2024) and other AMP or pattern recognition proteins (PRPs) as lipopolysaccharide and β -1,3-glucan-binding protein (LGBP) for triggering the innate immunity (Tassanakajon *et al.*, 2018).

The high transcriptional response of LGBP and MnSOD are consistent with the antimicrobial and antioxidant activity of HT reported in previous research (Zhu *et al.*, 2021). In this study we reported gene expression with higher values in shrimp fed D1 compared to D2 and D3, but some values were significantly lower than shrimp fed CD, suggesting a stabilization of PO system related genes and therefore maintaining the shrimp physiological homeostasis.

CONCLUSIONS

This present study determined that low fishmeal diets with 0.1 % supplementation of HT promote growth performance, hepatopancreas condition and transcriptional response of immune related genes of shrimp. Nevertheless, further studies should be conducted to elucidate the possible molecular mechanisms related to high doses of tannins and their effects on the condition of the hepatopancreas and other organs of shrimp, as well as the effects on shrimp nutritional metabolism.

DECLARATIONS

Supplementary Material

This work includes no supplementary material.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, J.R.G.G., R.C.H.; methodology, J.R.G.G., K.P.C.G., J.C.G.N., R.A.B.L., C.R.J., J.C.I.G., F.L.V., L.Z.R.A., G.I.B.C.; software, J.R.G.G., L.Z.R.A., C.R.J.; formal analysis, J.R.G.G., R.C.H.; investigation, R.C.H, J.R.G.G.; resources, R.C.H., J.R.G.G.; data curation, J.R.G.G., L.Z.R.A.; writing—original draft preparation, R.C.H., J.R.G.G.; writing—review and editing, J.R.G.G., G.I.B.C.; visualization, R.C.H., J.R.G.G., C.R.J., G.I.B.C.; supervision, R.C.H., and J.R.G.G.; project administration, R.C.H.; funding acquisition, R.C.H.

DATA AVAILABILITY

The data for this study is available from the corresponding author by reasonable request.

ETHICAL APPROVALS AND PERMITS FOR STUDIES INVOLVING ANIMALS

The animals' use was conducted with ethical standards and approved by the Ethics and Biosafety Committee of the Institution (2022–01).

CONSENT FOR PARTICIPATION AND PUBLICATION FOR STUDIES INVOLVING PEOPLE

Not applicable.

USE OF AI TOOLS

The authors did not employ any AI tools in this work.

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