Dietary Resin Acids Oil Enhances Immunity, Stress Response, and Intestinal Integrity in Juvenile Red Tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*) Challenged with *Aeromonas hydrophila*

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ABSTRACT

The effects of dietary resin acid oil (RAO; Progres®, AB Vista, UK) on intestinal integrity, stress response, and immunity of red tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*) were evaluated during an *Aeromonas hydrophila* immersion challenge. The experiment followed a completely randomized design (CRD) with three treatments and three replicates. A total of 90 red tilapia (14.65–15.07 g) were randomly distributed into nine aquaria and fed diets containing RAO at 0 ppm (control), 350 ppm (R350), and 700 ppm (R700) three times daily for two weeks. Dietary RAO supplementation significantly reduced serum cortisol and markedly increased hemoglobin concentrations compared to the control ($p<0.05$). Hemoglobin levels in the control, R350, and R700 groups were 3.92±0.10, 4.29±0.02, and 4.38±0.10 g·dL^{-1}, respectively. Corresponding cortisol levels were 131.90±27.90, 64.74±3.99, and 63.55±8.66 µg·mL^{-1}, with RAO groups showing values less than half that of the control ($p<0.05$). Intestinal *Aeromonas* counts were significantly lower in R700 group (6.06±0.43 CFU·mL^{-1}) than in the control (7.20±0.09 CFU·mL^{-1}) and the R350 groups (7.02±0.14 CFU·mL^{-1}) ($p<0.05$). Histological analysis revealed that the RAO-fed fish exhibited longer intestinal villi and reduced tissue degeneration in both the intestine and liver under pathogen stress. These findings demonstrate that dietary RAO at 350–700 ppm improves the stress response and protects intestinal and hepatic tissues of red tilapia. Therefore, RAO shows promise as a natural alternative for disease management in aquaculture.

Keywords: *Aeromonas hydrophila*, Natural resin acid oil, Red tilapia, Stress conditions

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is one of the most widely cultivated aquaculture species worldwide, while hybrid red tilapia (*O. niloticus* × *O. mossambicus*) is predominantly cultured in Asia. They are naturally contaminated with the bacterium (*Aeromonas hydrophila*) in many of the places where they are farmed (Sirimanapong *et al*., 2018). *Aeromonas* is an aquatic bacterium belonging to the family Aeromonadaceae. This bacterium is a gram-negative, with straight or curved rods, and motile by polar flagella. These organisms have both fermentative and respiratory metabolism and are chemoorganotrophic. The oxidase reaction is positive. *Aeromonas* is an extremely uncommon cause of bacterial skin and soft tissue infection. *A. hydrophila* and other motile aeromonads are among the most abundant bacteria in freshwater to seawater environments worldwide and have been identified as occasional pathogens of of fish globally. Moreover, *A. hydrophila* has been reported as an environmental biomarker for TiLV-infected tilapia detection (Lu *et al*., 2021). In addition, bacterial infection has primary and secondary effects on immune response and disease resistance in fish.

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It is possible that inflammation and infection cause damage in red tilapia tissues, affecting the immune system. Thus, reducing these responses helps the fish effectively protect themselves against opportunistic pathogens.

In Thailand, the most valuable freshwater fish is the red tilapia, it is a hybrid fish species that holds significant economic value in aquaculture. Among freshwater species, red tilapia is the second most farmed fish worldwide and is an important high-value export. However, the production costs of red tilapia vary widely, due to climate and environmental conditions, resulting in sudden deaths or contagious diseases in the fish populations. To ensure successful farming and maximize production, it is crucial to understand the factors that can impact the health, immunity, and disease response of these juvenile fish (Montaser *et al.*, 2021). One such influential factor is stress. Stress in fish can arise from a variety of sources, including environmental changes, handling procedures, overcrowding, poor water quality, and fluctuations in temperature or salinity. These stressors can disrupt the delicate balance of physiological processes in fish, leading to significant impacts on their overall well-being and performance. The effects of stress on fish physiology have been extensively studied in various species, but there is still a need for specific research on red tilapia. Understanding how stress conditions affect the physiological responses of red tilapia juveniles is essential for implementing effective management strategies in aquaculture and ensuring the sustainable production of this valuable species.

Natural resin acids of Scots pine and Norway spruce are currently widely used as a class of feed additives for land animals and poultry, according to international research on growth performance and gut inflammation; broiler chickens and litter quality (Lipiński *et al*., 2021a), turkey (Lipiński *et al*., 2021b), piglet (Hasan *et al*., 2019; Shah Hasan *et al*., 2019; Uddin *et al*., 2021a). The resin acid component consists of abietic acid (33%), dehydroabietic acid (18%), pimaric acid (14%), including various minor resin acids (35%) and possess antibacterial properties (Roy *et al*., 2018a).

This study investigates the effects of dietary RAO supplementation on the physiological responses of juvenile red tilapia under *A. hydrophila*-induced stress, focusing on immunity, stress response, and intestinal integrity.

MATERIALS AND METHODS

Experimental design

The experiment was conducted using a completely randomized design (CRD) with three treatments and three replications: (a) Control: control diet without resin acid supplements (0 ppm), (b) R350: diet with resin acid at 350 ppm, and (c) R700: diet with resin acid at 700 ppm.

Animal and experimental procedures with Aeromonas immersion challenge stress

The experiment was carried out at the Nutrition and Aquafeed Laboratory, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Thailand, to demonstrate the impacts of RAO under *Aeromonas* immersion challenge stress. Healthy juvenile red tilapia originated from a local private farm, Ayutthaya province, Thailand. Fish were acclimated for one week on a control diet in a 1,000-L tank, then starved for 24 h before weighing. Ninety juveniles (14±0.50 g) were randomly distributed into nine 100-L aquaria (10 fish each) with 60 L of freshwater (0–1 ppt).

Fish were immersed in *A. hydrophila* suspension (1.07×10^9 CFU·mL^{-1}, 140 mL·aquarium^{-1}) to reach a final concentration of 2.5×10^6 CFU·mL^{-1} for two weeks. They were fed the experimental diets three times daily (9:00 a.m., 12:00 a.m., and 4:00 p.m.) at 1–2% body weight. Water temperature (28±2.4 °C), dissolved oxygen (5±0.4 mg·L^{-1}), and pH (7.5±0.3) were maintained with continuous aeration.

The experiment protocol was approved by the Kasetsart University Institutional Animal Care and Use Committee (IACUC approval number: ACKU61-FIS-040)

Diets preparation and composition

Experimental diets were formulated according to NRC nutrient requirements (National Academies of Sciences, 2011) to be isonitrogenous (32% crude protein) and isolipidic (6% crude lipid). The ingredients (Table 1) were ground to 250 µm, mixed, and pelleted (3 mm diameter). Pellets were subsequently dried at 80 °C for 8 h using a hot air blower, cooled, and stored in a plastic bag at room temperature until used. Proximate composition (moisture, protein, fat, fiber, ash, energy, calcium, and phosphorus) was evaluated following the Association of Official Analytical Chemists (AOAC, 2023) methods.

Resin acid oil (RAO) was prepared from 8% natural resin acid extract derived from Norway spruce (*Picea abies* L.) and Scots pine (*Pinus sylvestris* L.) which contained abietic acid (33%), dehydroabietic acid (18%), pimaric acid (14%), and minor resin acids (35%) (Roy *et al*., 2018b). The extract was mixed with fish oil and coated onto the basal diet to achieve concentrations of 0, 350, and 700 ppm in the control, R350, and R700 diets, respectively.

Data collection

After a two-week immersion challenge with *A. hydrophila*, the total number of surviving fish was recorded to calculate the survival rate, indicating the efficacy of RAO on *Aeromonas* resistance, fish health, immunity and stress response. Surviving fish were deprived of feed for 24 h, and three fish from each replicate were randomly selected and euthanized using clove oil (0.40 $mL \cdot L^{-1}$) as an anesthetic (Fernandes *et al*., 2016).

Table 1. Experimental diet formulation and proximate composition of red tilapia feeds (%).

Ingredients	**Control**	**R350**	**R700**
Fish meal	7.00	7.00	7.00
Poultry meal	10.00	10.00	10.00
De-oiled rice bran	8.00	8.00	8.00
Soyabean meal	35.00	35.00	35.00
Corn protein	10.00	10.00	10.00
Tapioca	24.50	24.50	24.50
Tuna fish oil	2.50	2.50	2.50
Soyabean oil	2.50	2.50	2.50
Vitamin-mineral premix	0.50	0.50	0.50
Total	**100.00**	**100.00**	**100.00**
Resin acid (top-up)	0.00	0.035	0.07
Proximate composition (AOAC, 2023)			
Moisture (%)	12.24	12.24	12.24
Protein (%)	32.12	32.12	32.12
Lipid (%)	6.38	6.38	6.38
Fiber (%)	3.12	3.12	3.12
Ash (%)	9.89	9.89	9.89
Energy ($Cal \cdot kg^{-1}$)	4,376.80	4,376.80	4,376.80
Phosphorous (%)	1.08	1.08	1.08
Calcium (%)	2.46	2.46	2.46
Nitrogen free extract (%)	36.25	36.25	36.25

Blood, serum, liver, and intestine samples were collected for biochemical, bacterial, and histopathological analyses. Blood samples were collected from the caudal vein. The first portion, collected in tubes containing ethylenediamine tetraacetic acid (EDTA), was used for hematological analysis. The remaining portion was centrifuged to obtain serum for biochemical and hormonal assays.

Fish health and immunity

Haematological analysis: Red blood cell (RBC) and white blood cell (WBC) counts were performed using a Neubauer hemocytometer. Haemoglobin concentration (Hb) was measured using the Drabkin or Cyanmethemoglobin method (Drabkin and Austin, 1932), while haematocrit (Htc) was determined by centrifugation following Morris *et al*. (2001).

Immunity variables: Serum protein concentration was measured using the Lowry method (Lowry *et al*., 1951). Immunoglobulin M (IgM) was determined by precipitating non-serum proteins with 12% polyethylene glycol and calculating IgM according to Wu *et al*. (2022). Lysozyme activity (LSZ) was measured using an enzymatic assay with *Micrococcus lysodeikticus* (Sigma-Aldrich, St. Louis, MO, USA) as the substrate.

Oxidative stress parameters: Superoxide dismutase (SOD) activity was analyzed using a SOD Determination Kit (Sigma-Aldrich, Buchs, Switzerland), and glutathione (GT) using an GT Determination Kit (Sigma-Aldrich, St.Loius, MO, USA). These methods have been validated and used in previous studies (Limwachirakhom *et al*., 2022).

Stress response: Heat shock protein (Hsp70), a key stress-related protein, was quantified using an ELISA kit (CSB-E16327Fh, Cusabio, USA). Serum insulin-like growth factor 1 (IGF-1) and cortisol, representing hormonal responses to stress, were also determined by ELISA (CSB-E12122Fh and CSB-E08487f, respectively; Cusabio, USA).

Aeromonas count from liver and intestine: Tissue samples from the liver and intestine were homogenized in sterile saline (0.45N) and spread plate on *Aeromonas* selection agar after serial dilution (10^{-1}, 10^{-3}, and 10^{-6}). Plates were incubated at 30 °C for 24–36 h, and colony-forming units ($CFU \cdot mL^{-1}$) were recorded (Vanamala *et al*., 2022).

Liver and Intestine morphology: After the two-week challenge, three surviving fish per aquarium were dissected using sterile scissors and forceps. Liver and intestine tissues were fixed in 10% buffered formalin, processed using standard histological procedures, dehydrated in ethanol, cleared with xylene, and embedded in paraffin. Transverse sections (6 µm thick) were cut and mounted onto glass slides. The sections were deparaffinized with xylene and stained with hematoxylin and eosin (H&E). Samples were examined under a light microscope equipped with Optika camera and Optika Lite Software (version 2.1). The experiment involved assessment of middle intestine damage, focusing on villus height (Montaser *et al*., 2021).

Intestinal tissue damage was assessed following Mbugani *et al.* (2022) (Table 2; Figure 1), based on villus height and histopathological grading. The degree of intestinal damage was classified as follows: ++++ (severe), +++ (moderate), ++ (mild), + (minimum), and - (normal), considering the structural integrity of the striated border, mucosa, submucosa, muscular layer, and overall tissue architecture.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics. Data are expressed as mean±standard deviation (SD). One-way analysis of variance (ANOVA) was used to test for differences among treatments, and Duncan's multiple range test was applied to identify significant differences at $p<0.05$. Regression analysis was also conducted to examine possible linear relationships between variables.

Table 2. Histopathological grading criteria for intestinal tissue damage in red tilapia.

Score	Description
-	Normal
+	Minimal occurrence
++	Mild occurrence
+++	Moderate occurrence
++++	Severe occurrence

Note: Modified from (Mbugani *et al*., 2022)

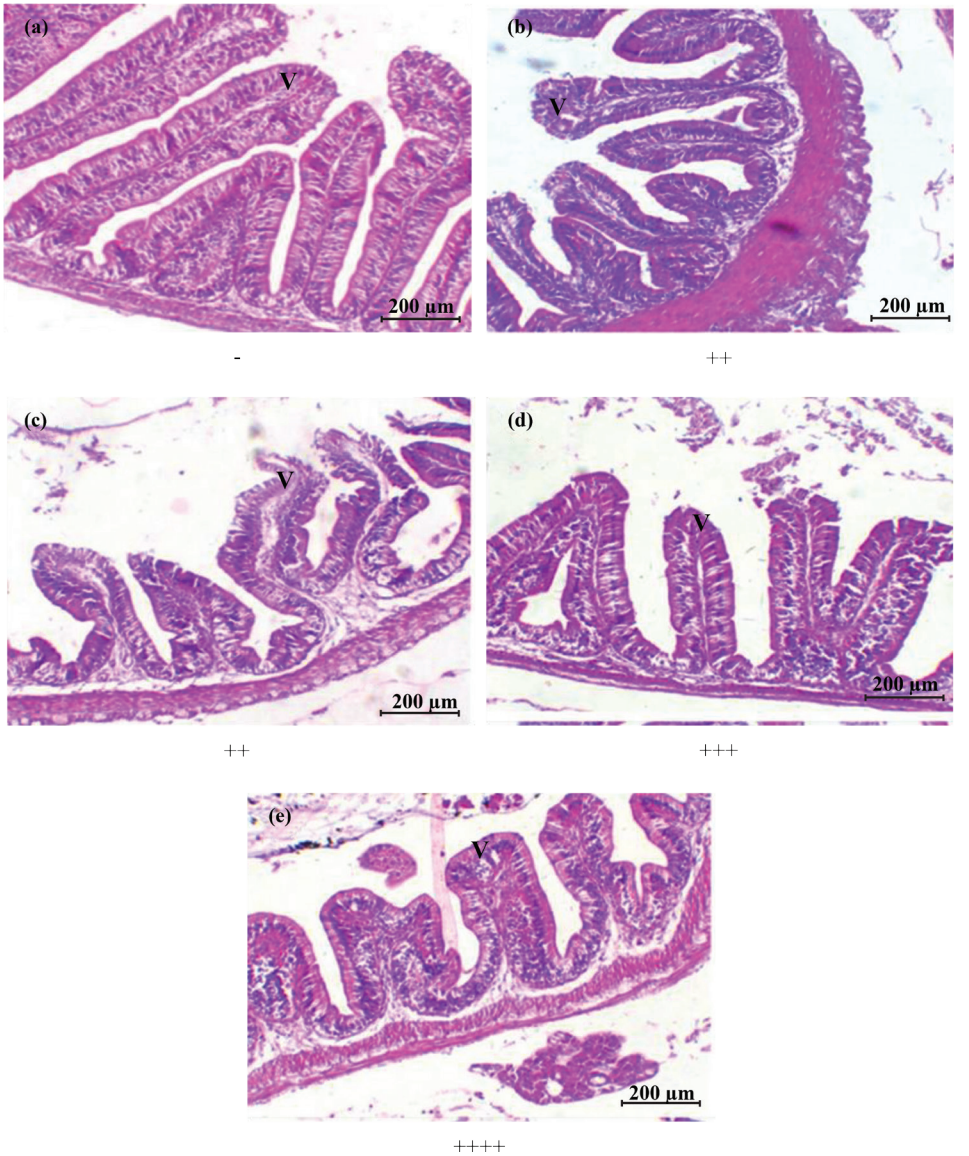

Figure 1. Representative micrographs showing histopathological alterations in the intestine of red tilapia after *Aeromonas* challenge: (a) normal (-), (b) minimal (+), (c) mild (++), (d) moderate (+++), and € severe (++++) damage.

RESULTS

The stress response of juvenile red tilapia after *A. hydrophila* immersion challenge is presented in Table 3. Survival rates did not differ significantly among treatments ($p \geq 0.05$), although fish fed RAO diets showed a numerical increase. The survival rates were 73.33±5.77%, 80.00±0.00%, and 86.67± 0.46% in the control, R350 and R700 groups, respectively.

Hematological variables, including red blood cell count, white blood cell count, and hematocrit, did not differ significantly among treatments ($p \geq 0.05$). However, hemoglobin levels were significantly higher in fish fed R350 and R700 diets compared with the control ($p < 0.05$), with values of 3.92±0.10, 4.27±0.02, and 4.38±0.10 g·dL^{-1}, respectively.

Immunity variables, serum protein, immunoglobulin M (IgM), and lysosome activity, did not differ significantly among groups ($p \geq 0.05$). Similarly, oxidative stress parameters (superoxide dismutase, glutathione, and HSP70) and IGF-1 levels were not significantly different ($p \geq 0.05$).

In contrast, cortisol levels were significantly higher in the control group and markly reduced in both the R350 and R700 groups ($p < 0.05$). Cortisol concentrations were 131.90± 27.90 in the control group, whereas the values were less than half in the R350 and R700 groups (64.74±3.99 and 63.55±8.66 μg·mL^{-1}, respectively). These findings indicate that dietary RAO improved hematological parameters and effectively reduced stress hormone levels in red tilapia under bacterial challenge.

Table 3. Hematological, immunological, antioxidant, and hormonal parameters of juvenile red tilapia (*Oreochromis* spp.) fed diets containing different levels of resin acid oil (RAO) after *Aeromonas hydrophila* immersion challenge for two weeks.

Immunity	**Control**	**R350**	**R700**	**p-value**
Initial body weight (g·fish^{-1})	14.91±0.92	14.73±0.68	14.87±1.27	0.971
Final body weight (g·fish^{-1})	15.78±0.91	16.16±0.93	16.66±0.46	0.453
Survival (%)	73.33±5.77	80.00±0.00	86.67±11.55	0.171
Hematological variables				
Red blood cell count (10^5 cell·mL^{-1})	3.57±0.25	4.20±0.62	4.17±0.21	0.181
White blood cell count (10^4 cell·mL^{-1})	1.17±0.58	1.67±0.29	0.83±0.29	0.115
Hematocrit (%)	22.00±2.65	23.33±3.21	28.33±2.08	0.061
Hemoglobin (g·dL^{-1})	3.92±0.10^{b}	4.27±0.02^{a}	4.38±0.10^{a}	0.001
Immunity variables				
Serum protein (mg·mL^{-1})	0.55±0.05	0.61±0.02	0.53±0.03	0.092
Immunoglobulin M (g·L^{-1})	0.22±0.04	0.25±0.03	0.28±0.03	0.188
Lysosome activity (mg·mL^{-1})	480.0±26.5	590.0±95.4	453.3±100.2	0.173
Antioxidative stress variables				
Superoxide dismutase (mg·mL^{-1})	1.78±0.09	1.94±0.21	2.05±0.13	0.159
Glutathione(mg·mL^{-1})	23.41±0.85	26.76±1.76	23.38±2.44	0.101
HSP70 (mg·mL^{-1})	58.77±6.72	53.00±5.33	51.76±7.02	0.415
Hormone				
IGF-1 (ng·mL^{-1})	1,135.0±34.0	1,258.1±137.5	1,254.8±49.4	0.220
Cortisol (ug·mL^{-1})	131.90±27.90^{a}	64.74±3.99^{b}	63.55±8.66^{b}	0.004

Note: Data are expressed as mean±SD (n = 3); Means in the same row with different superscript letters are significantly different ($p < 0.05$).

Aeromonas counts in red tilapia after the *A. hydrophila* challenge are presented in Table 4. The bacterial load in the liver was similar among treatments ($p \geq 0.05$), whereas the intestinal *Aeromonas* count was significantly lower in the R700 group (6.06±0.43 CFU·mL^{-1}) compared with the control (7.20±0.09 CFU·mL^{-1}) and R350 (7.02±0.14 CFU·mL^{-1}) ($p<0.05$). These results indicate that dietary RAO, particularly at 700 ppm, effectively inhibited intestinal pathogenic bacteria in red tilapia.

Histological observations of intestine structures are presented in Figure 2 (n = 27). The gut histomorphometry results showed a marked reduction in intestinal tissue damage, particularly longer villi, in fish fed RAO diets as summarized in Table 5. The improvement was associated with increasing dietary RAO levels, with the R700 group showing significant greater villus length ($p<0.05$). The degree of intestinal damage is presented in Table 6 and illustrated in Figure 2.

Histology analysis revealed microstructureal deterioration of the intestinal tissue following *Aeromonas* immersion challenge. In the R700 group (Figure 2a), the intestinal structures displayed minimal damage (+), with only slight peeling at the villus tips and an average villus length of 287.13± 59.66 µm. The R350 group (Figure 2b) exhibited mild damaged (++), characterized by localized necrosis, loose tissue, and partial villus loss, with villus length averaging 148.54±25.20 µm. In contrast, the control group (Figure 2c) showed moderate damage (+++), including exfoliation of the mucosal surface, thinning and shortening of villi, with an average villus length of 118.76± 12.61 µm.

These findings indicate that dietary RAO supplementation, particularly at 700 ppm, help preserve intestinal structures and maintain villus integrity in red tilapia under bacterial stress.

In Figure 3, the liver structure of fish in the R350 and R700 groups exhibited well-organized hepatocytes arranged in distinct cellular cords with clearly defined nuclei. Hepatocytes in the R350 groups showed mild vacuolization and reduced cytoplasmic transparency, indicating moderate lipid accumulation. In contrast, the R700 group exhibited more pronounced vacuolization, suggesting greater lipid and glycogen storage. Both RAO supplemented groups also showed markedly expanded sinusoidal gaps, indicating efficient hepatic perfusion. Cytoplasmic changes of hepatocytes of both treatment groups revealed vacuolization with nuclei displaced toward the cell periphery, consistent with enhanced nutrient storage. In comparison, the control group showed deformed hepatocyte structures and lower nutrient deposition than the R350 and the R700 groups.

Table 4. *Aeromonas hydrophilla* counts (log CFU·mL^{-1}) in the liver and intestine of juvenile red tilapia fed diets containing different levels of resin acid oil (RAO) after two weeks of bacterial challenge.

Tissue	Control	R350	R700	p-value
Liver	4.59±0.29	4.35±0.16	4.14±0.29	0.172
Intestine	7.20±0.09^{a}	7.02±0.14^{a}	6.06±0.43^{b}	0.004

Note: Data are expressed as mean±SD (n = 3); Means in the same row with different superscript letters are significantly different ($p<0.05$).

Table 5. Internal villus length (µm) of red tilapia fed diets containing different levels of resin acid oil (RAO) after *Aeromonas hydrophila* immersion challenge stress for two weeks.

Intestinal morphology	Control	R350 (ppm)	R700 (ppm)	*p*-value
Normal villi length, µm	118.76±12.61^{b}	148.54±25.20^{b}	287.13±59.66^{a}	< 0.001

Note: Data were expressed as mean±SD. Means in the same row with different superscript letters are significantly different ($p<0.05$).

Table 6. Histopathological grading of intestinal tissue damage in juvenile red tilapia fed diets containing different levels of resin acid oil (RAO) after *Aeromonas hydrophila* challenge for two weeks.

Treatment	Condition (damage grade)
Control	+++ (moderate)
R350	++ (mild)
R700	+ (minimal)

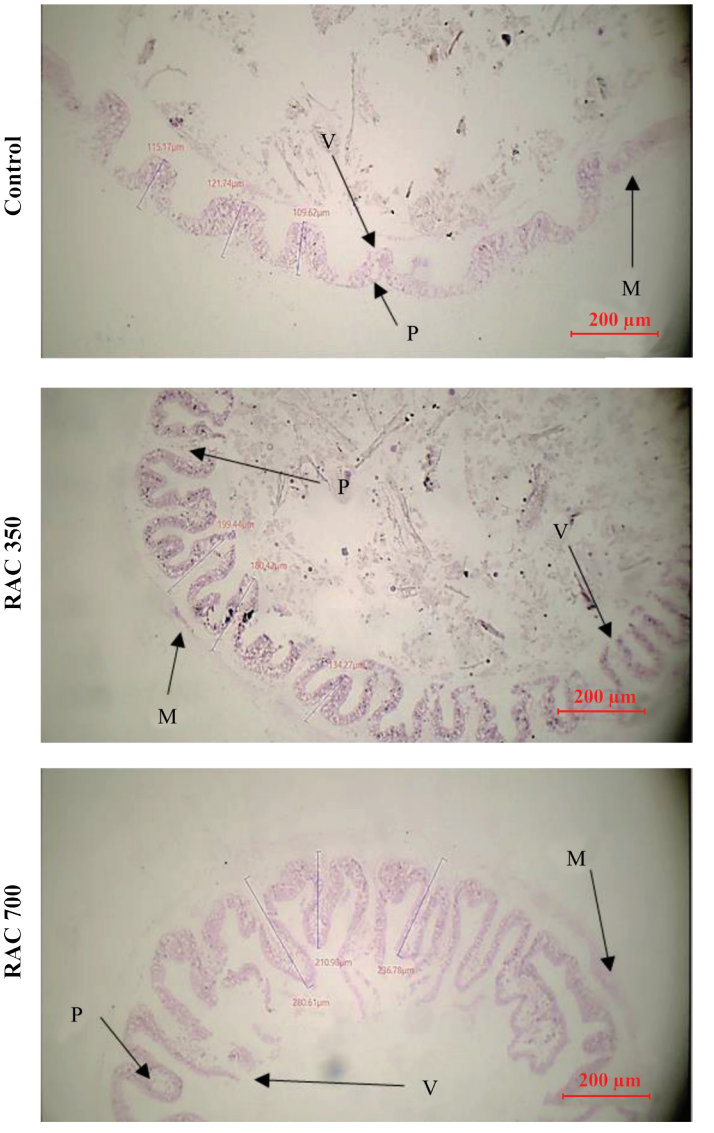

Figure 2. Light micrographs of the middle intestine of red tilapia fed diets containing resin acid oil (RAO) after *Aeromonas hydrophila* immersion challenge (H&E stain; scale bar = 200 µm; magnification ×100). (a) Control – moderate damage (+++), characterized by villus exfoliation and shortened villi; (b) R350 – mild damage (++), showing partial villus necrosis and loose tissue; (c) R700 – minimal damage (+), with intact villi and preserved epithelial structure.

Note: V = intestinal villi; P = lamina propria/sub mucosa; M = tunica muscularis

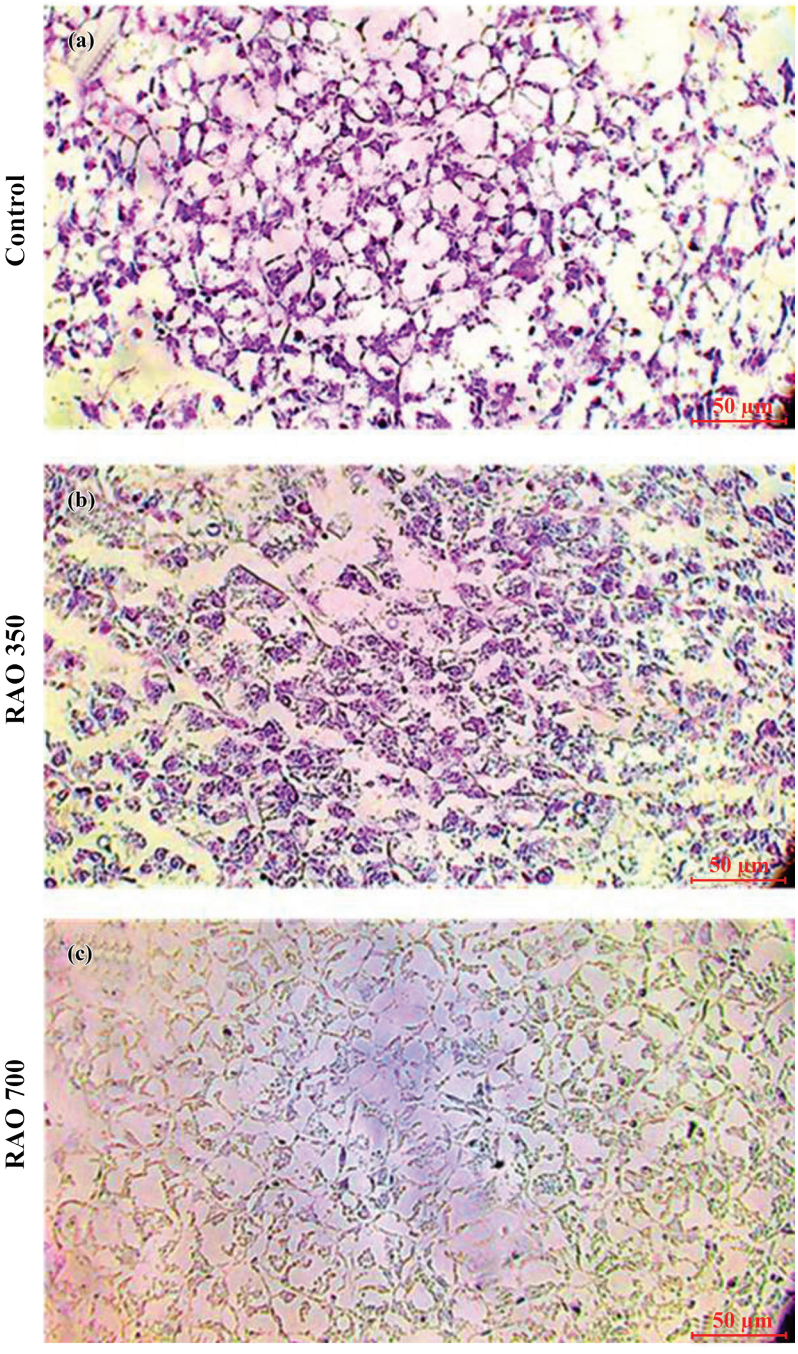

Figure 3. Light micrographs of liver tissue of red tilapia fed diets containing different levels of resin acid oil (RAO) after *Aeromonas hydrophila* immersion challenge (H&E stain; scale bar = 50 µm, magnification ×400). (a) Control – irregular hepatocyte arrangement with deformed cellular structures and reduced nutrient deposition; (b) R350 – mild vacuolization and decreased cytoplasmic transparency, indicating moderate lipid accumulation; (c) R700 – pronounced vacuolization and peripheral nuclear displacement, suggesting enhanced lipid and glycogen storage and improved hepatic perfusion.

DISCUSSION

The stress response of red tilapia fed diets supplemented with RAO at concentrations of 350–700 ppm was evaluated following an immersion challenge with *A. hydrophila*. Final body weight and survival rates did not differ significantly among treatments, likely due to reduced feed intake and the short experimental duration under stress conditions, which limited nutrient assimilation. However, the observed trend of increasing survival in fish fed RAO diets suggests that RAO primarily enhanced immunological and physiological resilience rather than growth performance.

Although red blood cell, white blood cell, and hematocrit values were not significantly affected, hemoglobin levels were significantly higher in fish fed R350 and R700 diets. This indicates improved oxygen-carrying capacity and possibly enhanced metabolic efficiency. Similar effects have been reported in juvenile rainbow trout (*Oncorhynchus mykiss*) fed diets containing bioactive additives (Kennedy *et al*., 1995; Landman *et al*., 2006; Nijiati *et al*., 2021). No significant changes were observed in serum protein, IgM, lysozyme activity, or antioxidant variables. This may be attributed to reduced feed intake during the pathogen challenge, resulting in insufficient RAO consumption to elicit a strong antioxidant response. Nonetheless, previous studies have demonstrated that RAO possess immunomodulatory and antioxidant properties that protect tissues from oxidative stress and enhance disease resistance (Kiczorowska *et al*., 2017; Kim and Lillehoj, 2019: Uddin *et al*., 2021b).

Cortisol levels were reduced to less than half in fish fed RAO-supplemented diets compared to the control, indicating RAO's ability to alleviate physiological stress in red tilapia. This finding aligns with the known anti-inflammatory, antistress and antibacterial properties of bioactive resin acid compounds (Ali *et al*., 2023). Lower cortisol concentrations indicate reduced activation of the hypothalamic–pituitary–interrenal (HPI) axis, leading to less immunosuppression and greater stability in hematological and immune parameters (Esmaealzadeh *et al*., 2022). The relative constancy of hematological and humoral immunity variables (serum protein, lysozyme, and IgM) implies that cellular immune defense may have taken precedence under bacterial stress.

Bacterial counts in the liver and intestine further supported the antimicrobial action of RAO. While liver bacterial loads did not differ significantly among treatments, intestinal bacterial counts were significantly lower in the R700 group compared to both the control and R350 groups. This indicates that a higher RAO dose effectively suppressed intestinal colonization by pathogenic bacteria, likely due to the antibacterial properties of resin acid constituents. The dose-dependent reduction suggests modulation of intestinal microbiota and enhanced mucosal protection, contributing to improved disease resistance (Ayub *et al*., 2023).

Histological examination of the intestine revealed that RAO supplementation preserved villus structure and epithelial integrity. Fish fed 700 ppm RAO exhibited the longest villi, indication a greater absorptive surface area and improved nutrient uptake capacity, consistent with findings with juvenile largemount bass and broiler chicken (Taylor *et al*., 2021; Liu *et al*., 2022). The R700 group showed minimal tissue damage, the R350 group exhibited moderate lesions, and the control group displayed severe degradation. These results demonstrate that RAO supplementation enhances intestinal resilience against stress-induced inflammation and bacterial infections, likely through its anti-inflammatory and epithelial-protective effects (Spisni *et al*., 2021; Shahbakht *et al*., 2024). Similar studies also reported increased goblet cell density and improved mucosal branching in animals receiving resin acid or related supplements (El-Katcha *et al*., 2024; Fang *et al*., 2024). Goblet cells secrete mucus that protects the intestinal lining and supports barrier function, indicating an enhanced mucosal defense system (Paone and Cani, 2020; Zhang and Wu, 2020).

Liver histology reflected adaptive metabolic responses. Both RAO-treated groups showed well-organized hepatocytes arranged in distinct cords with clear nuclei, indicating healthy hepatic architecture. Mild vacuolization was observed in

the R350 group, suggesting moderate lipid and glycogen storage, whereas the R700 group exhibited more pronounced vacuolization and peripheral nuclear displacement, indicating greater energy reserve accumulation (Mattioli *et al*., 2020). Expanded sinusoidal gaps in both groups indicated improved hepatic perfusion and nutrition transport. These structural adaptations suggest that RAO supplementation enhances metabolic efficiency and circulation while reducing oxidative and inflammatory stress (Yang *et al*., 2023; Gracia-Sancho, 2024).

CONCLUSIONS

Dietary supplementation with RAO at 350–700 ppm improved the stress tolerance of juvenile red tilapia under *A. hydrophila* challenge. RAO enhanced oxygen-carrying capacity by increasing hemoglobin levels, significantly reduced cortisol concentrations to less than half of the control, and lowered intestinal *Aeromonas* colonization, particularly at 700 ppm. The 700 ppm RAO diet also preserved intestinal villus structure and epithelial integrity, supporting better nutrient absorption and gut health under bacterial stress. These physiological and structural improvements highlight the potential of RAO as a dietary supplement to enhance resistance to *Aeromonas* bacterial infections and overall performance in tilapia aquaculture.

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