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Saponin in white shrimp *Penaeus vannamei* diet possible cause of hemocytic enteritis, a negative effect in growth performance and survival

Saponina en dieta de camarón blanco *Penaeus vannamei* posible causa de enteritis hemocítica y efectos negativos sobre el crecimiento y supervivencia

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ABSTRACT

The optimization of production techniques and intensification of shrimp culture has led to diversified nutrition and health strategies. While the inclusion of additives to improve results is common, they must be properly evaluated. This study aims to perform a comprehensive evaluation of saponin on juvenile white shrimp (*Penaeus vannamei*). Four saponin concentrations (0 %, 2 %, 4 %, and 6 %) were included in a commercial diet and fed to shrimp for 28 days under controlled culture conditions. The diet without saponin (0 %) showed the most significant difference results ($p < 0.05$) when compared to the other concentrations. Survival rates decreased as saponin inclusion increased, along with the observed digestive tract damage (0 % > 2 % > 4 % > 6 %). Contrary to expectations, the evaluated concentrations in this study did not produce improvements in shrimp; instead, they resulted in problems in the digestive tract. Furthermore, greater fungi proliferation was observed in the diet with higher saponin inclusion.

KEY WORDS: Nutrition, *Penaeus vannamei*, saponins, histopathology, hemocytic enteritis.

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R E S U M E N

La optimización de técnicas de producción e intensificación en la camaronicultura han diversificado las estrategias en nutrición y salud. La inclusión de aditivos para mejorar resultados es cada vez más común y es de gran importancia evaluarlos adecuadamente. Este estudio realizó un análisis integral sobre el efecto de saponina en juveniles de camarón blanco *Penaeus vannamei*. Se incluyeron 4 concentraciones de saponina (0 %, 2 %, 4 % y 6 %) en una dieta comercial y se alimentaron durante 28 días bajo condiciones controladas de cultivo. El alimento sin saponina (0 %) mostró los mejores resultados, el crecimiento fue significativamente diferente (p <0.05) respecto a las demás concentraciones. La sobrevivencia mostró reducción conforme la inclusión de saponina se incrementó, al igual que el daño en tracto digestivo (0 % > 2 % > 4 % > 6 %). Las concentraciones evaluadas en este estudio no produjeron mejoras en los camarones, por el contrario, provocaron problemas en su tracto digestivo y en el alimento con mayor proliferación de hongos a mayor inclusión de saponina.

P A L A B R A S C L A V E : Nutrición, *Penaeus vannamei*, saponinas, histopatología, enteritis hemocítica.

Introduction

During the last decade, diseases have significantly affected shrimp farming, leading to substantial losses worldwide (Abdel-Latif *et al.,* 2023; Flegel, 2019). Strategies such as improvements in biosecurity, health management, and genetic selection have been implemented, notably reducing the risks of infectious outbreaks (Alday-Sanz *et al.,* 2018). However, despite these efforts, controlling and preventing the spread of certain diseases remains challenging. This is particularly evident in the cases of white spot syndrome disease (WSSD) and acute hepatopancreatic necrosis disease (AHPND), which continue to cause significant losses due to the lack of efficient treatments (Han *et al.,* 2019; WOAH, 2021). Given this background, nanotechnology and bioactive substances from natural extracts are being explored as additives in specialized foods to promote stress tolerance in shrimp farming and combat diseases, showing promising results (Abdel-Latif *et al.,* 2023; Sharawy *et al.,* 2022; Tacon, 2017).

Improvements in animal health under optimal nutritional conditions promote favorable performance under adverse culture and management conditions in organisms in general (Servin *et al.,* 2021). Given the trend of using vegetable protein in aquaculture (El-Naby *et al.,* 2023), particularly in shrimp diets, evaluating sublethal effects that could affect intestinal homeostasis and lead to deterioration in their health is vital. This is because soybean or sunflower flours contain

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considerable saponins amounts in their composition (Jannathulla *et al.,* 2018), which could pose a health risk for organisms if not considered during the diet formulation. Similarly, some phytogenic products containing essential oils, amino acids, organic acids, steroids, and/or triterpenes have been evaluated as dietary supplements for similar purposes (Abdel-Latif *et al.,* 2023; Francis *et al.,* 2002; Kesselring *et al.,* 2021).

The particular case of saponins, which are present in plants, has shown that their biological activity produces benefits in humans when moderately consumed. However, in other animals, beneficial, adverse, and even detrimental effects have been reported (Bureau *et al.,* 1998; Francis *et al.,* 2002; Francis *et al.,* 2005; Wang *et al.,* 2022). Bureau *et al.* (1998) observed that the addition of 1.5 and 3.0 g of *Quillaja saponins* per kg of feed for chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*) decreased growth and caused damage to the intestinal tract. Conversely, in white shrimp (*P. vannamei*), saponins have been attributed with benefits in growth, improvements in digestive processes, intestinal microbiota modulation, immune system stimulation, and/or increased survival; however, the benefit in promoting digestive tract health was limited (Akbary *et al.,* 2023; Servin *et al.,* 2021). Huang *et al.* (2022) evaluated the effect of bioactive compounds in the culture water with shrimp, concluding that adding them once a week over 70 days improved the specific growth rate and average daily growth, with no differences in survival compared to the control group. Krogdahl *et al.* (2015) and Gopan *et al.* (2020) reported that in diets with low saponins concentration, the immune system was stimulated, but at high concentrations, growth was affected and damage to the integrity of the intestinal mucosa occurred due to interactions with cell membranes. Recently, saponins have been identified as a causative agent of distal gut enteritis in salmon (Krogdahl *et al.,* 2015). Given the scarcity of existing information on shrimp, this study aimed to comprehensively evaluate the effect of different concentrations of saponin extracts on juvenile white shrimp (*P. vannamei*).

Material and Methods

Inclusion of different saponin concentrations in commercial feed

Commercial feed (containing 35 % protein and 9 % lipids; Purina®) and saponin were pulverized using a Nixtamatic® grinder. They were then mixed with a household blender (Blazer home®) to create four different saponin treatments. Initially, one treatment did not include saponin, while the other three were supplemented with 20, 40, and 60 g/Kg of saponin, resulting in concentrations of 0 %, 2 %, 4 %, and 6 %, respectively. Subsequently, each mixture was combined with 250 mL of water to form a homogeneous mass. Finally, the mixture was reconstituted into pellets using a meat grinder (Torrey®) and stored in Ziploc® bags under refrigeration at 4 °C, following the procedure outlined by Loya-Rodriguez *et al.* (2023).

Acclimatization of organisms

Juvenile white shrimp (*P. vannamei*) sourced from a local larval laboratory (El Rosario, coordinates: 22°54’28.3” N, 106°05’43.5” W) were acclimated in 300 L light gray fiberglass tanks filled with filtered seawater (salinity 33.79 ± 0.5 g/L, temperature 29.90 ± 1.14 °C) and provided with constant aeration. Shrimp were fed to satiety three times a day. qPCR (IQ REAL™ Quantitative Systems) analyses were conducted on a representative sample from the batch to rule out the presence of white spot syndrome virus (WSSV), Taura Syndrome Virus (TSV), and acute hepatopancreatic necrosis disease (AHPND), following the methodology outlined by Dangtip *et al.* (2015).

Experimental design and system

After obtaining negative test results for the aforementioned diseases and completing the acclimatization period, a total of 640 juvenile shrimp weighing 0.23 ± 0.1 g and measuring 35.44 ± 0.23 mm on average were randomly distributed into 16 experimental units (40 organisms per unit). Each unit consisted of round fiberglass tanks with a useful capacity of 50 L, featuring black walls and a white bottom. Each concentration (0 %, 2 %, 4 %, and 6 %) was tested in four replicates. The shrimp were fed three times a day (at 08:00, 12:00, and 16:00) for 28 days. Culture conditions included filtered water (10 μm), constant aeration using diffuser stones, and a photoperiod of 12 hours of light followed by 12 hours of darkness. Before the first daily feeding, each experimental unit was siphoned to remove feces, uneaten food, or any waste material. Daily recordings were made of temperature (29.90 ± 1.14 °C), salinity (33.79 ± 0.5 g/L), and dissolved oxygen (3.18 ± 0.7 mg/L) using a YSI 85 Multimeter. Other physicochemical parameters such as nitrite, nitrate, ammonium, and pH were measured using commercial kits (API Saltwater Master Test Kit). The reaction time of the shrimp to feeding was recorded at the moment they caught the pellet and began consuming it (consumption reaction).

At the end of the 28-day experimental period, individual tests were conducted on all shrimp across all concentrations and replicates. Subsequently, the following parameters were calculated:

Growth (Pan *et al.*, 2007)

$$Equation\ 1: Weight\ gain\ (g) = \frac{(Final\ weight - Initial\ weight)}{(Duration\ days)}$$

$$Equation\ 2: Increase\ in\ length\ (mm) = \frac{(Final\ length - Initial\ length)}{(Duration\ days)}$$

Specific Growth Rate (Stadtlander *et al.*, 2013).

$$Equation\ 3: SGR\ (\%\ day^{1}) = \left[\frac{(Ln\ final\ weigh) - (Ln\ Initial\ weight)}{Days}\right] x\ 100$$

Survival (Li *et al.*, 2007)

$$Equation\ 4: S\ (\%) = \frac{Final\ number\ of\ organisms}{Initial\ number\ of\ organisms} X100$$

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Histopathological analysis

The initial control involved randomly sampling 17 shrimp to assess their health before initiating feeding with the established saponin concentrations. On day 22 of feeding, a high mortality episode occurred, prompting the random collection of eight shrimp from each concentration (two shrimp per replicate) for fixation and subsequent histopathological analysis. Similarly, on day 28 of feeding, eight shrimp from each concentration were fixed in Davidson's solution and processed histologically for evaluation. Each shrimp was longitudinally cut, and each section was dehydrated in ethanol solutions at 70 %, 80 %, 96 %, and 100 % before being embedded in paraffin wax following the method defined by Bell & Lightner (1988). Sections of 5 μm thickness were obtained from each shrimp using a manual microtome (Leica RM 2125RT), stained with hematoxylin-eosin-floxin, and mounted on slides (Lightner, 1996). Each sample was examined under an Olympus microscope (CX31) to identify tissue alterations. The histopathological condition observed in the controls (both before and after feeding) was used as a reference to detect tissue alterations and assess effects on shrimp health. Consequently, any observed alterations in the shrimp after feeding with different saponin concentrations were considered indicative of the saponin effect at the respective experimental concentration (Frías-Espericueta *et al.,* 2008[a]).

Statistical analysis

Data obtained for initial weight and length, as well as final weight and length, were subjected to Kruskal-Wallis statistical analysis followed by a Dunn's *post hoc* test. Gained weight, length increment, and specific growth rate data were analyzed using a one-way ANOVA with a Tukey *post hoc* test. Survival data were analyzed using a Kaplan-Meier test with a Holm-Sidak *post hoc* test. All analyses were conducted with a 0.05 significance level using Sigma Plot software.

Results and Discussion

Crustaceans detect food through chemoreceptors (aesthetascs), so diets must be attractive for rapid identification and consumption (Junnathulla *et al.,* 2021). In the present study, the feed with different inclusions was accepted by the shrimp; however, acceptance varied among the tested concentrations. The consumption reaction was immediate for the feed without saponin (0 %). Shrimp fed with the 2 % concentration exhibited a consumption reaction of < 2 seconds, while those fed with the 4 % concentration showed a reaction time of >4 seconds. Shrimp fed with the 6 % concentration had a reaction time of >15 seconds, and in some cases, the feed was not consumed. In this regard, Jumah *et al.* (2020) demonstrated in *P. monodon* that the inclusion of *Quillaja saponin*, at a concentration of 0.6 g/kg in the diet, affects attractiveness compared to food with lower inclusion levels. Similarly, Junnathulla *et al.* (2021) reported that the soybean meal inclusion in shrimp diets, which contains tannins and saponins, reduced feed attractiveness, leading to reduced consumption and response time. It has been reported that saponins can be

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toxic to certain organisms, and their toxicity is concentration-dependent (Francis *et al.,* 2002). *Oncorhynchus tshawytscha* and *Oncorhynchus mykiss* fed with diets supplemented with 1.5 and 3.0 g/Kg of *Quillaja saponin* showed reduced consumption, growth suppression, and digestive tract damage (Bureau *et al.,* 1998).

In this research it was observed that feed with 6 % saponin presented higher fungal growth when kept without adequate refrigeration, the high-purity saponin used for this study turned out to be highly hygroscopic (Figure 1).

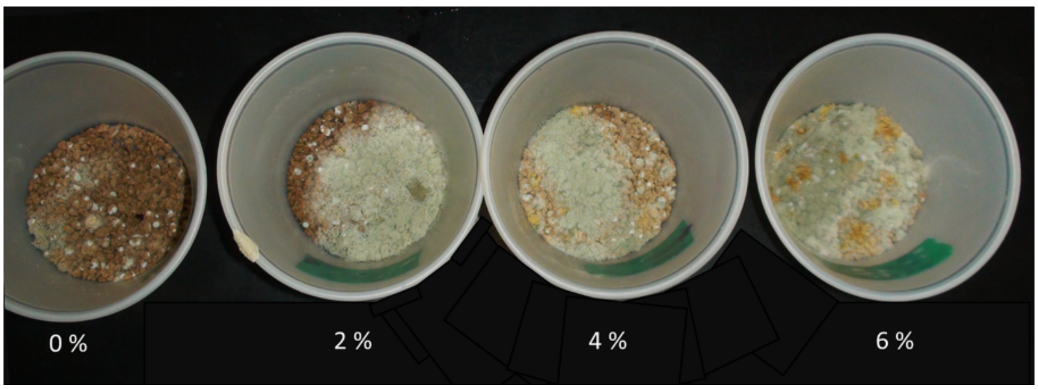

Figure 1. Fungal growth in specialized shrimp feed with different concentrations of saponins included (0 %, 2 %, 4 % and 6 %).

According to the obtained results, the saponin addition to the shrimp diet did not influence the physicochemical parameters of the water under open flow conditions, as no significant variations were observed in the physicochemical parameters evaluated, and the values were within the recommended range for shrimp (Instituto Nacional de Pesca, 2018; https://www.gob.mx/inapesca/acciones-y-programas/acuaculturacamaron-blanco-del-pacifico, March 2023). These results were expected due to the constant water flow, which allowed for a total replacement every 7.5 hours.

Weight and length data at the beginning (IW and IS, respectively) and at the end (FW and FS, respectively) of the evaluation were analyzed using a Kruskal-Wallis test (α=0.05), which indicated that the initial data did not show significant differences (*p>0.05*) between concentrations at the beginning of the test, but exhibited significant differences at the end (*p < 0.05*) (Table 1). Data on weight gain (WG), length increment (SI), and specific growth rate (SGR % d-1) were analyzed using one-way ANOVA followed by Tukey's *post hoc* analysis. The analysis of these results (Table 1) suggests that shrimp fed without saponin (0 %) exhibited higher growth in both weight and length (WG=0.82 g and SI=20.98 mm) compared to the 2%, 4%, and 6% saponin treatments. This finding aligns with previous studies by Jannathulla *et al.* (2018) with *P. vannamei* and Chen *et al.* (2011) with Japanese flounder, who reported a negative effect on the growth parameters of the organisms as saponin concentration increased, even when

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dealing with saponin concentrations lower than those used in the present study (0.8 to 6.5 g/Kg). These results exhibit the tolerance and resilience of organisms to saponin concentrations of up to 20 g/Kg, without showing significantly different effects on survival compared to the control. In contrast, Huang *et al.* (2022) reported that *P. vannamei* juveniles treated weekly with different concentrations (0 to 5 mL/m^3) of bioactive compounds based on saponins in the culture water showed better growth than untreated shrimp.

Table 1. Results of juvenile white shrimp *Penaeus vannamei* fed four concentrations of saponin (Conc.) for 28 days.

Conc	IW (g)	FW (g)	WG (g/d)	IS (mm)	FS (mm)	SI (mm/d)	SGR (%/d)	S (%)
0 %	0.23±0.01	1.05±0.43^c	0.82±0.10^c	35.06±4.79	56.04±8.19^c	20.98±1.21^c	5.44±0.31^c	86.25±4.79^c
2 %	0.24±0.01	0.78±0.38^b	0.54±0.06^b	35.54±5.01	50.96±7.83^b	15.42±1.48^b	4.19±0.33^b	86.25±2.50^c
4 %	0.24±0.01	0.61±0.36^a	0.37±0.05^a	35.55±4.35	46.96±8.15^a	11.41±1.08^a	3.36±0.28^a	52.50±6.12^b
6 %	0.24±0.01	0.71±0.33^{ab}	0.47±0.10^{ab}	35.48±5.58	50.73±7.38^{ab}	15.25±1.56^b	3.82±0.48^{ab}	18.13±1.25^a

IW=initial weight; WG=weight gained; SI=increase in size; FS=final size; SGR=specific growth rate; S=survival. 0 % = without saponin inclusion; 2 % = with 2 % saponin inclusion; 4 % = with 4% saponin inclusion and 6 % = with 6 % saponin inclusion. Letters indicate homogeneous concentrations obtained in the corresponding *post hoc* analyses (Dunn/Tukey/Holm-Sidak) where those with no significant differences between them ($p > 0.05$) share equal letters.

In the present study, an effect on shrimp survivability was observed depending on saponin concentration (Table 1), with significant differences ($p < 0.05$) distinguished in shrimp fed 0 % and 2 % compared to other concentrations. The results indicate that survival decreased as saponin inclusion increased (0 % > 2 % > 4 % > 6 %), with the 6 % concentration exhibiting the highest mortality between days 8 and 14 of feeding, reaching 25 % mortality by day 11. Similar results were observed for the 4 % concentration by day 22. These two concentrations demonstrated markedly negative effects compared to the 0 % and 2 % concentrations. On day 28 of feeding, survival rates were 18.13 % for the 6 % concentration, and 52.50 % for the 4 % concentration, while for the 2 % and 0 % concentrations, they were 86.25 % (see Figure 2).

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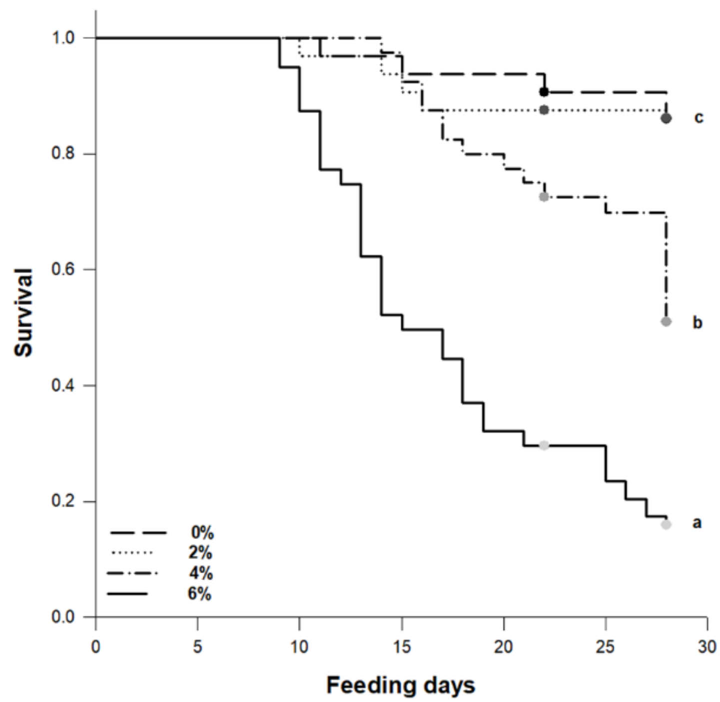

Figure 2. Kaplan-Meier survival curve in *Penaeus vannamei* juveniles fed with different saponin concentrations (0 %; 2 %; 4 %, and 6 %).

Letters indicate homogeneous concentrations obtained in the *post hoc* analysis (Holm-Sidak) where those with no significant differences between them ($p > 0.05$) share equal letters.

Histopathological evaluation

Before beginning saponin feeding, shrimp were observed to be healthy with a normal appearance and behavior, showing no disease signs, a condition that was confirmed by initial PCR analysis and histological evidence (Figure 3). No histopathological changes were observed in tissues or organs. All analyzed shrimp exhibited digestive tracts without damage to the stomach, intestine, or hepatopancreas (Figures 3a and b). The hepatopancreas appeared normal, without tubular epithelium damage, with an evident abundance of secretory vacuoles in B cells and lipid vacuoles in R cells (Figure 3b). The gills of all shrimp showed normal epithelium, except for 3 out of 17 shrimp evaluated, which exhibited focal necrosis in secondary filaments.

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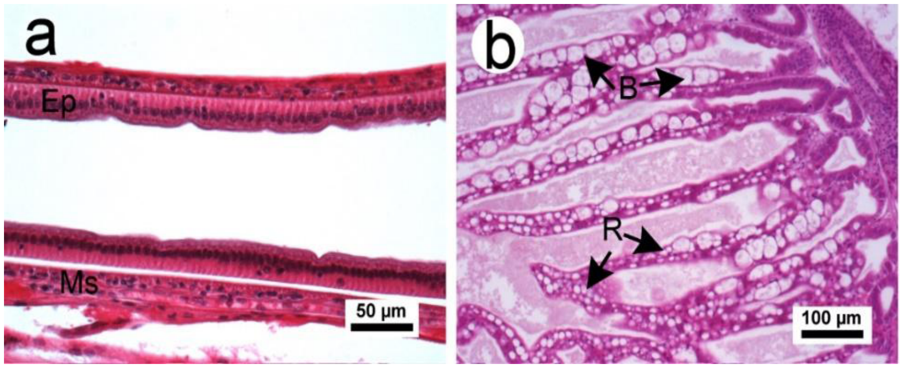

Figure 3. Microphotographs of juvenile white shrimp *Penaeus vannamei* before being fed with different saponin concentrations included in the food.

Middle intestine with epithelium (Ep) and muscular (Ms) layers without apparent pathological (a) and hepatopancreatic tubules with normal microanatomy. Hematoxylin-eosin-phloxin staining.

The effects of saponins on animal health depend on the species, compound origin, and exposure route (Couto *et al.,* 2015). Meanwhile, the effect of saponins on the intestinal tract of animals has been attributed to increased permeability in intestinal cell membranes (Francis *et al.,* 2005). In the present study, shrimp fed with added 4 % and 6 % of saponin developed severe midgut enteritis, while hepatopancreatic tissue (Figure 4) and zootechnical parameters, including survival at the end of the experiment, were affected. These results are consistent with Krogdahl *et al. (*2015), who reported that diets with concentrations between 2 and 10 g/Kg of soy saponins caused enteritis in salmonids, altering digestive and immunological functions, a condition closely related to obtained observations made in the present study. However, shrimp seem to exhibit greater tolerance to saponin inclusion in the diet compared to fish (Couto et al., 2015; Krogdahl *et al.,* 2015; Yasir *et al.,* 2021). From day 22, shrimp fed saponins (2 %, 4 %, and 6 %) showed damage to the intestine and hepatopancreas, including severe development of hemocytic enteritis in the midgut and tubular epithelium atrophy (Figure 4).

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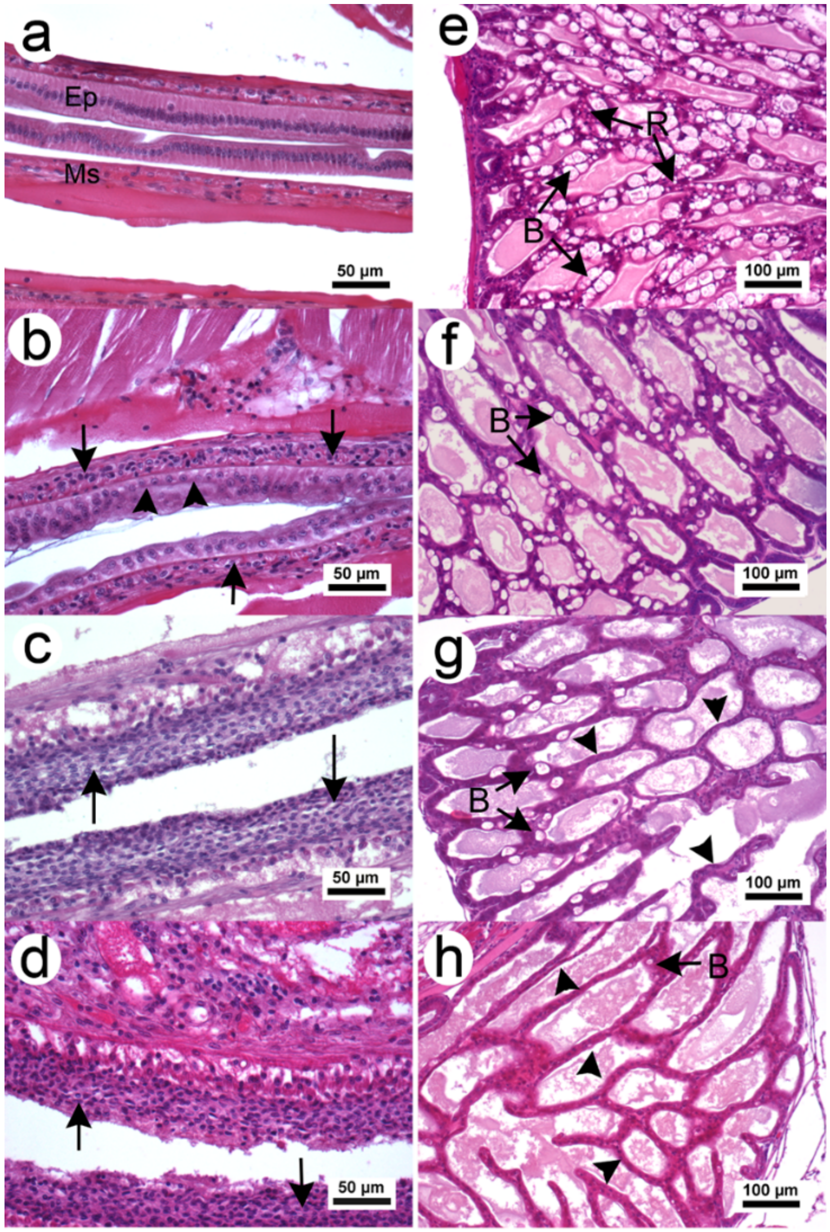

Figure 4. Microphotographs of the intestine (a – d) and hepatopancreas (e – h) of juvenile white shrimp *Penaeus vannamei* fed for 22 days with inclusions of 0 % (a, e), 2 % (b, f), 4 % (c, g) and 6 % (d, h). a.

Midgut without pathological changes in the epithelium (Ep) and muscle (Ms) layers; **b.** Intestine with mild hemocytic infiltration (arrow) and an epithelium with reduced size cells (arrowhead); **c – d**. Intestine with severe hemocytic enteritis damage (arrow); **e.** Normal hepatopancreatic tubules and abundant secretory vacuoles and lipids in B and R cells (arrows); **f.** Lipid-depleted tubules and secretory vacuoles in R and B cells (arrow); **g – h.** Hepatopancreas with tubular epithelium atrophied (arrowhead) and few or no secretory vacuoles and lipids. Hematoxylin-eosin-phloxin staining.

Shrimp fed without saponins (0 %) were observed without apparent pathological changes in the digestive tract (Figure 4a), and only 1 out of the 8 analyzed showed mild hemocytic infiltration. Intestinal damage was not severe with the inclusion of 2 % saponin compared to those fed 4 % and 6 % saponin. However, 3 out of the 8 analyzed showed incipient signs of enteritis, such as hemocytic infiltration in the muscle layer with slight modification in the intestinal epithelium height (Figure 4b). Hemocytic enteritis was observed in 5 out of the 8 and 7 out of the 8 shrimp analyzed at 4 % and 6 % concentrations, respectively (Figure 4c, d). These results differ from those reported by Huang *et al.* (2022), who observed that the addition of saponin-based extracts in the water did not significantly influence the structures of the digestive tract in shrimps. However, the thickness of the muscle layers and the height of the intestinal villi tended to be greater when compared to the control, possibly due to the selected administration route.

Although exposure to saponins has been associated with inflammatory processes in the digestive tract of fish (Bureau *et al.,* 1998; Francis *et al.,* 2002; Krogdahl *et al.,* 2015), currently, there are no reports on the development of enteritis in *P. vannamei* shrimp fed diets containing saponins. Chen *et al.* (2011) observed negative effects on the intestinal tract of Japanese flounder fed concentrations of 3.2 and 6.4 g/Kg of saponin, finding decreased intestinal goblet cells, as well as hyperplasia of intestinal villi and decreased lamina propria in the intestinal mucosa as saponin concentrations increased. The negative effects of saponins are attributed to their astringent and irritant properties, which increase the permeability of intestinal cells, facilitating the entry of antigens and toxins (Böttger & Melzing, 2013; Francis *et al.,* 2002; Knudsen *et al.,* 2008). Alterations in the intestinal mucosa, such as increased intraepithelial lymphocytes and supranuclear vacuoles, were associated with sublethal effects by the inclusion of 1 and 2 g/Kg saponin in a diet for juvenile gilt-head bream (*Sparus aurata)*, potentially compromising function and/or predisposing to the entry of opportunistic pathogens (Couto *et al.,* 2014). Recently, Huang *et al.* (2022) observed modifications in the tissue architecture of shrimp intestines cultured in waters treated with saponin products, such as the presence of supranuclear vacuoles and increased undulations of the intestinal epithelium, findings that differ from a healthy intestinal tract (Bell & Lightner, 1988). Similar results were obtained in this study, including incipient signs of inflammation in the submucosa and sections of epithelium with shape modifications, such as reduced epithelial cell height in shrimp fed 2 % saponin, suggesting that these modifications could be associated with sublethal effects as mechanisms to maintain intestinal homeostasis.

In the present study, it was observed that the hepatopancreatic epithelium of shrimp exhibited affectation in its microanatomy, presenting damaged tubules with pyknotic cells, lipid reduction, and reduced secretory vacuoles in epithelial cells. Damage in the hepatopancreas and the number of affected shrimp increased in direct relation to the increase in the concentration of included saponin (Figure 4e - h). Shrimp fed without saponin (0 %) showed a normal tubular epithelium (Figure 4e), with abundant secretory vacuoles in B cells and lipid vacuoles in R cells, as observed in 6 out of the 8 analyzed shrimp. Only 2 out of the 8 shrimp presented minimal reductions in vacuoles in R cells. Shrimp fed 2 % saponin presented a tubular epithelium with reduced numbers of secretory vacuoles in B cells and lipid vacuoles in R cells, as observed in all 8 of the analyzed shrimp (Figure 4f). In comparison, shrimp fed 4 % and 6 % saponin developed severe atrophy of the tubular epithelium, and vacuoles in R and B cells were absent in 2 and 5

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out of the 8 analyzed shrimp, respectively (Figure 4g, h). Lipid depletion related to the saponins presence has already been documented in studies such as that by Jannathulla *et al.* (2018), who observed a decrease in cholesterol and triglycerides in the hemolymph related to the increase in the saponin percentage. These cell damage and lipid decrease are associated with the great capacity of saponins to alter cell membranes, interacting with cholesterol and, in some cases, causing alterations in cellular organelles (Schulz, 1990; Stewart *et al.,* 2016).

At 28 days (end of the experiment), the treatments with 4 % and 6 % saponin caused shrimp to exhibit histopathological alterations similar to those found on day 22 of feeding, including intestinal damage manifested as hemocytic enteritis and atrophy of the tubular epithelium in the hepatopancreas (Figure 5a, b). Shrimp fed with 2 % and 0 % saponin showed midgut without epithelial damage in all 8 of the analyzed shrimps. However, 2 out of the 8 shrimp fed with 2 % saponin and 1 out of the 8 shrimp fed with 0 % saponin showed a slight reduction of lipid vacuoles at the hepatopancreas level and focal tubular necrosis.

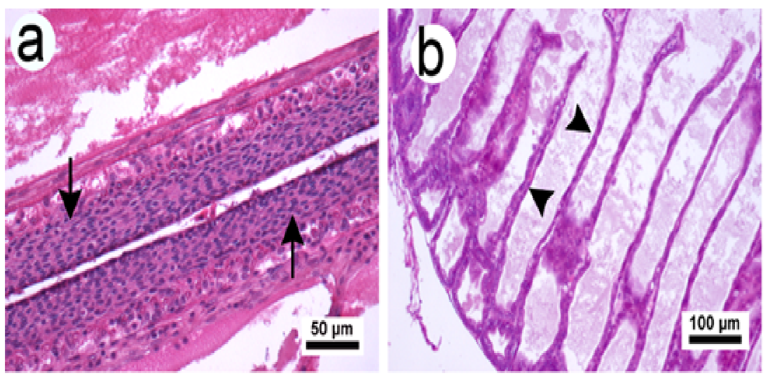

Figure 5. Microphotographs of white shrimp juvenile *Penaeus vannamei* after feeding period (28 days).

Intestine with severe hemocytic enteritis (a) shrimp fed 6 % and hepatopancreas (b) with atrophied tubular epithelium and scared secretor vacuoles and lipidic cells at day 28. Hematoxylin-eosin-phloxin staining.

In addition, it is important to consider that shrimp exposed to saponin-based compounds and pure saponin extracts added to the culture water also showed modifications in the microanatomy of the hepatopancreas, including a lower amount of reserve vacuoles (lipid vacuoles in R cells) and secretory vacuoles in B cells (Huang *et al.,* 2022), as well as detachment of the basal lamina, degradation of hepatopancreatic tubules, and decreased exoskeleton roughness (Nagesh *et al.,* 1999). This highlights the importance of further research to evaluate the sublethal effects of saponin on digestive tract microanatomy. Exposure to pathogens or toxic agents, including heavy metals exposure, has been implicated in causing hemocytic enteritis in shrimp (Lightner, 1996; Frías-Espericueta *et al.,* 2008[b]). However, its pathogenesis remains unclear.

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Conclusions

In the present study, shrimp fed with saponin developed severe hemocytic enteritis and damage to the hepatopancreas directly related to the tested concentration. These results underscore the necessity for further research focused on evaluating the related pathogenic mechanisms. It is important to consider that the inclusion of ingredients in foods should be thoroughly evaluated, considering aspects such as the effect of the product on the organism and water, as well as the impact it may have on the physicochemical and organoleptic properties of the diet.

Author contribution

Conceptualization of work, PCAC; methodology development, LOR, TFEA; software management, PCAC, LOR; experimental validation, PCAC, TFEA, LOR, PGDA; analysis of results, PCAC, TFEA, LOR, PGDA; Data management, PCAC, TFEA, LOR, PGDA; manuscript writing and preparation PCAC, TFEA, LOR, PGDA; writing, revising and editing, PCAC, LOR, PGDA; project manager and fund acquisition, PCAC.

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Own resources generated by the Laboratorio Nutrición, Alimentación y Alimento vivo alternativo CIAD-Mazatlán.

Ethical statements

The authors confirm that all procedures were performed following the relevant Mexican animal welfare law (NOM-062-ZOO-1999); although these guidelines do not include shrimp, we apply the same principles regarding animal welfare and care for other species."

Statement of Informed Consent

Not applicable.

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Conflict of interest

The authors declare that they have no conflicts of interest.

References

Abdel-Latif, H.M.R., Yilmaz, S., & Kucharczyk, D. (2023). Editorial: Functionality and applications of phytochemicals in aquaculture nutrition. *Frontier in Veterinary Science*, 10. https://doi.org/10.3389/fvets.2023.1218542

Akbary, P., Ajdari, A., & Ajang, B. (2023). Growth, survival, nutritional value and phytochemical, and antioxidant state of *Litopenaeus vannamei* shrimp fed with premix extract of brown *Sargassum ilicifolium*, *Nizimuddinia zanardini*, *Cystoseira indica*, and *Padina australis* macroalgae. *Aquaculture International*, 31, 681–701.https://doi.org/10.1007/s10499-022-00994-5

Alday-Sanz, V., Brock, J., Flegel, T.W., Mcintosh, R., Bondad-Reantaso, M.G., Salazar, M., & Subasinghe, R. (2018). Facts, truths and myths about SPF shrimp in Aquaculture. *Reviews in Aquaculture*, 12(1), 76-84.https://doi.org/10.1111/raq.12305

Bell, T., & Lightner, D.V. (1988). *A handbook of normal penaeid shrimp histology*. Baton Rouge, La.:World Aquaculture Society.

Böttger, S., & Melzig, M.F. (2013). The influence of saponins on cell membrane cholesterol. *Bioorganic & Medicinal Chemistry*, 21(22), 7118-7124.https://doi.org/10.1016/j.bmc.2013.09.008

Bureau, D.P., Harris, A.M., & Cho, C.Y. (1998). The effects of purified alcohol extracts from soy products on feed intake and growth of chinook salmon (*Oncorhynchus tshawytscha* and rainbow trout (*Oncorhynchus mykiss*) *Aquaculture*, 161(1-4),27-43. https://doi.org/10.1016/S0044-8486(97)00254-8

Chen, W., Ai, Q., Mai, K., Xu, W., Liufu, Z., Zhang, W., & Cai, Y. (2011). Effects of dietary soybean saponins on feed intake, growth performance, digestibility and intestinal structure in juvenile Japanese flounder (*Paralichthys olivaceus*). *Aquaculture*, 318(1–2), 95-100. https://doi.org/10.1016/j.aquaculture.2011.04.050

Couto, A., Kortner, T.M., Penn, M., Bakke, A.M., Krogdahl, Å., & Oliva-Teles, A. (2014). Effects of dietary phytosterols and soy saponins on growth, feed utilization efficiency and intestinal integrity of gilthead sea bream (*Sparus aurata*) juveniles. *Aquaculture*, 432, 295-303.https://doi.org/10.1016/j.aquaculture.2014.05.009

Couto, A., Kortner, T.M., Penn, M., Bakke, A.M., Krogdahl, Å., & Oliva-Teles, A. (2015). Dietary saponins and phytosterols do not affect growth, intestinal morphology and immune response of on-growing European sea bass (*Dicentrarchus labrax*). *Aquaculture Nutrition*, 21(6), 970-982. https://doi.org/10.1111/anu.12220

Dangtip, S., Sirikharin, R., Sanguanrut, P., Thitamadee, S., Sritunyalucksana, K., Taengchaiyaphum, S., Mavichak, R., Proespraiwong, P., & Flegel, T.W. (2015). AP4 method for two-tube nested PCR detection of AHPND isolates of *Vibrio parahaemolyticus*. *Aquaculture Reports*, 2, 158–162. https://doi.org/10.1016/j.aqrep.2015.10.002

Revista Bio ciencias
ISSN 2007-3380

El-Naby, A.S.A., Eid, A.E., Gaafar, A.Y., Sharawy, Z., Khattaby, A.A., El-sharawy M.S., & El Asely, A.M. (2023). Overall evaluation of the replacement of fermented soybean to fish meal in juvenile white shrimp, *Litopenaeus vannamei* diet: growth, health status, and hepatopancreas histomorphology. *Aquaculture International*. https://doi.org/10.1007/s10499-023-01234-0

Flegel, T.W. (2019). A future vision for disease control in shrimp aquaculture. *Journal of the World Aquaculture Society*, 50(2), 249-266.https://doi.org/10.1111/jwas.12589

Francis, G., Kerem, Z., Makkar, H.P.S., & Becker, K. (2002). The biological action of saponins in animal systems: A review. *British Journal of Nutrition*, 88(6), 587-605. https://doi.org/10.1079/BJN2002725

Francis, G., Makkar, H.P.S., & Becker, K. (2005). *Quillaja saponins*-a natural growth promoter for fish. *Animal feed science and technology*, 121(1-2), 147-157. https://doi.org/10.1016/j.anifeedsci.2005.02.015

Frías-Espericueta, M. G., Castro-Longoria, R., Barrón-Gallardo, G.J., Osuna-López, J.I., Abad-Rosales, S.M., Páez-Osuna, F., & Voltolina, D. (2008[a]). Histological changes and survival of *Litopenaeus vannamei* juveniles with different copper concentrations. *Aquaculture*, 278(1-4), 97-100. https://doi.org/10.1016/j.aquaculture.2008.03.008

Frías-Espericueta, M.G., Abad-Rosales, S., Nevárez-Velázquez, A.C., Osuna-López, I., Páez-Osuna, F., Lozano-Olvera, R., & Voltolina, D. (2008[b]). Histological effects of a combination of heavy metals on Pacific white shrimp *Litopenaeus vannamei* juveniles. *Aquatic toxicology*, 89(3), 152-157. https://doi.org/10.1016/j.aquatox.2008.06.010

Gopan, A., Lalappan, S., Varghese, T., Maiti, M.K., & Peter, R.M. (2020). Anti-Nutritional Factors in Plant-Based Aquafeed Ingredients: Effects on Fish and Amelioration Strategies Bioscience. *Biotechnology Research Communications*, 13(12), 01-09.

Han, J.E., Kim, J.E., Jo, H., Eun, J.S., Lee, C., Kim, J.H., Lee K.J., & Kim, J.W. (2019). Increased susceptibility of white spot syndrome virus-exposed *Penaeus vannamei* to *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease. *Aquaculture*, 512, 734333. https://doi.org/10.1016/j.aquaculture.2019.734333

Huang K.C., Lee J.W., Shiu Y.L., Ballantyne R., & Liu C.H. (2022). Micro-Aid Liquid 10 Promotes Growth Performance and Health Status of White Shrimp, *Litopenaeus vannamei*. *Journal of Marine Science and Engineering*, 10(1), 49. https://doi.org/10.3390/jmse10010049

Jannathulla, R., Dayal, J. S., Vasanthakumar, D., Ambasankar, K., & Muralidhar, M. (2018). Effect of fungal fermentation on apparent digestibility coefficient for dry matter, crude protein and amino acids of various plant protein sources in *Penaeus vannamei*. *Aquaculture Nutrition*, 24(4), 1318-1329. https://doi.org/10.1111/anu.12669

Jannathulla, R., Sravanthi, O., Khan, H.I., Moomeen, H.S., Gomathi, A., & Dayal, J.S. (2021). Chemoattractants: Their essentiality and efficacy in shrimp aquaculture. *Indian Journal of Fisheries*, 68(1), 151-159. https://doi.org/10.21077/ijf.2021.68.1.95994-20

Kesselring, J., Gruber, C., Standen, S., & Wein, S. (2021). Effect of a phytogenic feed additive on the growth performance and immunity of Pacific white leg shrimp, *Litopenaeus vannamei*, fed a low fishmeal diet. *Journal of the World Aquaculture*, 52(2), 303-315. https://doi.org/10.1111/jwas.12739

Jumah, Y.U., Tumbokon, B.L., & Serrano, A.E. (2020). Dietary *Quillaja saponin* improves growth and resistance against acute hyposalinity shock in the black tiger shrimp *Penaeus monodon* post larvae. *Israeli Journal of Aquaculture-Bamidgeh*, 72, 1-13. https://doi.org/10.46989/

Revista Bio ciencias
ISSN 2007-3380

IJA.72.2020.1227608

Knudsen, D., Jutfelt, F., Sundh, H., Sundell, K., Koppe, W., & Frøkiaer, H. (2008). Dietary soya saponins increase gut permeability and play a key role in the onset of soya bean induced enteritis in Atlantic salmon (*Salmo salar L.*). *British Journal of Nutrition*, 100(1), 120–129. https://doi.org/10.1017/S0007114507886338

Krogdahl, Å., Gajardo, K., Kortner, T.M., Penn, M., Gu, M., Berge, G.M., & Bakke, A.M. (2015). Soya saponins induce enteritis in Atlantic salmon (*Salmo salar* L.). *Journal of agricultural and food chemistry*, 63(15), 3887-3902. https://doi.org/10.1021/jf506242t

Li, E., Chen, L., Zeng, C., Chen, X., Yu, N., Lai, Q., & Qin, J.G. (2007). Growth, body composition, respiration and ambient ammonia nitrogen tolerance of the juvenile white shrimp, *Litopenaeus vannamei*, at different salinities. *Aquaculture*, 265(1-4), 385–390. https://doi.org/10.1016/j.aquaculture.2007.02.018

Lightner, D. V. (1996). A handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp. World Aquaculture Society, Baton Rouge, Louisiana.

Loya-Rodríguez, M., Palacios-González, D.A., Lozano-Olvera, R., Martínez-Rodríguez, I.E., & Puello-Cruz, A.C. (2023). Benzoic Acid Inclusion Effects on Health Status and Growth Performance of Juvenile Pacific White Shrimp *Penaeus vannamei*. *North American Journal of Aquaculture*, 85(2), 188-199. https://doi.org/10.1002/naaq.10286

Nagesh, T.S., Jayabalan, N., Mohan, C.V., Annappaswamy, T.S., & Anil, T.M. (1999). Survival and histological alterations in juvenile tiger shrimps exposed to saponin. *Aquaculture International*, 7, 159-167. https://doi.org/10.1023/A:1009239319468

Pan, L.Q., Zhang, L.J., & Liu, H.Y. (2007). Effects of salinity and pH on ion-transport enzyme activities, survival and growth of *Litopenaeus vannamei* postlarvae. *Aquaculture*, 273(4), 711–720. https://doi.org/10.1016/j.aquaculture.2007.07.218

Servin Arce K., de Souza Valente C., do Vale Pereira G., Shapira B., & Davies S.J. (2021). Modulation of the gut microbiota of Pacific white shrimp (*Penaeus vannamei* Boone, 1931) by dietary inclusion of a functional yeast cell wall-based additive. *Aquaculture Nutrition*, 27, 1114–1127. https://doi.org/10.1111/anu.13252

Sharawy, Z.Z., Ashour, M., Labena, A., Alsaqufi, A.S., Mansour, A.T., & Abbas, E.M. (2022). Effects of dietary *Arthrospira platensis* nanoparticles on growth performance, feed utilization, and growth-related gene expression of Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, 551, 737905. https://doi.org/10.1016/j.aquaculture.2022.737905

Schulz, I. (1990). Permeabilizing cells: Some methods and applications for the study of intracellular processes. *Methods in Enzymology, Academic Press*. 192, 280-300. https://doi.org/10.1016/0076-6879(90)92077-Q

Stadtlander, T., Khalil, W.K.B., Focken, U., & Becker, K. (2013). Effects of low and medium levels of red alga nori (*Porphyra yezoensis* Ueda) in the diets on growth, feed utilization and metabolism in intensively fed Nile Tilapia, *Oreochromis niloticus* (L.). *Aquaculture Nutrition*, 19, 64–73. https://doi.org/10.1111/j.1365-2095.2012.00940.x

Stewart, M.P., Sharei, A., Ding, X., Sahay G., Langer R., & Jensen K.F. (2016). *In vitro* and *ex vivo* strategies for intracellular delivery. *Nature* 538, 183–192. https://doi.org/10.1038/nature19764

Tacon, A. G. (2017). Biosecure shrimp feeds and feeding practices: guidelines for future development. *Journal of the World Aquaculture Society*, 48(3), 381-392. https://doi.

Revista Bio ciencias ISSN 2007-3380

org/10.1111/jwas.12406
Wang, Y., Jia, X., Guo, Z., Li, L., Liu, T., Zhang, P., & Liu, H. (2022). Effect of dietary soybean saponin Bb on the growth performance, intestinal nutrient absorption, morphology, microbiota, and immune response in juvenile Chinese soft-shelled turtle (*Pelodiscus sinensis*). *Frontiers in Immunology*, 13,1093567. https://doi.org/10.3389/fimmu.2022.1093567
WOAH – World Organization for Animal Health. (2021). Acute hepatopancreatic necrosis disease in: Manual of Diagnostic Tests for Aquatics Animals 2021. World Organization for Animal Health, Paris, France Available at: https://www.woah.org/fileadmin/Home/eng/Health_standards/aahm/current/2.2.01_AHPND.pdf
Yasir, I., Tresnati, J., Aprianto, R., Yanti, A., Bestari, A.D., & Tuwo, A. (2021). Effect of different doses of saponins and salinity on giant tiger prawn *Penaeus monodon* and Nile tilapia *Oreochromis niloticus*. In IOP Conference Series: Earth and Environmental Science (Vol. 763, No. 1, p. 012021). IOP Publishing. https://doi.org/10.1088/1755-1315/763/1/012021