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RESEARCH PAPER

Effects of Resin Acid on White Shrimp (*Litopenaeus vannamei*) Performance and Resistance to *Vibrio parahaemolyticus* under Oral Administration

Chonnikarn Piyavorasakul[1], Supawit Triwutanon[2], Srinoy Chumkam[3], Orapint Jintasataporn[1,*]

[1]Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Thailand.
[2]Faculty of Verterinary Medicine, Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand.
[3]Faculty of Agricultural Technology, Valaya Alongkorn Rajabhat University Under the Royal Patronage, Pathum Thani, Thailand.

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Corresponding Author
E-mail: ffisora@ku.ac.th

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Abstract

The effects of resin acids oil with carrier (RAC) in powder form, Progres®, supplementation on *Litopenaeus vannamei* growth performance, immune response, and disease resistance to severe Vibriosis, *Vibrio parahaemolyticus* AHPND were assessed. A completely randomized design (CRD) with three treatments of RAC supplemented—control 0, RAC350, and RAC700 ppm was applied to shrimp for 2 weeks. Juvenile white shrimp initial weights of 1.82±0.03 g., were created a random stocked 30 shrimp to each aquarium of 240 L with 15 ppt. saline water 120 L with total 810 shrimp. After 2 week feeding trial, shrimp were applied oral administration. The study found that short-term dietary RAC under normal condition, shrimp growth performance and survival rate were in the same range (P>0.05). The immunity of shrimp fed both treatments in term of lysozyme activity and superoxide dismutase activity was significantly increased (P<0.05). After stress condition, shrimp innate immunity significantly improved (P<0.05), and exhibited disease resistance against, Vibriosis count in both treatments decreased (P<0.05). The survival rate after challenge was low in Control and significantly high in both treatments (P<0.05). There were 66.67±5.77, 86.67±5.77 and 80.00±10.00%, respectively. Therefore, short-term dietary resin acid effects on improving immune response and resistance to *Vibrio* under stress conditions.

Introduction

Aquaculture has become a critical solution for meeting the rising global demand for seafood, with *Litopenaeus vannamei* (Pacific white shrimp)(Delgado, 2003; Naylor et al., 2023)being one of the most widely farmed and economically valuable species (Zhang et al., 2024). However, the intensification of shrimp farming has led to increased susceptibility to infectious diseases, particularly acute hepatopancreatic necrosis disease (AHPND) caused by *Vibrio parahaemolyticus* (Rajeev et al., 2021; Vaiyapuri et al., 2021). These outbreaks result in severe economic losses and raise significant concerns about the sustainability and biosecurity of shrimp aquaculture (Savary et al., 2012).

While conventional disease management strategies such as antibiotics and vaccines have been employed to reduce mortality, their overuse poses risks including antibiotic resistance, environmental pollution, and consumer health concerns (Ahmed et al., 2023; Hernando-Amado et al., 2019; Sharma et al., 2018). These challenges underscore the urgent need natural, sustainable alternatives to promote shrimp health and disease resistance without compromising environmental safety.

In recent years, attention has turned to plant-derived feed additives, such as essential oils, polyphenols, and resin extracts, due to their antimicrobial, immunostimulatory, and antioxidant properties (Baptista et al., 2020; Dhama et al., 2018;

Ghosh et al., 2021). Among there, resin acids, found in tree species like Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*), have shown promise in livestock by enhancing gut health and reducing pathogenic load (Hasan et al., 2019; Kettunen et al., 2015). There compounds have been marketed in several counties for animal nutrition and are reported to improve survival rates and reductions in *Vibrio* spp. In aquatic animals (Lipiński et al., 2021). However, despite these advances, the effects of resin acid supplementation in shrimp particularly in the context of *Vibrio parahaemolyticus* AHPND, remain poorly studied (Li et al., 2022; Servin, 2020; Zheng & Bossier, 2023).

Therefore, this study investigate the short-term effects of dietary resin acid supplementation on growth performance, immune response, bacterial load and disease resistance of *Litopenaeus vannamei* (Pacific white shrimp) under controlled challenge with Vibrio parahaemolyticus AHPND. By evaluating the potential benefits of resin acids as functional feed additives, this work aim to contribute to the development of sustainable and antibiotic-free approaches for shrimp health management in modern aquaculture.

Materials and Methods

Experimental Design

The experiment was a conducted as a completely randomized design (CRD) with three dietary treatments and nine replications per treatment. The diets were supplemented with resin acid extract with carrier in powder form (RAC; Progres® AB Vista, UK), containing 4% natural resin acid extract derived from Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies*). The experimental diets were as follows: (a) Control: a basal diet without resin acid supplemented, (b) T1 (RAC350): the basal diet supplemented with at 350 ppm resin acid, and (c) T2 (RAC700): the basal diet supplemented with at 700 ppm resin acid.

Animal Rearing and Experimental Procedures

The experiment was conducted at the Nutrition and Aquafeed Laboratory, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Thailand. Juvenile Pacific white shrimp (*L. vannamei*) were obtained from a specific pathogen-free (SPF) stock from a private farm in Samut Songkhram, Thailand. After acclimatization on a control diet in a common tank for two weeks,810 healthy juveniles were selected. Prior to the feeding trial, shrimps were starved for 6 hours, weighed, and randomly assigned to 27 glass tanks (240 L each; dimensions 36 × 18 × 24 inches) with an initial stocking density of 30 shrimp per tank (mean initial body weight 1.82±0.03 g.).

Each tank received a 20% water exchange with dechlorinated 15 ppt saline water every three days. Plastic tarps were placed over tanks to prevent shrimp form jumping out. Water quality parameters were maintain as follows: temperature 28±2.5ºC, dissolved oxygen 5±0.4 mg/L, pH 7.5±0.3, and ammonia 0.03±0.01 mg/L in a semi-closed system with aeration supplied by air pumps with a fine-bubble disc diffuser (kawaii). Shrimp were reared indoors. Shrimp were fed at 5–8% of their total body weight per day, divided into three feedings at 09:00, 12:00, and 16:00. The feeding protocol was followed for two weeks.

Diets Preparation and Dietary Treatments

Three isonitrogenous and inolipidic diets (35% crude protein and 7% crude lipid) were prepared: control, T1 (RAC350), T2 (RAC700). Each diet was based on commercial shrimp feed, top-coated with different levels of natural resin acid oil with carrier (powder form) and coated with 5% chitin showing in Table 1 (International et al., 2012). The resin acid with carrier in powder form (RAC), Progres® (AB Vista, UK) mixture constrained approximately 4% natural resin acid extract from Scots pine (*Pinus sylvestris* L.) and Norway spruce including. 33% abietic acid, 18% dehydroabietic acid, 14%) pimaric acid, and 35% other minor resin acids. (E551a, 500 g/kg) was included as an anti-caking agent (Roy et al., 2018). Proximate composition (moisture, protein, fat, fiber, ash, energy, calcium, and phosphorus) of each diet was analysis following Association of Official Analytical Chemists method (International et al., 2012). Shrimp were fed the experimental diet three times per day at 5-8% of their body weight.

Data Collection

After two-week feeding trial, shrimps were starved for 24 hours, counted, weighed to determine survival and growth. Six shrimp from each tank were cold-shocked for sampling of hemolymph, hepatopancreas, and intestine for biochemical, bacterial, and histopathological analysis.

Hematological, Biochemical and Antioxidative Stress Enzymes

Hemolymph was collected from the hemocoel (pseudo-heart). Total hemocyte count (THC) was measured by mixing hemolymph with trypan blue solution (Sritunyalucksana et al., 2005). Hemolymph protein concentration was determined by the Lowry method (Lowry et al., 1951). Phenoloxidas (PO) activity was measured (Söderhäll & Smith, 1983) and lysosome activity (Yao et al., 2008). Antioxidative stress enzymes, including superoxide dismutase (SOD) and glutathione, were quantified using Signa kits (SOD Kit 19160, Sigma-Aldrich, Buchs, Switzerland; Glutathione Kit G4376, Sigma-Aldrich, St. Louis, MO, USA)

Challenge

After the feeding trial, 10 shrimp from each replicate were transferred to 100 L glass aquaria for an oral administration challenge. Shrimp in each treatment group were orally exposed to *Vibrio parahaemolyticus* AHPND (1× 10^7 cfu/ml). The pathogen suspension was sprayed onto feed (20 ml/Kg feed) to achieve a final concentration (2 × 10^5 cfu/g feed). Shrimp were fed with pathogen-inoculated pellets for two weeks. Survival rate, histopathology of intestine and hepatopancreas, immune parameters, and bacteria counts in intestinal samples were determined.

Hepatopancreas and Intestine Morphology

After 2 weeks of challenge period, six shrimp from three replicate tanks per treatment were sampled. Hepatopancreas and intestines were dissected (n=3) using sterile tools, fixed in 10% buffered formalin, processed, and embedded in paraffin. Tissue sections every 4 µm were xylene deparaffinization and hematoxylin and eosin (H&E) staining (Montaser et al., 2021). Micrographs were taken using an Olympus microscope at 400× magnification. For every diet, hepatopancreases micrographs were counted 10 cells form each filed and 3 field per sample, nine per treatments (n=27). Microscopic examination of intestinal tissue slices revealed histological features such as intestinal wall architecture, epithelial cells, villi height, and signs of inflammation. The samples were viewed under a light microscope, an Optika camera and microscope. Villi height was measured with Optika software (version Light 2.1).

Degree of Intestinal Structure Damage

The degree of intestinal damage was focusing on the histology of anterior gut from second segment of shrimp body. The detail of intestinal structure damage in Table 2. and Figure 1. appears to show varying degrees of intestinal injury, which may be related to a biological or medical setting. A general description of the criteria used to evaluate the damage is included with the damage level, which is categorized into four levels: ++++ (severe, f), +++ (moderate, e), ++ (mild, d and c), + (minimal, b), and - (normal, a). Striated border is one of the observing cell features. Mucosa, this criterion refers to the mucous membrane that lines the structure and specifies any alterations or abnormalities found. Submucosa, this criterion focuses on the layer beneath the mucosa and identifies any changes or disruptions present. Muscular layer, this criterion refers to the muscular layer within the structure and indicates any alterations or damage found in this specific layer, and whole structure or overall structure, this criterion offers an overall assessment. The table describes the characteristics detected under each criterion for each damage level.

Statistical Analysis

All data were expressed as means ± standard deviation (SD). Differencess among treatments were analyzed using linear relationship between tha variable and one-way analysis of variance (ANOVA). When significant difference were detected (P<0.05), means were separated using multiple-comparisons (Duncan) test. Different superscript letters (a, b, c) indicat significant differences among treatment means.

Results

The effects of resin acids, Progres®, supplementation on shrimp growth performance are presented in Table 3. Short-term dietary RAC feeding for 2 weeks demonstrated that survival rate and growth performance in term of final weight, weight gain, specific growth rate were not significantly difference among treatment (P>0.05). Feed utilization in term of

Table 1. Experimental shrimp diet formulation and proximate composition

Ingredients (kg.)	**Control**	**RAO350**	**RAO700**
Soybean meal (49% CP)	35	35	35
Wheat flour (13.2 % CP)	30	30	30
Fish meal (70.7% CP)	15	15	15
Squid meal and Squid paste	10	10	10
Premix (vitamins and minerals)	5	5	5
Emulsifier and preservation	4	4	4
Fish oil	1	1	1
Total	100	100	100
Resin acid (top-up) (%)	0	0.035	0.070
Proximate composition (AOAC, 2000)			
Moisture (%)	6.75	6.75	6.75
Protein (%)	35.98	35.98	35.98
Lipid (%)	5.12	5.12	5.12
Fiber (%)	3.07	3.07	3.07
Ash (%)	13.62	13.62	13.62
Energy (Cal/kg)	4045.94	4045.94	4045.94
Phosphorous (%)	1.69	1.69	1.69
Calcium (%)	3.12	3.12	3.12
Nitrogen Free Extract (%)	35.46	35.46	35.46

feed intake and feed conversion ratio, remained in the same range (P>0.05).

The immunity results in Table 4, summarized the immune responses and Vibriosis count under both normal and stress condition fllowing oral administration of pathogen to white shrimp. Under normal condition, shrimp immunity in terms of total hemocyte count (THC), hemolymph protein, phenol oxidase (PO)activity, and glutathione levels did not differ significantly among treatment groups (P>0.05). However, lysosome activity was lowest in the control and increased in shrimp fed RAC350, followed by RAC700 and then control (P<0.05), with values of 153.33±5.77, 196.67±25.17, and 180.00±10.00 unit/mL, respectively. Superoxide dismutase (SOD) activity was significantly increased in shrimp fed RAC diets (P<0.05), with values of 1.73±0.19, 2.51±0.21, 2.58±0.13 unit/mL for control RAC350, and RAC700, respectively. Vibrio counts were significantly higher in the contaol group compared to TAC350 and RAC700 (P<0.05), with values of 2.32±0.17, 1.94±0.09, 1.76±0.20 log cfu/ml, respectively.

After two weeks of oral administration by *Vibrio parahaemolyticus* AHPND, the survival rate of shrimp fed RAC350 (86.70±5.77) and RAC700 (80.0±10.00) was significantly higher than of the control group (66.67±5.77) (P<0.05). Total hemocyte count differed significantly, with values of 17.67±4.04, 24.00±2.65, and 27.33±2.08 10^5 cell/ml in control, RAC350, and RAC700 groups, respectively (P<0.05). Hemolymph protein level were significantly higher in RAC700 (5.46±0.14 mg/mL) than RAC350 (4.10±0.12 mg/mL) and control (3.72±0.09 mg/mL) (P<0.05). Phenol oxidase activity was a significantly increased in both RAC groups compared to the control, with values of 81.88±2.56, 94.50±6.08, and 112.41±7.22 unit/mg protein, respectively (P<0.05). Lysozyme activity after the challenge test was not significantly different among groups (P>0.05). SOD activity was highest in the control (5.51±0.35 unit/ml) and significantly higher that RAC350 (4.70±0.27 unit/ml) and RAC700 (3.29±0.11 unit/ml) (P<0.001). Total glutathione levels did not differ significantly among treatments (P>0.05), with values of 19.04±3.94,

Table 2. Degree of intestinal structure damage

Damage degree	Criteria	Striated border	Mucosa	Submucosa	Muscular layer	Whole structure
−	Normal	Arranged closely	Folds and villi and crypts normal	Tight intercellular space	Cells arranged in order	Clear and complete
+	Minimal	Arranged closely	Shorter folds short villi	Large spaces	Space enlarged	Loose structure
++	Mild	Swelling	Less folds, short villi	Large spaces	Vacuolation and dispersed	Loose tissue
+++	Moderate	Degeneration	Folds almost disappeared, short villi	Not compact or dense	Marked vacuolation, Nucleolytic	Necrosis
++++	Severe	Exfoliation, thin villi structure	Disintegration of villi, some necrosis	Dissolution	Separation	Necrosis

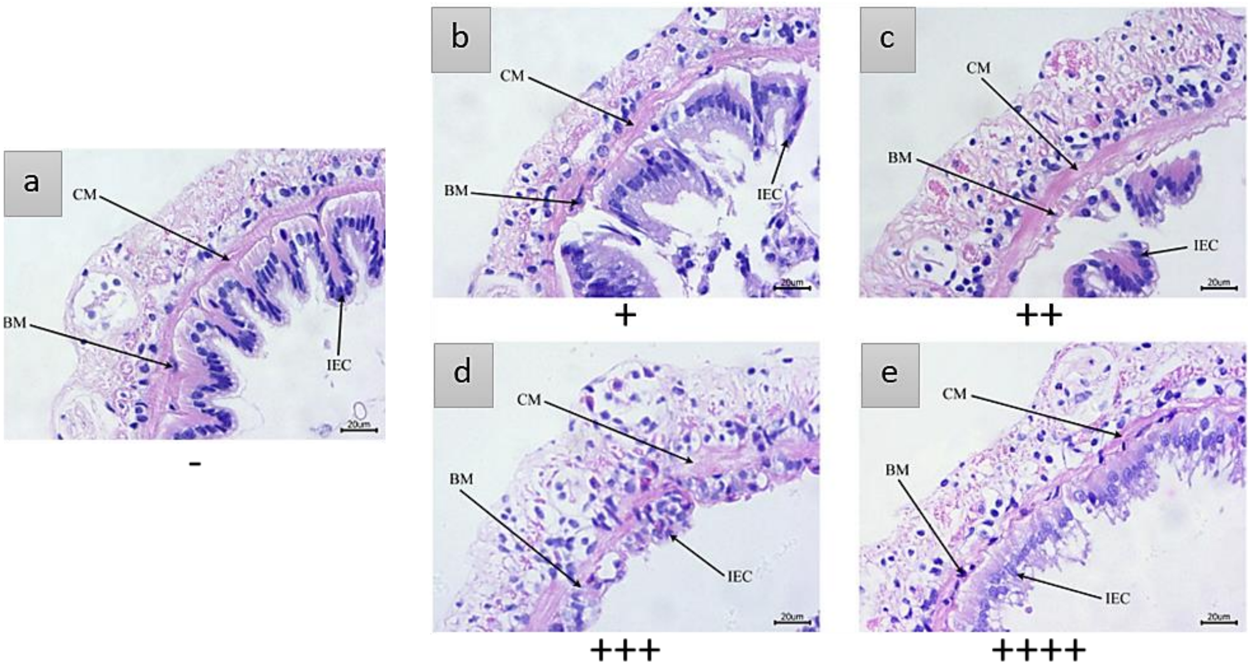

Figure 1. Degree of intestine structure damage. Note: Figures a, b, c, d, e, the arrows in figure CM, IEC, and BM indicate different parts of the intestine, following CM=Circular muscle, IEC=Intestinal epithelial cells, BM=Basement membrane (Huang et al., 2019).

16.59±0.58, and 16.81±0.54 nM/ml for control, RAC350, and RAC700, respectively (P>0.05). After oral administration challenge with *Vibrio paraheamolyticus* AHPND (2 × 10^5 cfu/g feed), vibrio count in both resin acid groups were significantly lower than in the control group (P<0.05), with values of 4.96±0.19, 4.61±0.08, 4.57±0.015 log cfu/mL in control, RAC350 and RAC700, respectively. These findings indicated the efficacy of RAC350 and RAC700 in promoting shrimp health and immunity under sever AHPND challenge. Furthermore, the survival rate was significantly higher in both RAC groups than in the control (P<0.05).

Figure 2 displayed the survival rate of shrimp under both normal and after challenging condition. It can see that after dietary supplement, all groups had high survival rates under normal condition of control group had good rate but non-significant difference also lower than both treatment groups (P>0.05). On the other hand, all group showed a significant drop in survival after the oral challenge test (P<0.05). In contrast to the control group, which had a survival rate of 66.67±5.77%, the RAC350 and RAC700 groups demonstrated a significantly higher survival rate of 86.67±5.77% and 80.0±10.0% respectively. The hepatopancreases section after Vibriosis oral administration challenge, presented in Table 5, showed significant difference in R cells count, with 17.20±1.81 cell/field in control, 22.80±3.29 cell/field in RAC350, and 12.10±1.97 cell/field in RAC700 (P<0.05). F cells count did not differ significantly among treatments (P>0.05). B cells number were significantly higher in RAC700 (5.60±1.43) compared to control (4.20±0.79) and RAC350 (4.20±1.40) (P<0.05). No significant difference was observed in F and E cells treatments (P>0.05).

Histological analysis of the hepatopancreas in RAC700-fed shrimp demonstrated healthier tissue with smooth edges, enlarged B cells. RAC350-fed shrimp exhibited enlarged and abundant R cell associated with lipid utilization and antioxidative enzyme synthesis. The control group showed degeneration of B cell and R cells in Figure 3.

Intestinal morphology result in Table 6 revealed thatt villi length in RAC350 was longest in RAC350-fed shrimp (53.21±10.25 µm), followed by RAC700 (42.59±6.75 µm) and, control (41.52±5.18 µm), with significant difference (P<0.01).

Histopathological examination of the intestine (Table 7 and Figure 4) after the Vibriosis stress challenge

Table 3. Growth performance of white leg shrimp fed different levels of resin acid oil with carrier for eight weeks under normal condition

Growth performance	Control	RAC350(ppm)	RAC700 (ppm)	P value
Survival rate (%)	95.56±1.92	97.78±1.92	97.78±3.85	0.548
Growth performance				
Initial body weight (g/shrimp)	1.81±0.04	1.84±0.01	1.80±0.03	0.412
Final body weight (g/ shrimp)	2.51±0.28	2.72±0.32	2.83±0.03	0.322
Body weight gain (g/ shrimp)	0.69±0.29	0.88±0.31	1.03±0.03	0.320
Average daily gain (g/shrimp/d)	0.05±0.02	0.06±0.02	0.07±0.00	0.242
Specific growth rate (%/d)	2.29±0.84	2.76±0.83	3.24±0.12	0.311
Feed intake (g/ shrimp)	1.95±0.04	1.91±0.04	1.91±0.08	0.558
Feed conversion ratio	3.14±1.14	2.41±1.03	1.85±0.12	0.285
Shrimp production (g/Aq.)	71.87±8.49	79.77±10.55	83.10±2.95	0.285

Remarks: Data were expressed as mean±SD and means in the same row were not significantly different from each other (P>0.05).

Table 4. Health and immunity of shrimp fed resin acids under normal condition for two weeks then applied oral administration challenge test by *V. parahaemolyticus* AHPND

Immunity	**Control**	**RAC350 (ppm)**	**RAC700 (ppm)**	**P value**
Under normal condition				
Survival rate (%)	95.56±1.92	97.78±1.92	97.78±3.85	0.548
Total hemocyte count (10^5 cell/ml)	32.00±1.00	34.33±2.08	35.33±1.53	0.101
Hemolymph protein (g/dL)	5.14±0.17	5.17±0.06	5.22±0.22	0.853
phenol oxidase activity (unit/mg protein)	144.61±8.41	168.16±12.71	174.31±25.38	0.160
Lysosome activity (unit/mL)	153.33±5.77^{a}	196.67±25.17^{b}	180.00±10.00^{ab}	0.042
Superoxide dismutase (unit/mL)	1.73±0.19^{a}	2.51±0.21^{b}	2.58±0.13^{b}	0.002
Glutathione(mM/mL)	31.97±1.61	33.56±0.85	33.95±0.96	0.189
Vibriosis count from intestine (log cfu/ml)	2.32±0.17^{b}	1.94±0.09^{a}	1.76±0.20^{a}	0.011
After oral administration challenge test				
Survival rate (%)	66.67±5.77^{a}	86.67±5.77^{b}	80.0±10.00^{b}	0.042
Total hemocyte count (10^5 cell/ml)	17.67±4.04^{a}	24.00±2.65^{b}	27.33±2.08^{b}	0.021
Hemolymph protein (g/dL)	3.72±0.09^{a}	4.10±0.12^{b}	5.46±0.14^{c}	< 0.001
phenol oxidase activity (unit/mg protein)	81.88±2.56^{a}	94.50±6.08^{b}	112.41±7.22^{c}	0.002
Lysosome activity (unit/mL)	133.33±15.28	136.67±23.09	153.33±20.82	0.469
Superoxide dismutase (unit/mL)	5.51±0.35^{c}	4.70±0.27^{b}	3.29±0.11^{a}	< 0.001
Glutathione(mM/mL)	19.04±3.94	16.59±0.58	16.81±0.54	0.415
Vibriosis count from intestine (log cfu/ml)	4.96±0.19^{b}	4.61±0.08^{a}	4.57±0.015^{a}	0.040

Remarks: Data were expressed as mean±SD and means in the same row with different letters (^{a,b,c}) were significantly different from each other (P<0.05).

revealed severe damage (+++) in the control group (a), including exfoliation, thin villi structure, disintegration of villi, dissolution, separation, and necrosis. In contrast, RAC700 (c) and RAC350 (b) groups showed moderate damage (++), such as degeneration, reduced folds, shortened villi, loose submucosa, vacuolation, and nucleolytic necrosis.

Discussion

This study investigated the short-term effects of dietary resin acids on the growth, immunity, gut health of white leg shrimp (*Litopenaeus vannamei*), particularly under *Vibrio parahaemolyticus* AHPND challenge. While limited literature exists regarding the short-term impact of resin acids on shrimp growth, this findings suggest that two weeks of dietary supplementation did not significantly affect growth performance under normal condition, non-stress condition. This aligns with previous observations that short duration and low-stress environments may not sufficiently stimulate physiological change in shrimp (Apajalahti et al., 2020). However, despite the lack of growth promotion, dietary RAC enhanced certain immune parameters and conferred protective effects during disease stress.

Notably, upon Vibriosis oral administration challenge, shrimp fed RAC diets demonstrated significantly higher survival rate and reduced pathogen loads, highlighting the immunomodulatory potential of resin acids. These impacts may stem from their well-documented antimicrobial properties and ability to serve as natural alternatives to antibiotics (Aikaterini Termentzi, 2011; Rodney Croteau, 2000). Although direct impact of resin acid on shrimp growth remains unclear, studies on other aquatic species suggested potential role in nutrient absorption, improve feed conversion efficiency, and modulate the gut microbiota, which could indirectly positively influence growth (Apajalahti et al., 2020; Khan et al., 2020; Uddin et al., 2021).

Resin acid supplements also appeared to enhance innate immune responses particularly under stress. Increased total hemocyte count (THC), elevated hemolymph protein levels, and higher phenol oxidase (PO) activity were observed in treated groups, indicating a strengthened immune defense. These parameters are

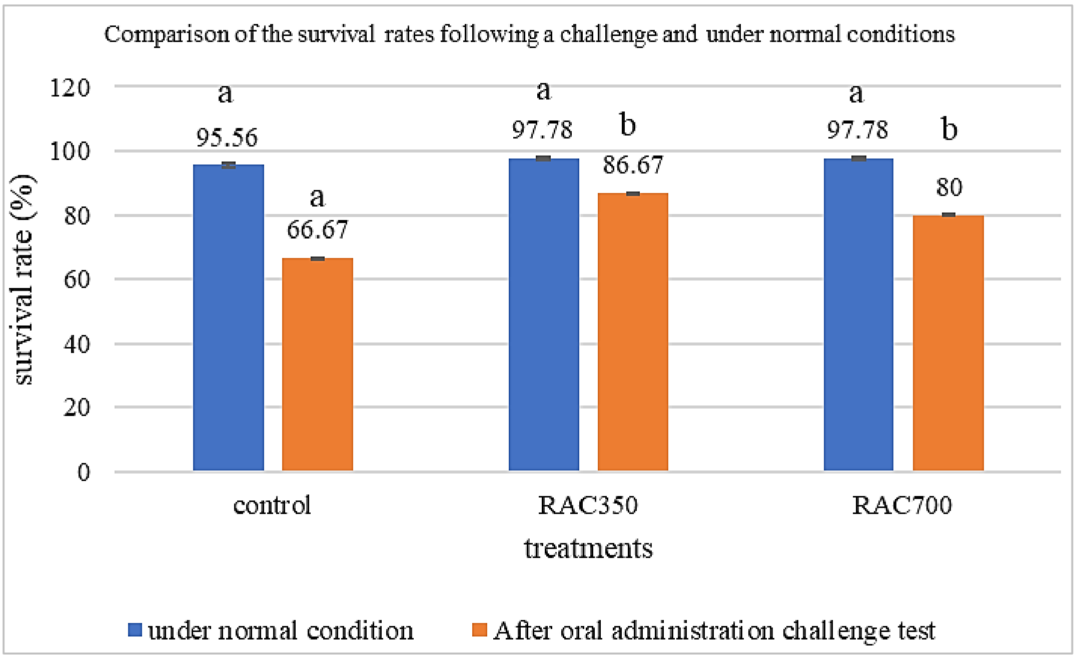

Figure 2. Comparison of the control and treatment groups' survival rates (%) under normal circumstances and following oral administration challenges.

Table 5. Different cell types in hepatopancreases of shrimp fed RAC after oral administration with *V. parahaemolyticus* AHPND for other two weeks

Cell type in the hepatopancreas	Control	RAC350 (ppm)	RAC700 (ppm)	P value
After oral administration challenge (cell/field; n=10)				
R cell	17.20±1.81^{b}	22.80±3.29^{c}	12.10±1.97^{a}	<0.001
F cell	3.10±0.88	2.90±0.99	3.80±1.32	0.166
B cell	4.20±0.79^{a}	4.20±1.40^{a}	5.60±1.43^{b}	0.025
E cell	4.30±1.06	4.70±0.95	4.40±1.17	0.686

Remarks: Data were expressed as mean ± SD and means in the same row with different letters (^{a,b,c}) were significantly different from each other (P<0.05).

key indicators of immune function in shrimp, involved in pathogen recognition, phagocytosis, and synthesis of immune-related proteins. These proteins have critical roles in a variety of immunological processes such as opsonization, coagulation, and antibacterial action between shrimp and pathogen like Vibriosis (Chang et al., 2015; Du et al., 2012) Enhanced PO activity and lysozyme leverls suggest activation of the prophenoloxidase system and antimicrobial enzyme production, both crucial in combating Vibriosis.

Additionally, the antioxidant enzyme superoxide dismutase (SOD) showed increased activity in shrimp fed RAC under normal conditions, consistent with a strengthened oxidative stress defense system. SOD plays a critical role in neutralizing reactive oxygen species (ROS), which are generated during immune activation and can cause tissue damage if uncontrolled (Ighodaro & Akinloye, 2018; Li et al., 2017). The decline in SOD activity post-challenge may reflect enzyme consumption during infection, yet the initial upregulation enhanced a preventive role of RAC antioxidative stress defense against ROS, helping to maintain cellular integrity during immune activation (Bhattacharya, 2015). Therefore, during stress and disease infection, the interplay between THC, hemolymph protein concentration, PO, Lysozyme, and SOD activity reflects the orchestrated immune response of shrimp to pathogen exposure, with each parameter contributing to different aspects of immune defense and regulation, reflecting aspects of immune cell mobilization, protein-mediated defense, and protection against oxidative stress (Tassanakajon et al., 2013). In addition, supplementation RAC, particularly at 350 to 700 ppm, led to a reduction in the intestinal concentration of *Vibrio parahaemolyticus* in the gastrointestinal tract of shrimp (Aribah & Wahyudi, 2022; Soto-Rodriguez et al., 2015). This reduction in intestinal pathogen load could be attributed to the effects of resin acids on shrimp health and immunity. Resin acids may enhance immune responses, reduce inflammation, and modulate gut microbiota composition, all of which can contribute to a healthier gut environment less conducive to pathogen colonization.(Al Azad et al., 2016; Bjanes & Nizet, 2021; Jiravanichpaisal et al., 2006).

The hepatopancreas histology further confirmed RAC's role in immune and metabolic support. An increased number of R cells, which are involved in lipid storage and energy metabolism, were observed in RAC350-fed shrimp, suggesting enhanced energy reserves for immune responses. Moreover, the RAC700 group showed a significantly higher count of B cells,

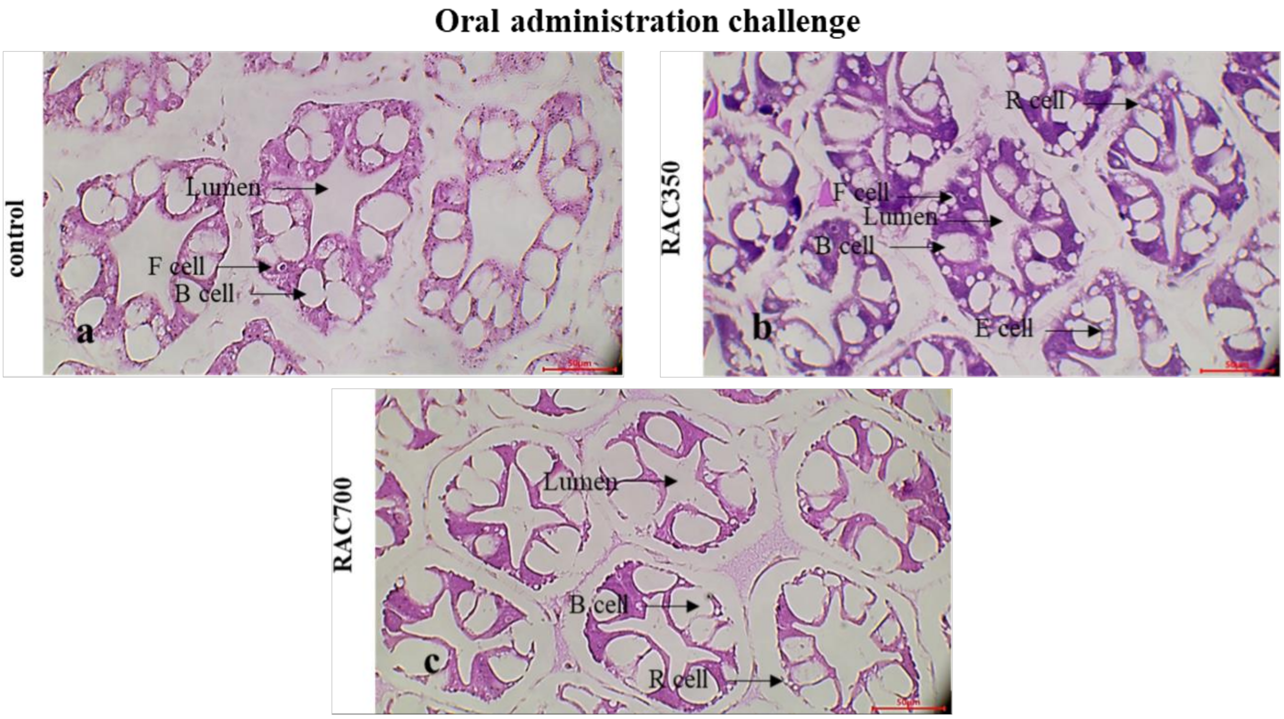

Figure 3. Light micrograph of hepatopancreas of shrimp fed RAC after oral administration challenge with Vibrio parahaemolyticus AHPND for two weeks. (Stain H&E, Bar = 200 µm, × 400).

Table 6. Intestinal villi length of shrimp fed RAC diet for two weeks then oral administration challenges for two weeks

Intestinal morphology	Control	RAC350 (ppm)	RAC700 (ppm)	p-value
Oral administration villi length, µm	41.52 ± 5.18^{a}	54.53 ± 6.10^{b}	42.59 ± 6.75^{a}	<0.001

Remark: Values represent means±SD of ten replicates and values in the same row with different letters are significantly different (P<0.01).

which are responsible for digestive enzyme production. This implies better digestive efficiency and nutrient assimilation under stress conditions (Dall & Moriarty, 1983; Štrus et al., 2019). While F and E cell numbers remained statistically unchanged, observed trends suggest physiological remodeling in response to infection and dietary intervention.

Intestinal morphology findings provided additional insights into the protective effects of resin acids. RAC-fed shrimp showed significantly longer villi and less structural damage following oral Vibriosis challenge. This suggests that resin acids may help maintain gut integrity and nutrient absorption capacity under pathogenic stress. These effects are likely mediated by modulation of matrix metalloproteinases (MMPs), particularly MMP-7, which is involved in collagen breakdown during inflammation. Resin acids have been shown to inhibit MMP activity, thereby preserving epithelial barrier function and reducing inflammatory damage (Aguirre et al., 2019; Kolpe et al., 2019; Vuorenmaa et al., 2017).

During the oral administration stress challenge, shrimp hepatopancreas plays an important role in responsible to stress condition. An increasing of R cells in the hepatopancreas which work for lipid digestion, absorption, and accumulation includes glycogen deposition, indicates that RAC350 and RAC700 contribute to immunological responses and defensive mechanisms against infections and foreign particles and in directly participate in phagocytosis, engulfing and digesting foreign substances such as bacteria, viruses, and cellular debris (Lim et al., 2017; Santa, 2023; Vogt, 2019). Moreover, a notable increased in B cells population in the hepatopancreas which produce and secrete digestive enzyme, create the enzymes and digestive fluids required to break down meal particles into smaller molecules that the shrimp can absorb and use including responsible for nutrient accumulation in tricellular digestion, transportation of digested nutrient exhibit the healthy shrimp condition (Dall & Moriarty, 1983; Štrus et al., 2019). The rising in F cell which responsible for protein synthesis and mineral deposition suggest the physiology response cause by enteric inflammation diseases. A drastic increase in E cell which play crucial roles in the mitotically to replace other cell type, killing invaded germs and promotion inflrammation to insolate and control diseases or allergic reactions demonstrate the development to specific cell type for defensive mechanism (Folci et al., 2021).

In addition, then focusing on histopathology of intestine after oral administration, the results suggest that RAC groups offer protective effects on intestinal morphology and structure in shrimp after challenged

Table 7. Degree of anterior intestine damage after *V. parahaemolyticus* oral administration challenge test in shrimp

Condition/Treatment	Control	RAC350 (ppm)	RAC700 (ppm)
Oral administration challenge	+++	++	++

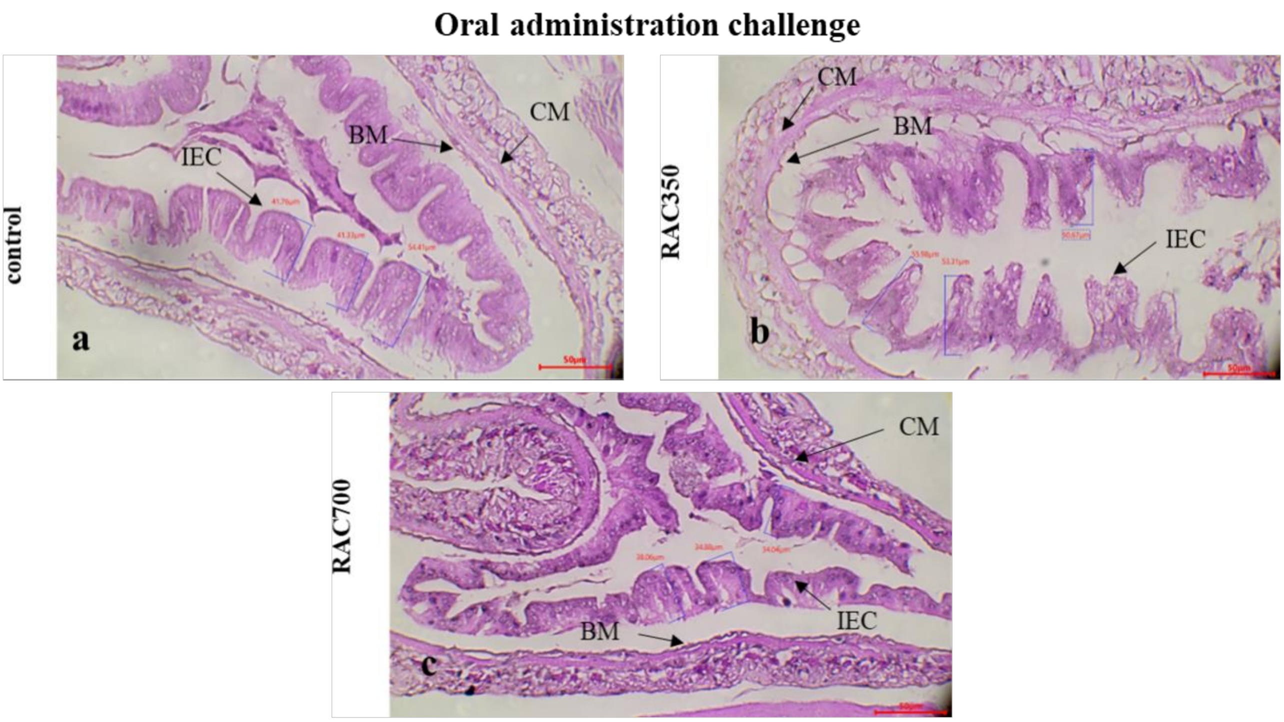

Figure 4. Light micrograph of intestine of shrimp fed RAC after oral administration with *Vibrio parahaemolyticus* AHPND for two weeks (Stain H&E, Bar= 50 µm, × 100), CM is circular muscle, IEC is intestine epithelial cells, BM is basement membrane.

with *Vibrio parahaemolyticus* orally. The longer microvilli and villi length indicate a potential role in preserving the absorptive surface area of the intestine. The observed moderate damage in the RAC350 and RAC700 suggests a particular protective effect against severe intestinal damage induced by the oral administration stress challenge. Resin acids introduced to feeds, resulting in a significant reduction in inflammation-associated collagen breakdown by particular matrix metalloproteinase enzymes (MMPs) in the intestinal epitheliums, this outcome suggested improved gut barrier functioning and increased resilience to diverse stressors on the gut membrane in memory species (Aguirre et al., 2019). These findings highlight the potential benefits of resin acids supplementation in mitigating the negative effects of pathogenic challenges on shrimp intestinal health and function. MMPs are essential for physiological processes. Resin acids reduce MMP7 activity in the intestinal, disrupting epithelial barrier integrity and activating α-defensins. Reducing MMP7 activity could improve intestinal health by enhancing barrier integrity and controlling inflammation in the gut (Aguirre et al., 2019). Researchers at the University of Ghent discovered that diet-derived resin acids can inhibit the collagen-degrading activity of matrix metalloproteinases (MMPs) in intestinal epithelium, consequently supporting epithelial integrity and barrier activities. This inhibits the invasion of inflammatory (Kolpe et al., 2019; Vuorenmaa et al., 2017). Hence, shrimp fed RAC350 presented the better gut heath under stress condition of oral administration.

Conclusions

Short-term feeding trail resin acids to shrimp promote a robust immune response, making them more resistant to *V. parahaemolyticus* AHPND. While short-term resin acid supplementation did not significantly enhance growth under non-stress conditions, it did confer immunological and structural benefits that enhanced shrimp resilience to Vibriiosis infection. The observed improvements in immue parameters, hepatopancreatic cell health, gut morphology suggest that resin acids act via multiple mechanisms- antimicrovial activity, immunomodulation, and oxidative stress control. These findings support the potential of RAC350 and RAC700 as functional feed additives to improve shrimp health and disease resistance in aquaculture. Future reseach recommends to investigate to other bacteria species with environmental stress in short and long term comparision.

Ethical Statement

Not applicable.

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Author Contribution

C.P. and OJ. designed the research, carried out the research, and wrote the article; O.J., S.T., S.C and S.H. conceptualized the central research idea, provided the theoretical framework, and supervised research progress; O.J. and S.H. conducted the review, conducted the revisions, and approved the article's submission. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors no conflict of interest.

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