Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27(5): 759 – 780 (2023)
www.ejabf.journals.ekb.eg

Benefits of Natural Immunostimulants Derived from Fruit By-Products Added to the Diet of *Litopenaeus vannamei*: Growth, Intestinal Microbiota, Digestive Enzyme Activities, Immune Responses and Economic Values

Ola A. Ashry[1*], Hafz M. Khouraiba[1], Zaki Z. Sharawy[2], Mervat A. Mohamed[1]

[1]Department of Animal Production and Fish Resources, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt

[2] National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt

[*]**Corresponding Author: oaashry707@gmail.com**

ARTICLE INFO

Article History:
Received: Sept. 25, 2023
Accepted: Sept. 29, 2023
Online: Oct. 25, 2023

Keywords:
Immunostimulants,
Fermented fruit waste,
Intestinal microbiota,
Digestive enzymes,
Gene expression,
Litopenaeus vannamei

ABSTRACT

The purpose of this study was to evaluate the effect of two fermented fruit wastes (FFW) as feed additives on the growth rate, economic analysis, intestinal microbiota, digestive enzymes and gene expression in the postlarvae (PL) of *Litopenaeus vannamei*. Three diets (38% crude protein, 9% crude lipid) consisting of T_0 (control), T_1 (2.5% fermented grape bagasse) and T_2 (2.5% fermented banana peels) were devised and given to the shrimp PL (n = 80 PL/tank in triplicate) for 45 days. The findings showed that adding dietary FFW supplements to shrimp diets significantly improved their survival rate (S%), final body weight (FBW), weight gain (WG) and specific growth rate (SGR). The feed conversion ratio (FCR) was also reduced by the FFW. There was an increase in the total bacterial count (TBC) in water and shrimp intestines, while total vibrio count (TVC) was reduced by the FFW. The addition of FFW could considerably enhance the activities of the investigated enzymes in the intestine, hepatopancreas and stomach, especially in T_2 followed by T_1 ($P< 0.05$). Furthermore, the levels of all studied genes in the shrimp's hepatopancreas given fermented banana peels were considerably greater ($P< 0.05$) than those of the control group. The results indicated that the shrimp fed with fermented banana peels presented the highest growth rate, better digestibility, and growth-related and immune-related genes. In conclusion, FFW can be used in modest amounts as immunostimulants in shrimp diets to promote the growth and health of shrimp.

INTRODUCTION

Developing cost-effective and secure methods for the prevention and stimulation of animal immune systems is a high priority in contemporary aquaculture. Consequently, better aquaculture production would enhance both animal health and the immune system's ability to fight off diseases. A functional nutrient or feed additive can improve shrimp performance via immune-nutrition in aquaculture including shrimp farming (**Rosas *et al.*, 2022**).

Immunostimulants are dietary additions that strengthen defence mechanisms and resistance against specific microorganisms. Additionally, the application of immunostimulants as an alternative to chemicals, medication and antibiotics are environmentally friendly and possibly available globally. It increases fish and shrimp immunity by enhancing the intrinsic (or nebulous) immune response (**Sakai, 1999; Rattanavichai *et al.*, 2017; Rosas *et al.*, 2022**). In this way, the use of immunostimulants for the treatment of diseases in fish and shrimp are regarded as a potential and additional option (**Sakai, 1999**).

Fruit waste or by-products are an important resource of bioactive substances, including terpenes, phenolic compounds, and β-glucans, etc. These substances have been linked to a variety of biological functions, as for example, antioxidants, antimicrobials, immune system modulators and gut microbiota. These qualities have been linked to better health in aquatic animals (**Leyva-López *et al.*, 2020**). The most consumed fruits worldwide are grapes and bananas, with production totals of 78 million and 120 million tonnes in 2020, respectively (**FAO, 2022**). Grape waste is a high-quality by-product with pharmaceutical and nutritional potential. Grape bagasse (GB) is the primary by-product of processing grapes, consisting of skins, seeds and stems. It makes up about 25% of the weight of the harvested grapes in the stages of grape processing. Grape bagasse contains beneficial essential oils with high unsaturated fatty acids [oleic acid (18:1n-9) and LA], tannins, polyphenols and flavonoids recognized for their antibacterial, antioxidative and immunomodulatory properties **(Roberts *et al.*, 2008; Currie *et al.*, 2019)**.

About 35% of the weight of a banana is made up of its peel, which is not edible. Banana peels are rich in bioactive substances such as tannins, flavonoids, phlobatannins, glycosides, alkaloids, anthocyanins and terpenoids. These substances are believed to have a wide range of biological and pharmacological effects, including antidiabetic, antihypertensive, anti-inflammatory and antibacterial activities **(Pereira & Maraschin, 2015; Vu *et al.*, 2019)**. However, **Van Hai (2015)** reviewed that medicinal plants can be taken as a whole plant or a specific portions such as leaf, root or seed, an extract chemicals, a routine or feed additives, a combination with prebiotics, and other immunostimulants.

Research has revealed that fruit by-products are effective immunostimulants that can replace antibiotics and chemical drugs. Various fruit wastes and their extract had a positive effect on the growth rate, survival, intestinal microbiota, digestive tract and immune responses in giant freshwater prawn (**Rattanavichai & Cheng, 2015**), rohu (**Giri *et al.*, 2016; Harikrishnan *et al.*, 2021),** red tilapia (**Mones & Angeles, 2017**), and white leg shrimp (**Rosas *et al.*, 2022**). The cost-effectiveness and eco-friendliness of fruit by-products make it an excellent feed supplement for fish and shrimp farming.

The fermentation process is being formulated globally as a supplementary diet for aquaculture development and production. Numerous fermented fibrous agricultural wastes, including papaya processing waste, apple pomace and banana peels have been

employed as feed additives and immunostimulants for fish or shrimp **(Fatmawati *et al.*, 2018).** This low-protein and high-carbohydrate agricultural waste can provide cheap fish energy. Nevertheless, carnivorous fish exhibit less carbohydrate amylolytic activity than herbivorous aquatic animals. Therefore, it will be beneficial to add amylase activity to the carbohydrate diet. Nutrient enrichment has been attempted by the fermentation process which can boost protein content, necessary metabolic enzymes, anti-nutritional factors (ANTFs), and substances that inhibit pathogenic microorganisms. Many fungi have been shown to have either amylolytic or cellulolytic activities, allowing them to be utilized to ferment wastes high in carbohydrates while producing the protein sources required for feed components after fermentation (**Hidalgo *et al.*, 1999; Fatmawati *et al.*, 2018).**

L. vannamei is one of the most frequently cultivated shrimp species worldwide due to its great flavor, rapid growth, and well adaptation to salt (**Chen *et al.*, 2018**). Pacific white shrimp (*L. vannamei*) individuals were produced in an amount exceeding 4.9 million tonnes worldwide in 2018 **(FAO, 2020)**. Although Egypt produces 14262 tons of shrimp annually from natural fisheries, *L. vannamei* shrimp output has increased year over year from 2015 to 2018 compared to the years before (**CAPMAS, 2020**).

This research is important for replacing antibiotics in shrimp cultivation with a novel and unconventional product. Thus, the goal of this study was to assess the effects of two dietary feed additives rich in polyphenols that were derived from various fruit wastes (natural products from both fermented grape bagasse and banana peels) on the growth rate, economic analysis, intestinal microbiota, digestive enzymes and gene expression of white shrimp, *L. vannamei.*

MATERIALS AND METHODS

1. Experimental diets

1.1. Fruit waste fermentation and diet preparation

Fruit wastes (FW) from grapes and bananas were collected from local markets, immediately washed, and minced into small sizes. One kg from each fruit waste was mixed with *Saccharomyces cerevisiae* inoculums (2.5 x 10^6 cfu/g) with aeration in 20L containers at 37℃ as an optimal growth temperature for *S. cerevisiae* for up to 6 days according to previously described methods (**Ashry *et al.*, 2023**). The end products were dried with a 70℃ fan until constant weight. Both fermented grape bagasse and fermented banana peels were dried, crushed into a smooth powder, and kept at 4°C until needed. The chemical composition of raw grape and banana (dried) before the fermentation process (**AOAC, 1995**), as well as fermented fruit wastes are shown in Table (1). The commercial diet (38% CP and 9% CL) was directly sprayed with fermented fruit wastes (FFW) at a concentration of 2.5% (25g/kg). The fermented grape bagasse (FGB) and fermented banana peel (FBP) powders were dissolved in 50ml water and mixed

thoroughly in a cooled slurry of diet in a domestic mixer, dried in an airflow oven at 60–70^{o}C, and divided into the adjusted pellet size.

Table 1. Proximate composition (%) of fruit by-products before and after fermentation

Parameter (%)	GB	FGB	BP	FBP
Moisture	**75.85 ±1.85**	**80.75±0.72**	**78.41 ±2.87**	**89.23±0.95**
Crude protein (CP)	**10.30±0.96**	**17.85±0.12**	**7.67 ± 0.38**	**19.48±0.17**
Crude lipid (CL)	**4.48 ± 0.65**	**1.93±0.06**	**1.54 ± 0.49**	**3.80±0.07**
Ash	**3.53 ± 0.55**	**16.33±0.05**	**3.89 ± 0.60**	**21.47±0.01**
Fiber	**35.79±0.59**	**31.35±0.10**	**26.60 ± 0.83**	**21.62±0.34**
Carbohydrate	**45.89 ± 1.53**	**32.54±0.31**	**60.30 ± 1.06**	**33.63±0.58**
Energy Kcal (kJ/g diet)	**12.14±0.21**	**10.60±0.16**	**12.85±0.51**	**11.92±0.21**

2. Shrimp handling and experimental design

This research took place at the National Institute of Oceanography and Fisheries (NIOF) laboratory in Suez branch, Egypt. *L. vannamei* was obtained from the commercial shrimp hatchery, Berket Ghalioun, Kafr Al-Sheikh, Egypt. The shrimp specimens were acclimatized in an indoor fiberglass tank (6 m^2, 5 tons) at a temperature of 28–29 °C and pH of 7.8–8, with a salinity of 30-32 ppt for 7 days. During this time, they were fed twice daily at 8:00 and 20:00 with a commercial feed (38% CP, 9% CL).

In the feeding trial, 750 shrimp PL were selected with a mean weight of 0.05±0.09g. In the experimental units, shrimp PLs were directly divided into fiberglass cylindrical tanks of 200 liters. Previously, filtered seawater (salinity 32ppt) was utilized to fill the tanks, treated with tap water, and then dechlorinated with gentle aeration. Eighty PL shrimp were randomly allocated among nine tanks to create three experimental groups, each was given one of the following diets: a. control group fed with only the commercial diet without fermented fruit waste additives (T_0); b. group fed the commercial diet with added fermented grape bagasse (T_1), and c. group fed the commercial diet with added fermented banana peel (T_2). All tanks were kept on a photoperiod cycle of 12 hours of light and 12 hours of darkness. Approximately, 40-55% of the water in each tank was exchanged every 3 days for removing excess feed and faeces. Black plastic sheets were employed to cover the tanks to ensure minimal light penetration to mimic the natural habitat, minimizing the stress on the shrimp, and reducing escapes. The shrimp were fed daily three times at 8:00, 14:00 and 20:00 at 10% of initial weight (adjusted gradually to 5% at the end of the experiment) for 45 days. The daily feeding rations for each group were calculated and adjusted by estimating the average biomass sampled biweekly. During the experiment, water quality parameters such as water temperature, salinity, pH, ammonia (NH_3) and nitrite (NO_2) were periodically monitored. A multi-parameter device was used to measure the temperature and salinity of water daily between 9:00 a.m. and

10:00 a.m. However, ammonia (NH_3), nitrite (NO_2), and pH were biweekly measured using colourimetric analysis kits.

3. Determination of microbial community

Samples of water tanks and shrimp intestines were examined twice a month to determine the total bacteria count (TBC) and *Vibrio* spp. (TVC) count (colony forming units; CFU) using trypticase soy agar (TSA) and Thiosulphate Citrate Bile Salts Sucrose Agar (TCBS Agar), respectively **(Liu *et al.*, 2010).** Each sample from the experimental tank was serially diluted (1/10) with sterile saline solution (1.0% NaCl) until it was diluted to a concentration of 10^{-4} and 10^{-3}, and then plated (0.1 mL) in triplicate for TBC and TVC counts, respectively. Each colony in the incubated plates was then counted between 30 and 300 and expressed as a bacterial colony-forming unit (cfu) (at 37 and 28^{o}C for 24h, respectively) (**Ganesh *et al.*, 2010; Kumar *et al.*, 2014**).

The ratio of total *Vibrio* bacteria (TVC) to total bacteria count (TBC) (TVC: TBC) was selected for microbial analysis during the experiment time. The ratio of TVC: THB was obtained by the formula of **Sharawy *et al*. (2022**) as follows:

- **TVC/TBC (%) =** 100 × (Total *vibrio* count/total bacterial count)

4. Digestive enzymes activities

On the last day of the feeding experiment, the digestive enzymes (protease, amylase, lipase, and cellulase) were measured. Nine shrimp per group (3 shrimp/tank) were randomly chosen to examine the activity of the digestive enzymes in the hepatopancreas, stomach and intestine. The shrimp organs were divided, homogenised in sterile deionized water, and then separately weighed and homogenized with chilled buffer phosphate (0.65%, pH 7, 1:10 w/v). The supernatant was used for enzyme assays after being centrifuged (3000 g for 1 minute at 4°C). Samples were put in sterile tubes and kept cold at −80°C until they were needed. Protease, lipase, amylase and cellulase activities were determined by the casein digestion method (**Cherry & Crandall, 1932; Drapeau, 1976)** and DNS (Dinitro-salicylic acid) method **(Rick & Stegbauer, 1974; Marsden *et al.*, 1982).**

5. RNA isolation

For growth and immune gene expression assays, three shrimp specimens from each replication were randomly sampled at the final stage of the trial. Expressions of growth hormone (*GH*), insulin-like growth factor 1 (*IGFI*), beta-glucan-binding protein (*β-BGP*) and prophenoloxidase **(***Proph)* were determined from the hepatopancreas tissue as described by **Hassan *et al.* (2022)**. Hepatopancreas tissue was carefully dissected and

preserved at –80°C for the extraction of RNA by TRIzol®. The NanoDrop (BioDrop, United Kingdom) was used to assess the concentration and purity of RNA (quantified at 260 nm at the ratio of 260/280).

Isolated RNA was processed using RT-PCR beads following the company's guidelines for cDNA synthesis. The cDNA was tested for antioxidant and immune-related genes via qRT-PCR) (Thermo Fisher equipment). The programme began with a 15-minute heating session (95°C), then proceeded to 40 cycles for 15 seconds (95°C) with annealing temperatures ranging from 60 to 65°C based on the gene. Table (2) shows the primer sequences for the several genes used in this investigation. At the end of each run, the PCR products were subjected to a melt curve analysis. Lastly, a dissociation curve was generated by capturing fluorescence signals at 60°C and monitoring the temperature every 7 seconds until it approached 95°C. After standardisation with the *β-actin* gene, data were represented as fold change and compared to a control. The relative quantification of targeted genes was performed using the comparative threshold cycle (ct) with a fold change of $2^{\wedge-\Delta\Delta ct}$ of **Rao *et al.* (2013)**.

Table 2. The primer sequences used in qRT-PCR

Genes	Sequence	Amplicon size (bp)	Reference
GH **XM027360152**	**F: AATTTGCGCTTGCACTACGG R: ATCCGGTTGAGGTGTAGCTG**	**100**	**Designed by NCBI tool**
IGFI **KP420228**	**F: GTGGGCAGGGACCAAATC R: TCAGTTACCACCAGCGATT**	**123**	**Designed by NCBI tool**
β-GBP **AY249858**	**F: ACGAGAACGGACAAGAAGTG R: TTCAGCATAGAAGCCATCAGG**	**137**	**Wang *et al.*, 2008**
Proph **AY723296**	**F: CGGTGACAAAGTTCCTCTTC R: GCAGGTCGCCGTAGTAAG**	**122**	**Wang *et al.*, 2008**
β- actin **(house-keeping gene) AF300705**	**F: GCCCATCTACGAGGGATA R: GGTGGTCGTGAAGGTGTAA**	**121**	**Yang *et al.*, 2013**

6. Growth evaluation of the shrimp

Weight gain (WG), specific growth rate (SGR), survival (S%) and feed conversion ratio (FCR) were the performance indicators for *L. vannamei* which were calculated using **Tekinay and Davis (2001)** formula:

- $\textbf{Weight gain (WG)} = \text{Final weight (g)} - \text{Initial weight (g)}.$
- $\textbf{Specific growth rate (SGR, \%/day)} = \frac{(\text{Log final weight} - \text{log initial weight})}{\text{Time}} \times 100$
- $\textbf{Survival (\%)} = \frac{\text{Final shrimp no.}}{\text{Initial shrimp no.}} \times 100$
- $\textbf{Feed conversion ratio (FCR)} = \frac{\text{Diet consumed (g)}}{\text{WG (g)}}$

7. Economic value

Based on the local market prices, the following equations were used to establish the cost of the tested diets in L.E.:

- **Feed cost** = Amount of feed consumed (kg) × Price of 1kg (L.E.)
- **Total operating cost** = Feed cost + Shrimp cost
- **Total net production (kg)** = Weight gain × Survival number
- **Total income** = Total net production (kg)× Price of 1kg (L.E.)
- **Net return** = Total income – Total operating cost (L.E).

8. Data analysis

The values of water quality parameters, microbial load in the water and shrimp, digestive enzyme activity, gene expression, growth and survival were analyzed by one-way analysis of variance, followed by Duncan's Multiple Range Test to determine differences between treatments. All significant tests were at $P< 0.05$ levels. All analyses were carried out using IBM SPSS 19.0 software. All the results are presented as mean ± standard error (SD).

RESULTS

1. Water quality

As shown in Table (3), the results of the water quality parameters demonstrate that there were no significant differences ($P > 0.05$) among any of the parameters measured. Furthermore, the various groups' water quality measurements all stayed within the ranges allowed for shrimp production **(Van Wyk & Scarpa, 1999; Boyd & Clay, 2002)**.

Table 3. Water quality parameters of *L. vannamei* through 45 days of feeding on different fermented fruit waste supplements

Parameter	Treatments		
	T_0	T_1	T_2
Salinity ppt	32.01±0.12	32.13±0.33	32.07±0.27
Temp ^oC	28.06±1.1	27.90±1.04	27.87±1.02
pH	7.81±0.18	7.69±0.14	7.66±0.16
NH_3 (mg/L)	0.20±0.06	0.18±0.04	0.17±0.03
NO_2 (mg/L)	0.22±0.06	0.17±0.08	0.16±0.08

No significant variations among any measured water parameter.

2. Nutritional evaluation of experimental diet

The 45-day experimental diets were used to evaluate the different growth indices of *L. vannamei*. All growth indices and survival rates (S%) of *L. vannamei* PL showed superiority in different fermented fruit wastes (FFW) than the control group; results are presented in Table (4). On the other hand, the feed conversion ratio (FCR) marginally declined and ranged between 1.20 and 1.37, with the highest and lowest values in the T_0 and T_2, respectively. However, no significant differences were detected between the control and fermented grape bagasse groups (Table 4). The survival rate (S%) was significantly different among the experimental groups and control ($P< 0.05$), recording ranges between 82.08% and 85.83%. All the results indicated that FFW could improve the growth rate of *L. vannamei*.

Table 4. Growth efficiency and feed utilization of *L. vannamei* fed with different fermented fruit waste supplements

Parameter	Treatments		
	T_0	T_1	T_2
FBW (g)	**2.33±0.06^c**	**2.64±0.08^b**	**3.10±0.19^a**
WG (g)	**2.28±0.06^c**	**2.59±0.08^b**	**3.05±0.19^a**
SGR (%/day)	**8.50±0.06^c**	**8.77±0.06^b**	**9.13± 0.13^a**
FCR	**1.37±0.06^a**	**1.31±0.02^a**	**1.20±0.04^b**
S (%)	**82.08±0.72^c**	**84.17±0.72^b**	**85.83±0.72^a**

Averages in the same row with different superscript letters varied significantly from the control.

3. Determination of microbial load

The overall bacteria count in the water tanks and the shrimp intestine rose ($P< 0.05$) when FFW was added to the shrimp diet, as shown in Fig. (1). In the water, the T_1 has significantly increased in TBC and TVC, while there was no significant difference ($P> 0.05$) in T_0 and T_1 groups. For the shrimp intestine when compared with the control, TVC count, and TVC/TBC% decreased in different fruit wastes, and there was no significant difference ($P> 0.05$) in TBC between T_1 and T_2 groups. In both the control and other experimental groups, the TVC count in the gut of shrimp was greater than that of water. Furthermore, the TVC/TBC% in the water and shrimp intestine was significantly increased ($P< 0.05$) in the control group compared to groups with different fermented fruit wastes.

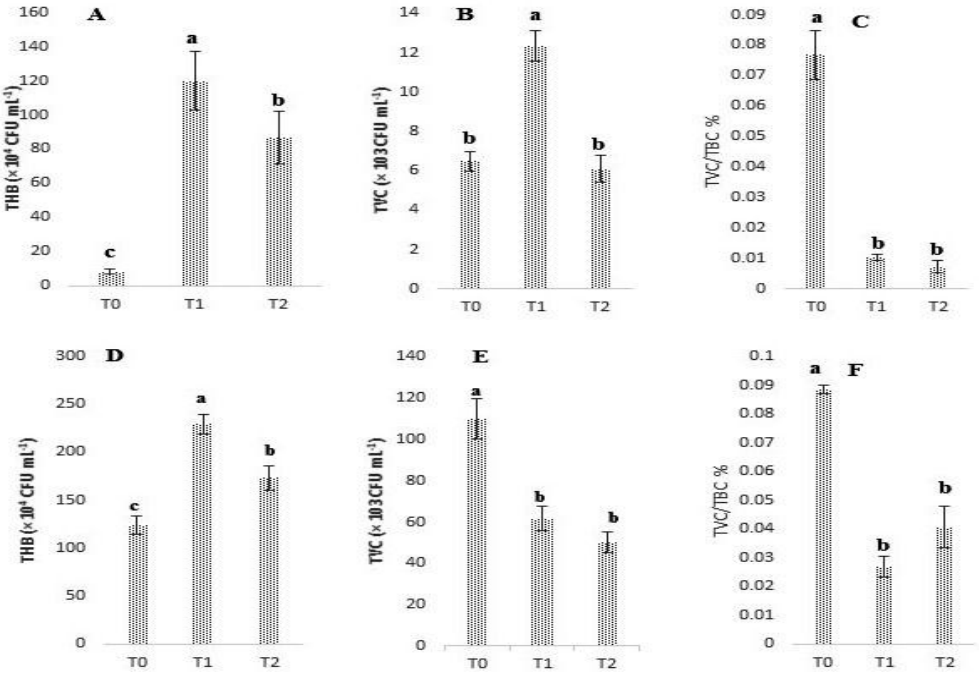

Fig. 1. In water, (A) total bacteria count (TBC), (B) *Vibrio* spp. (TVC), and (C) TVC/TBC (%) for shrimp intestine; (D) total bacteria count (TBC), (E) *Vibrio* spp. (TVC), and (F) TVC/TBC (%); of *L. vannamei* fed on different fermented fruit waste

Values for each treatment are means ± SD (range) of three replicate tanks. The means in the same row having different letters varied significantly (*P< 0.05, one-way ANOVA).

4. Digestive enzymes activities

The positive impact of shrimp feeding with FFW on protease activity is seen in Table (5). The protease activities of shrimp fed FFW were considerably higher (P< 0.05) than those of the control group in the hepatopancreas, stomach and intestine. The results of shrimp lipase and amylase activities are shown in Tables (6, 7). The activities of lipase and amylase in the hepatopancreas, intestine and stomach were considerably improved (P < 0.05) in the T_2, followed by the T_1 groups in comparison to the control group. While, the cellulase activity had significantly increased (p< 0.05) in the control group in the hepatopancreas, stomach and intestine compared to the fermented fruit waste supplements, as shown in Table (8). The results indicate that the fermentation process could improve the digestive enzymes of *L. vannamei*.

Table 5. The protease activity of shrimp fed different fruit wastes for 45 days

Enzyme	**Protease**		
Tissue	**T_0**	**T_1**	**T_2**
Hepatopancreas	**296.02±0.13^{c}**	**308.19±0.16^{b}**	**378.04±0.08^{a}**
Stomach	**256.21±0.03^{c}**	**286.48±0.35^{b}**	**304.15±0.18^{a}**
Intestine	**270.17±0.07^{b}**	**316.23±0.22^{a}**	**316.09±0.10^{a}**

Averages in the same row with different superscript letters varied significantly from the control.

Table 6. The lipase activity of shrimp fed different fruit waste for 45 days

Enzyme	Lipase		
Tissue	**T_0**	**T_1**	**T_2**
Hepatopancreas	**112.50±0.48^c**	**141.78±0.23^b**	**144.46±0.18^a**
Stomach	**70.63±0.55^c**	**110.06±0.06^b**	**130.33±0.49^a**
Intestine	**32.11±0.19^c**	**62.09±0.10^b**	**72.20±0.15^a**

Averages in the same row with different superscript letters varied significantly from the control.

Table 7. The amylase activity of shrimp fed different fruit waste for 45 days

Enzyme	Amylase		
Tissue	**T_0**	**T_1**	**T_2**
Hepatopancreas	**12.65±0.65^c**	**28.53±0.21^a**	**16.84±0.37^b**
Stomach	**70.63±0.55^c**	**110.06±0.06^b**	**130.33±0.49^a**
Intestine	**40.48±0.20^c**	**55.52±0.06^b**	**66.5±0.11^a**

Averages in the same row with different superscript letters varied significantly from the control.

Table 8. The cellulase activity of shrimp fed different fruit waste for 45 days

Enzyme	Cellulase		
Tissue	**T_0**	**T_1**	**T_2**
Hepatopancreas	**7.67±0.03^a**	**1.87±0.01^b**	**1.32±0.02^c**
Stomach	**1.59±0.02^a**	**0.71±0.01^b**	**0.75±0.01^c**
Intestine	**8.75±0.21^a**	**3.06±0.06^c**	**4.81±0.01^b**

Averages in the same row with different superscript letters varied significantly from the control.

5. Gene expression

FFW groups considerably up-regulated the expression levels of *GH, IGFI, β-BGP*, and *Proph* in the hepatopancreas tissue, as represented in Fig. (2). The expression levels of *GH* and *IGFI* were considerably higher ($P< 0.05$) in the hepatopancreas of shrimp fed on a fermented banana peel (T_2) group. However, there were no significant differences in the expression ($P> 0.05$) in T_0 and T_1 groups in *GH* and *IGFI* genes, as present in Fig. (2). In contrast, T_2 followed by T_1 were considerably higher in the levels of gene expression (*β-BGP* and *Proph*) in the hepatopancreas of shrimp, compared to the control group, as present in Fig. (2).

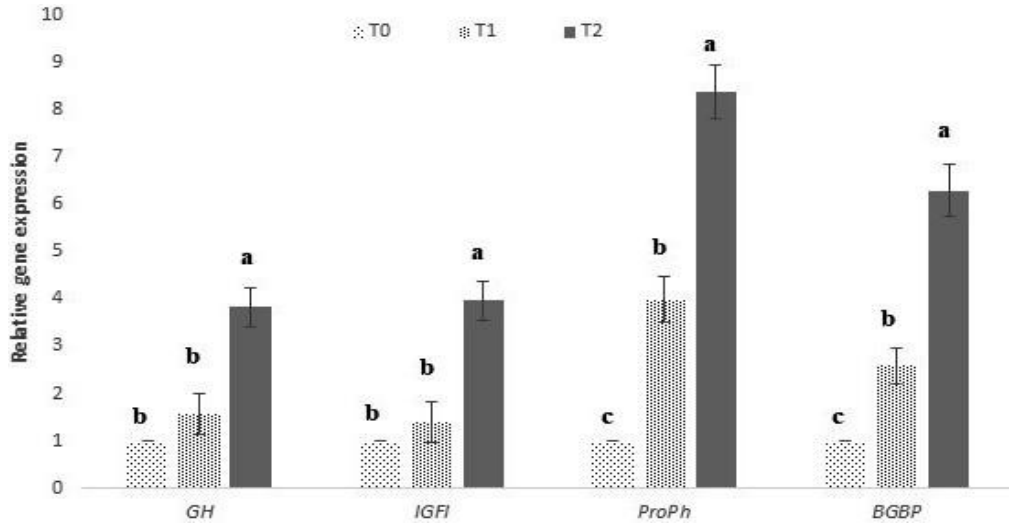

Fig. 2. Expression of *GH, IGFI, β-BGP*, and *Proph* in the hepatopancreas tissue of *L. vannamei* fed on different fermented fruit waste supplements

6. Economic value

The results of the economic assessment of the diets are shown in Table (9). The calculation was performed using the price at which the dietary elements might be purchased on the local market in 2020. According to the findings of the economic analysis, shrimp-fed fermented banana peels followed by fermented grape bagasse had the greatest total operating costs, total income, and net returns, whereas the shrimp-fed control diet recorded the lowest value. The inclusion of fermented grape bagasse and fermented banana peel in the diet led to the most dramatic effects, with decreases in costs of 38.75% and 35.26%, respectively, when compared to the control.

Table 9. Economic feasibility including fruit wastes in *L. vannamei* diets for 45 days

Treatment	Feed cost (LE/Kg)	Shrimp cost (LE/240PL)	Total operating cost	Total income	Net return
T_0	4.62 ± 0.53^{b}	72	327.38 ± 3.17^{b}	802.84 ± 1.87^{b}	475.45 ± 1.56^{b}
T_1	5.14 ± 0.52^{ab}	72	370.58 ± 4.23^{ab}	960.07 ± 1.42^{ab}	589.49 ± 1.06^{ab}
T_2	5.65 ± 0.27^{a}	72	412.23 ± 4.09^{a}	1171.63 ± 1.36^{a}	759.41 ± 1.00^{a}

Significant values (mean ± SD) are those in the same column with different superscript letters (P<0.05). The cost of 1 kg Grape bagasse and Banana peel were 3.5 and 4 L.E., respectively. Shrimp (1000PL) cost was 300 L.E and 1Kg of diet was 21 L.E.

DISCUSSION

Fruit waste can be recycled since it has high nutrients and is a rich source of compounds in the aquafeed industries (**Paini *et al.*, 2021**). According to **Fierascu *et al.* (2020)**, thousands of bioactive substances are produced from fruit waste and are used as

dietary supplements and immunostimulants, including polyphenols, pectins, carotenoids, etc. Additionally, the fermentation process is used to improve the nutritional quality of fruit wastes by lowering fiber and antinutritional factors (**Mones & Angeles, 2017; Fatmawati *et al.*, 2018**). Consequently, this study demonstrated that dietary FFW in shrimp diets could influence the growth rate of shrimp (*L. vannamei*), as seen by the lowered FCR and increased S%, WG, and SGR in FFW groups. Similar findings about the effectiveness of various fruit wastes in fostering growth were noticed in fish and shrimp aquaculture (**Saravana *et al.*, 2013; Sun, Yuan & Fang, 2014;Rattanavichai & Cheng, 2015; Rattanavichai *et al.*, 2017; Rosas *et al*,. 2022)**.

The right dosage of fermented plants improves the growth indicators of fishes and shrimps. The supplementation of fermented grape bagasse (FGB) and fermented banana peel (FBP) at a rate of 2.5% for 45 days enhanced FBW, WG, SGR, FCR and S in *L. vannamei*. These findings follow the results presented on FBP in different fish and prawn species, for example, prawn (**Rattanavichai & Cheng, 2015; Rattanavichai *et al.*, 2017**), rohu (**Giri *et al.*, 2016**), and red tilapia (**Mones & Angeles, 2017)**. However, the study of **Karaket *et al.* (2021)** postulated that the addition of ripe bananas to supplementary diets did not affect growth rates and may be utilised in red tilapia farming as an immunostimulant to improve fish health. This indicates that the fermentation process enhances the nutritional value of fruit waste by transforming insoluble proteins into soluble components and raising the quantities of lysine as well as vitamins B and C.

According to research by **Harikrishnan *et al.* (2021)**, *Labeo rohita* fed with 200mg of grape pomace flour (GPF) showed considerably improved growth rates, immune defence systems and antioxidant status compared to fish given 300mg of GPF. However, **Rosas *et al.* (2022)** found that it is possible to add up to 2.5% of grape bagasse to shrimp diets, which has a substantial impact on growth and feed conversion rate. Furthermore, even at this level of inclusion, there was no noticeable difference in survival between all treatments and the control (ranging between 80.5 to 91%). This explains why significant differences in FBW, WG, SGR, FCR and S% between the T_1 and T_2 groups were found in the current study. This reason may be attributed to the abundance of nutrients in banana peels, including soluble and insoluble fibers, vitamins, minerals, high levels of B vitamins (B-12 and B-6) as well as polyphenols, carotenoids, sugar (sucrose, fructose, glucose), and other bioactive molecules that have a positive impact on both human and animal health **(González-Montelongo *et al.*, 2010; Mohapatra *et al.*, 2010).**

The growth-promoting effect of fermented fruit wastes on fish and shrimp is possibly connected to the fact that the antimicrobial properties in fruit wastes and Brewer's yeast, *Saccharomyces cerevisiae,* which regulate intestinal digestive enzyme composition and intestinal microbiota and then facilitate nutrient absorption and digestion (**Roy & Lingampeta, 2014; Saleem & Saeed, 2020**). Thus, fermented fruit peels, known as eco-enzyme, could be an alternative method for pulp irrigation. In the current study, FFW as an immunostimulant supplement has significant effects on culture pond water bacteria

and gastrointestinal bacteria on the growth and development of shrimp. According to a similar model, **MacLennan *et al.* (2002)** reported that aquatic animal's growth was found to be aided by nutritional supplements that have detectable impacts (direct and indirect) on the microbiome.

In the water tank, both bacteria counts of TBC and TVC were significantly increased in T_1 followed by T_2. This is due to the use of red grapes as the substrate for making functional fermented beverages having selected lactic acid bacteria regarded as the most important bacteria concerning food fermentation, pharmaceutical and special dietary applications (**Di Cagno *et al.*, 2010**). **Kurt and Cekmecelioglu (2021)** found that the use the fermented grape pomace could be a good source of *Bacillus subtilis*, which can manufacture cellulase well. On the contrary, **Chuchird *et al.* (2017)** found no differences in the total bacteria and *Vibrio* counts in control and phytobiotics from the yeast cell wall, spent hops, and grape pomace, which demonstrated that polyphenols' antibacterial abilities were not a crucial factor.

Acar *et al.* (2015), **Baba *et al.* (2016)** and **Salem *et al.* (2019)** found that the dietary citrus peel supplements had the potential to decrease the total bacterial count, including *Staphylococcus* spp., *Vibrio* spp., *Salmonella* spp. in fish guts. For evidence, numerous studies have examined how prebiotics affect the microbiota of the gut (**Merrifield *et al.*, 2011; Kühlwein *et al.*, 2013**). Additionally, various research articles have noticed that consuming medicinal plants and their compounds increases the viral and bacterial resistance of fish and shrimp (**Pourmozaffar *et al.*, 2019**). For instance, *Labeo rohita*'s resistance to *Aeromonas hydrophila* was enhanced by dietary banana peel flour (**Giri *et al.*, 2016**). That was attributed by the authors to the antibacterial properties of the active substances found in banana peels. As a result, the effectiveness of ingesting fruit peel to lower the bacterial count in the current investigation might be related to its naturally active antimicrobial compounds, such as ketones, aldehydes, and phenols (**Jing *et al.*, 2014**). Therefore, maintaining a well-balanced and healthy gut flora, as seen when yeast and herbs (YAH) are included in the shrimp diet may lead to more flexible and strong shrimps that are more inclined to react to stressful circumstances (**Servin, 2020**). In addition, the different fermented fruit wastes as phytogenic (immunostimulant) supplements may have antibacterial activity against *Vibrio* spp. and may improve the intestinal microbiota.

The results of this study showed that FFW could increase the activities of all enzymes in the hepatopancreas, stomach and intestine of shrimp by aiding the digestive enzymes in breaking down the nutrients in the feed, thereby increasing feed efficiency (**Zhang *et al.*, 2021; Ghafarifarsani *et al.*, 2022)**. The improvement of growth performance of *L. vannamei* PL in this study may be associated with the improvement of digestive enzymes induced by fermented fruit wastes as immunostimulant supplements, especially in FBP. Protease activities showed an increase in hepatopancreas and intestine in shrimp *L.*

vannamei fed with FBP followed by FGP. This reason may be due to the fact that bananas are easily digestible fruits, rich in components that induce digestion as well as serving as appetite stimulants.

Equivilant to the amylase activities that showed an increase in the stomach and intestine of shrimp (*L. vannamei*) feeding with fermented fruit wastes, **Zamani *et al.* (2021)** also found that after supplementing with grape seed oil in rainbow trout diets, total alkaline protease, trypsin and lipase activities reduced, but growth performance and α-amylase activity improved. Many by-products including fruit waste, contain polyphenols, anthocyanin, pigments, essential oils, minerals, fatty acids and bioactive peptides with potential applications as food antioxidants, polyunsaturated fatty acids, and a variety of nutrients. (**Rafiq *et al.*, 2018; Gandhi *et al.*, 2020).**

Banana peels are abundant in potassium, polyphenols, flavonoids, catecholamines, fibre and vital amino acids **(Vu *et al.*, 2019)**. Interestingly, the amount of polyphenols in banana peels is around three times more than in the fruit's flesh **(González-Montelongo *et al.*, 2010)**. Thus, the use of diets containing these bioactive components has enhanced prawn growth, with positive outcomes like a strengthened immune system and expanded digestive ability. These results are in agreement with the findings reported by **Rattanavichai and Cheng (2015)** and **Rattanavichai1 *et al.* (2017)**. Besides, **Fatmawati *et al.*** (**2018**) showed the possibility of using fermented banana peel as a plant food additive for aquatic animals' feed, which led to an increase in the protein content, reducing the fiber content of the amyloid enzyme to increase the digestibility of the feed. The increase in the activity of digestive enzymes could be driven by the fermentation activity of probiotic yeast (*S. cerevisiae*). Therefore, adding probiotics to the meal improves the efficiency of nutritional absorption. (**Gibson & Roberfroid, 1995**). **Sumon *et al.* (2018)** showed that, the probiotic-treated food greatly enhanced shrimp growth, suggesting that it may improve shrimp health and nutrition metabolism, which is consistent with the current study.

Like other crustaceans, *L. vannamei* lacks specialized immunity; alternatively, they rely on innate immune processes, which include cellular and humoral responses to defend themselves against infections (**Vazquez *et al.*, 2009**). Most medicinal plants, including banana peel and grape bagasse have immunostimulant qualities that can be used to enhance health and prevent disease **(Rattanavichai & Cheng, 2015; Rosas *et al.*, 2022)**. Furthermore, the use of fruit by-product as phytogenic and immunostimulants in aquatic animals were reported to enhance growth, immunity and resistance to pathogens infection **(Saravana *et al.*, 2013; Sun *et al.*, 2014; Chuchird *et al.*, 2017)**. This is in line with our finding that the highest value of *GH, IGFI, β-GBP* and *Proph* genes were observed in shrimps fed the diet with FBP (Fig. 2) **(Rattanavichai & Cheng, 2014, 2015; Rattanavichai *et al.*, 2015).**

Our results showed no significant difference ($P > 0.05$) between T_1 and T_0 groups in *GH* and *IGFI*, but growth performance in T_1 was higher than control. The study of **Rosas**

***et al.* (2022)** found that shrimp development and feed conversion could be altered by the addition of up to 2.5% of grape bagasse (GB). On the other hand, since GB has anti-nutritional qualities, it is advised to investigate levels of safe insertion (was 2.5% GB in our study). This indicates that there have been some unfavorable impacts on animal growth that have been attributed to the high levels of polyphenols (PPS) in the grape. Furthermore, the grape is an abundant source of tannin (**Vallet *et al.*, 1994)**, while elevated concentrations of such compounds may slow down nitrogen absorption, which can impact growth rates. According to **Butler and Rogler (1992)**, tannins do not affect feed digestibility, but enzymes' capacity to metabolize protein is remarkable. This indicates that fermented grape bagasse did not affect growth-related genes but positively affected immune-related genes. These results are consistent with **those of Harikrishnan *et al.* (2021)** who found that, fish (*Labeo rohita*) fed grape pomace flour showed that immune, antioxidant and anti-inflammatory-related gene mRNA expression was significantly upregulated in head kidney tissues. Thus, grape and their by-products comprise diverse phenolic compounds such as RVS, quercetin, procyanidins and other compounds that may be associated with anti-inflammatory and antioxidant activity **(Xia *et al.*, 2010)**. In another study, **Chuchird *et al.* (2017)** when compared to other groups, it was shown that the immunostimulatory impact of the yeast cell wall greatly increased the shrimp's total hemocyte numbers, phagocytosis actions, phenoloxidase operations and bactericidal activities. This outcome is thought to be the result of both catechin and proanthocyanidin, as well as the yeast cell wall, which includes an important quantity of β-glucans, a potent immunostimulant **(Ringø *et al.*, 2012)**.

Since, it is possible, as was found in this study, to add fermented fruit wastes to the diets of shrimp (*L. vannamei*) without impairing growth, such an addition to the diet offers intriguing possibilities for sustainable aquaculture techniques. Rendering to the findings of the economic research, the highest total operating cost, total income and net return were observed for shrimp-fed fermented banana peels followed by fermented grape bagasse, while the shrimp-fed control diet recorded the lowest value. According to reports, the overall cost of feeding shrimp accounts for about 40% of the total cost of producing shrimp (**Kumar *et al.*, 2016**). In this study, it was found that adding fermented grape bagasse and fermented banana peel to the diet resulted in reductions in costs of 38.75% and 35.26%, respectively, as compared to the group receiving the control diet. Probiotics, exogenous digestive enzymes, prebiotics and phytobiotics are examples of feed additives with known advantages in aquaculture. These benefits include both positive direct and indirect impacts on living organisms and the environment, enhancing feed conversion, immunological boost, disease control and water quality that support the financial performance of fish farming. Moreover, it will be anticipated to lower feed efficiency, which will lower the cost of fish production (**Dawood *et al.*, 2022**).

The results indicate that the used fruit wastes (grape bagasse and banana peels) improved the intestinal microbial, digestive enzymes, shrimp growth rates and health.

Therefore, a primary objective for modern aquaculture is to create efficient and safe solutions for prophylaxis and stimulating the immune system (strengthening the immune system versus pathogens and promoting health), which will result in better aquaculture production. Selecting immune-nutrition and functional additives to the aquafeed promotes animal immunological resistance and improves shrimp performance in aquaculture.

CONCLUSION

Certainly, using fruit by-products is undoubtedly essential to promoting aquaculture that is more environmentally friendly and economically viable. The results of this study showed that the utilization of fruit wastes, which are available in large quantities at low cost upon improving its nutritional quality through the fermentation process to enhance protein and lower fiber and anti-nutritional factors is promising and effective. The fermented fruit wastes have the potential to be valuable feed additions for the long-term success of the shrimp aquaculture industry. The present study showed that the addition of 2.5% fermented fruit wastes to the shrimp diet can enhance shrimp development, decrease intestinal *Vibrio* count, increase digestive enzyme activity, and immunological response. In addition,the results indicate that using these natural compounds as feed supplements and immunostimulant affect the antioxidant enzymes' activities. However, fermented grape bagasse and fermented banana peel increased growth performance, feed utilization and survival rate. Thus, it can be used as a phytobiotic and immunostimulant for aqua life.

REFERENCES

Acar, Ü.; Kesbiç, O. S.; İnanan, B. E. and Yılmaz, S. (2019). Effects of dietary Bergamot (*Citrus bergamia*) peel oil on growth, haematology and immune response of European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture Research, 50*(11): 3305-3312.

AOAC, (1995). Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Ashry, O. A.; Khouraiba, H. M.; Mohamed, M. A. and Sharawy, Z. Z. (2023). Influence of different fermented fruit wastes phytobiotic as feed additive on zootechnical performance, bacteriological analysis, digestive enzymes, and immune response of Litopenaeus vannamei. *Egyptian Journal of Aquatic Biology & Fisheries*, *27*(4).

Baba, E.; Acar, Ü.; Öntaş, C.; Kesbiç, O. S. and Yılmaz, S. (2016). Evaluation of Citrus limon peels essential oil on growth performance, immune response of Mozambique tilapia *Oreochromis mossambicus* challenged with *Edwardsiella tarda*. Aquaculture, 465: 13–18.

Boyd, C. and Clay, J. (2002). Evaluation of Belize Aquaculture, Ltd: A Superintensive Shrimp Aquaculture System". Report prepared under the World Bank, NACA, WWF and FAO Consortium Program on Shrimp Farming and the Environment. Work in Progress for Public Discussion. (p. 17). Consortium

Butler, L. G. and Rogler, J. C. (1992). Biochemical mechanisms of the antinutritional effects of tannins. *ACS Symposium Series*, *506*: 298–304.

CAPMAS, (2020). Central Agency for Public Mobilization and Statistics, Issues many specialized statistical publication in different agriculture fields concerning (agriculture, fish, animal products adding to the agriculture ownership), the main source is the Ministry of Agriculture and Land Reclamation in addition to other organizations. Annual Bulletin of the estimated income from the agricultural sector.

Chen, S.J.; Guo, Y.C.; Espe, M.; Yang, F.; Fang, W.P.; Wan, M.G.; Niu, J.; Liu, Y.J. and Tian, L.X., (2018). Growth performance, haematological parameters, antioxidant status and salinity stress tolerance of juvenile Pacific white shrimp (*Litopenaeus vannamei*) fed different levels of dietary myo- inositol. *Aquaculture Nutrition*, *24*(5): 1527-1539.

Cherry, I. S.; and Crandall Jr, L. A. (1932). The specificity of pancreatic lipase: its appearance in the blood after pancreatic injury. *American Journal of Physiology-Legacy Content*, *100*(2): 266-273.

Chuchird, N.; Niyamosatha, H.; Rairat, T. and Keetanon, A. (2017). Research Article Effect of Dietary Phytobiotics Products on Growth, Immune Responses and *Vibriosis* Resistance in *Litopenaeus vannamei*.

Currie, K. L.; Purvis, M.; Bansemer, M.; Harris, J. O. and Stone, D. A. (2019). Dietary inclusion of Acti-Meal improves growth and feed utilisation of green lip abalone (*Haliotis laevigata*). *Aquaculture*, 498: 364-370.

Dawood, M. A.; Habotta, O. A.; Elsabagh, M.; Azra, M. N.; Van Doan, H.; Kari, Z. A. and Sewilam, H. (2022). Fruit processing by- products in the aquafeed industry: a feasible strategy for aquaculture sustainability. *Reviews in Aquaculture*, *14*(4): 1945-1965.

Dhillon, G. S.; Kaur, S. and Brar, S. K. (2013). Perspective of apple processing wastes as low-cost substrates for bioproduction of high value products: A review. *Renewable and sustainable energy reviews*, *27*: 789-805.

Di Cagno, R.; Mazzacane, F.; Rizzello, C.G.; De Angelis, M.; Giuliani, G.; Meloni, M.; De Servi, B. and Gobbetti, M., (2010). Synthesis of γ-aminobutyric acid (GABA) by *Lactobacillus plantarum* DSM19463: functional grape must beverage and dermatological applications. *Applied microbiology and biotechnology*, *86*: pp.731-741.

Drapeau, G. (1974). Protease from staphylococcus aureus. In: Lorand, B.L. (Ed.), Methods in Enzymology. Academic Press, NY, USA, p. 469 pp. Eka, H.D., TajulAris, Y., Wan Nadiah, W.A., 2010. Potential use of Malaysian rubber (Hevea brasiliensis) seed as food, feed and biofuel. Int. Food Res. J. 17: 527–534.

FAO, (2020). Sustainability in action. *State of World Fisheries and Aquaculture.* Rome, 200.

FAO, (2022). Food and agriculture organization of the United Nations. Crop Statistics; 2020. (accessed January 23, 2022)

Fatmawati, A.; Simangunsong, T. L.; Hadinata, S. and Adiarto, M. (2018). Solid-state fermentation of banana peels potential study for feed additive. In *Matec Web of Conferences: The 2nd International Conferences on Technology, Innovation, Society and Science-to-Business (ICTIS 2018)* (Vol. 215). EDP Sciences.

Fierascu, R. C.; Sieniawska, E.; Ortan, A.; Fierascu, I. and Xiao, J. (2020). Fruits by-products–A source of valuable active principles. A short review. *Frontiers in Bioengineering and Biotechnology*, 8: 319.

Ganesh, E.A.; Das, S.; Chandrasekar, K.; Arun, G. and Balamurugan, S. (2010). Monitoring of total heterotrophic bacteria and *Vibrio spp.* in an aquaculture pond. *Current Research Journal of Biological Sciences*, *2*(1): 48-52.

Gandhi, G.R.; Vasconcelos, A.B.S.; Wu, D.T.; Li, H.B.; Antony, P.J.; Li, H.; Geng, F.; Gurgel, R.Q.; Narain, N. and Gan, R.Y. (2020). Citrus flavonoids as promising phytochemicals targeting diabetes and related complications: A systematic review of in vitro and in vivo studies. *Nutrients*, *12*(10): p.2907.

Ghafarifarsani, H.; Hoseinifar, S. H.; Javahery, S.; Yazici, M. and Van Doan, H. (2022). Growth performance, biochemical parameters, and digestive enzymes in common carp (*Cyprinus carpio*) fed experimental diets supplemented with vitamin C, thyme essential oil, and quercetin. *Italian Journal of Animal Science*, *21*(1): 291-302.

Giri, S. S.; Jun, J. W.; Sukumaran, V. and Park, S. C. (2016). Dietary administration of banana (*Musa acuminata*) peel flour affects the growth, antioxidant status, cytokine responses, and disease susceptibility of rohu, *Labeo rohita*. *Journal of immunology research*, *2016*.

Gibson, G. R. and Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of nutrition*, *125*(6): 1401-1412.

González-Montelongo, R.; Lobo, M. G. and González, M. (2010). Antioxidant activity in banana peel extracts: Testing extraction conditions and related bioactive compounds. *Food Chemistry*, *119*(3): 1030-1039.

Harikrishnan, R.; Devi, G.; Van Doan, H.; Balasundaram, C.; Esteban, M. Á. and Abdel-Tawwab, M. (2021). Impact of grape pomace flour (GPF) on immunity and immune-antioxidant-anti-inflammatory genes expression in Labeo rohita against Flavobacterium columnaris. *Fish & Shellfish Immunology*, 111: 69-82.

Hassan, S.A.H.; Sharawy, Z.Z.; El Nahas, A.F.; Hemeda, S.A.; El- Haroun, E.; Abbas, E.M. (2022). Carbon sources improve water quality, microbial community, immune- related and antioxidant genes expression and survival of challenged Postlarvae in biofloc system. *Aquaculture Research, 53(17): 5902-5914.*

Hidalgo, M. C.; Urea, E. and Sanz, A. (1999). Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. *Aquaculture*, *170*(3-4): 267-283.

Jing, L.; Lei, Z.; Li, L.; Xie, R.; Xi, W.; Guan, Y.; Sumner, L.W. and Zhou, Z., (2014). Antifungal activity of citrus essential oils. *Journal of agricultural and food chemistry*, *62*(14): pp.3011-3033.

Karaket, T.; Somtua, C.; Ponza, P. and Areechon, N. (2021). Potential Benefits of Ripe Cultivated Banana (Musa sapientum Linn.) in Practical Diet on Growth Performance, Feed Utilization and Disease Resistance of Hybrid Tilapia (Oreochromis niloticus x O. mossambicus). *Turkish Journal of Fisheries and Aquatic Sciences*, *21*(10): 501-508.

Kühlwein, H.; Emery, M.; Rawling, M.; Harper, G.; Merrifield, D.; Davies, S. and Kühlwein, H. (2013). Effects of a dietary β- (1,3)(1,6)- D- glucan supplementation on intestinal microbial communities and intestinal ultrastructure of mirror carp (*Cyprinus carpio L.*). Journal of Applied Microbiology, 115: 1091–1106

Kumar, B.; Sharma, R.; Lakra, W. S.; Sharma, A.; Prakesh, S. and Sharma, M. M. (2016). Economic assessment of shrimp farming (*Litopenaeus vannamei*) in Gujarat—A profitable venture. *International Journal of Innovative Research in Science, Engineering and Technology*, *5*(8): 15334–15342.

Kumar, S.; Shyne Anand, P.S.; De, D.; Sundaray, J.K.; Ananda Raja, R.; Biswas, G.; Ponniah, A.G.; Ghoshal, T.K.; Deo, A.D.; Panigrahi, A. and Muralidhar, M. (2014). Effects of carbohydrate supplementation on water quality, microbial dynamics and growth performance of giant tiger prawn (*Penaeus monodon*). *Aquaculture international*, 22: 901-912.

Kurt, A. S. and Cekmecelioglu, D. (2021). Bacterial cellulase production using grape pomace hydrolysate by shake-flask submerged fermentation. *Biomass conversion and biorefinery*, 1-8.

Leyva-López, N.; Lizárraga-Velázquez, C. E.; Hernández, C. and Sánchez-Gutiérrez, E. Y. (2020). Exploitation of agro-industrial waste as potential source of bioactive compounds for aquaculture. *Foods*, *9*(7): 843.

Liu, K. F.; Chiu, C. H.; Shiu, Y. L.; Cheng, W. and Liu, C. H. (2010). Effects of the probiotic, *Bacillus subtilis* E20, on the survival, development, stress tolerance, and immune status of white shrimp, *Litopenaeus vannamei* larvae. *Fish and shellfish immunology*, *28*(5-6): 837-844.

MacLennan, A. H.; Wilson, D. H. and Taylor, A. W. (2002). The escalating cost and prevalence of alternative medicine. *Preventive Medicine*, 35: 166–173.

Marsden, W. L.; Gray, P. P.; Nippard, G. J. and Quinlan, M. R. (1982). Evaluation of the DNS method for analysing lignocellulosic hydrolysates. Journal of Chemical Technology and Biotechnology, 32(7- 12): 1016-1022.

Merrifield, D. L.; Harper, G.; Mustafa, S.; Carnevali, O. Picchietti, S. and Davies, S. J. (2011). Effect of dietary alginic acid on juvenile tilapia (*Oreochromis niloticus*) intestinal microbial balance, intestinal histology and growth performance. Cell and Tissue Research, 344: 135–146.

Mohapatra, D.; Mishra, S. and Sutar, N. (2010). Banana and its by-product utilisation: An overview. Journal of Scientific, & Industrial Research, 69: 323–329.

Mones, G. H. and Angeles, I. P. (2017). Performance of Red tilapia (*Oreochromis sp.*) fed diet with fermented banana (*Musa acuminata 9 Balbisiana*) peel at different stages of ripeness following Aeromonas hydrophila infection. *International Journal of Agricultural Technology*, *13*(7.1): 1041-1064.

Paini, J.; Benedetti, V.; Ail, S. S.; Castaldi, M. J.; Baratieri, M. and Patuzzi, F. (2021). Valorization of wastes from the food production industry: A review towards an integrated agri-food processing biorefinery. *Waste and Biomass Valorization*, 1-20.

Pereira, A. and M. Maraschin (2015): Banana (*Musa spp*.) from peel to pulp: Ethnopharmacology, source of bioactive compounds and its relevance for human health. J. Ethnopharmacol., 160:149–163.

Pourmozaffar, S.; Hajimoradloo, A.; Paknejad, H. and Rameshi, H. (2019). Effect of dietary supplementation with apple cider vinegar and propionic acid on hemolymph chemistry, intestinal microbiota and histological structure of hepatopancreas in white shrimp, *Litopenaeus vannamei. Fish & shellfish immunology*, 86: 900-905.

Rafiq, S.; Kaul, R.; Sofi, S.A.; Bashir, N.; Nazir, F. and Nayik, G. A. (2016). Citrus peel as a source of functional ingredient: A review. Journal of the Saudi Society of Agricultural Sciences, DOI: 10.1016/j.jssas.2016.07.006

Rao, A. R.; Baskaran, V.; Sarada, R. and Ravishankar, G. A. (2013). In vivo bioavailability and antioxidant activity of carotenoids from microalgal biomass—A repeated dose study. *Food research international*, *54*(1): 711-717.

Rattanavichai, W. and Cheng, W. (2015). Dietary supplement of banana (*Musa acuminata*) peels hot-water extract to enhance the growth, anti-hypothermal stress, immunity and disease resistance of the giant freshwater prawn, *Macrobrachium rosenbergii*. *Fish & Shellfish Immunology*, *43*(2): 415-426.

Rattanavichai, W.; Cheng, W. and Chang, C. C. (2017). Simplified processing method of banana (Musa acuminata) peels possess the improvement in immunological responses of Macrobrachium rosenbergii. *Aquaculture Research*, *48*(10): 5202-5213.

Rick, W. and Stegbauer, H. P. (1974). α-Amylase measurement of reducing groups. In *Methods of enzymatic analysis* (pp. 885-890). Academic Press.

Ringø, E.; Olsen, R. E.; Vecino, J. G.; Wadsworth, S. and Song, S. K. (2012). Use of immunostimulants and nucleotides in aquaculture: a review. *J Mar Sci Res Dev*, *2*(1), 104.

Roberts, J. S.; Kidd, D. R. and Padilla-Zakour, O. (2008). Drying kinetics of grape seeds. *Journal of Food Engineering*, *89*(4): 460-465.

Rosas, V. T.; Mureb, R. A.; Monserrat, J. M.; Wasielesky Jr, W. and Tesser, M. B. (2022). Inclusion of grape bagasse (Vitis sp.) in the diet of white shrimp (*Litopenaeus vannamei*) and its effects on growth and antioxidant system. *Aquaculture Research*, *53*(13): 4805-4813.

Sakai, M. (1999). Current research status of fish immunostimulants. *Aquaculture*, *172*(1-2): 63-92.

Saleem, M. and Saeed, M. T. (2020). Potential application of waste fruit peels (orange, yellow lemon and banana) as wide range natural antimicrobial agent. *Journal of King Saud University-Science*, *32*(1): 805-810.

Salem, M. E. S.; Abdel- Ghany, H. M.; Sallam, A. E.; El- Feky, M. M. and Almisherfi, H. M. (2019). Effects of dietary orange peel on growth performance, antioxidant activity, intestinal microbiota and liver histology of Gilthead sea bream (*Sparus aurata*) larvae. *Aquaculture Nutrition*, *25*(5): 1087-1097.

Saravana Bhavan, P.; Kirubhanandhini, V.; Muralisankar, T.; Manickam, N. and Srinivasan, V. (2013). EFFECTS OF FRUITS WASTES (APPLE, GRAPE AND ORANGE) INCORPORATIONS ON THE GROWTH OF THE FRESHWATER PRAWN MACROBRACHIUM ROSENBERGII. *Asian Journal of Science and Technology*, *4*(10): 075-081.

Servin, K. (2020). The evaluation of functional feeds additives in commercial conditions with the pacific white leg shrimp (Doctoral dissertation, University of Plymouth).

Sharawy, Z. Z.; Abbas, E. M.; Abdelkhalek, N. K.; Ashry, O. A.; Abd El-Fattah, L. S.; El-Sawy, M. A.; Helal, M. F. and El-Haroun, E. (2022). Effect of organic carbon source and stocking densities on growth indices, water microflora, and immune-related genes expression of *Litopenaeus vannamei* Larvae in intensive culture. *Aquaculture*, *546*: 737397.

Sumon, M. S.; Ahmmed, F.; Khushi, S. S.; Ahmmed, M. K.; Rouf, M. A.; Chisty, M. A. H. and Sarower, M. G. (2018). Growth performance, digestive enzyme activity and immune response of Macrobrachium rosenbergii fed with probiotic Clostridium butyricum incorporated diets. *Journal of King Saud University-Science*, *30*(1): 21-28.

Sun, H. Lv. H.; Yuan, G. and Fang, X. (2014). Effect of grape seed extracts on the melanosis and quality of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage. Food Science and Technology Research, 20(3): 671-677.

Tekinay, A.A. and Davies, S.J. (2001). Dietary carbohydrate level influencing feed intake, nutrient utilization and plasma glucose concentration in the rainbow trout, *Oncorhynchus mykiss*. *Turkish Journal of Veterinary and Animal Sciences*., 25: 657- 666.

Vallet, J.; Rouanet, J. M. and Besançon, P. (1994). Dietary grape seed tannins: Effects on nutritional balance and on some enzymic activities along the crypt-villus axis of rat small intestine. *Annals of Nutrition and Metabolism*, *38*:75–84.

Van Hai, N. (2015). The use of medicinal plants as immunostimulants in aquaculture: A review. Aquaculture, 446: 88–96.

Van Wyk, P. and Scarpa, J. (1999). Water quality requirements and management. In Farming marine shrimp in recirculating freshwater systems (pp. 141–161). Harbor Branch Oceanographic Institution.

Vazquez, L.; Alpuche, J.; Maldonado, G.; Agundis, C.; Pereyra-Morales, A. and Zenteno, E. (2009). Review: Immunity mechanisms in crustaceans. *Innate Immunity*, *15*(3): 179–188.

Vu, H. T.; Scarlett, C. J. and Vuong, Q. V. (2019). Changes of phytochemicals and antioxidant capacity of banana peel during the ripening process; with and without ethylene treatment. *Scientia Horticulturae*, *253*: 255-262.

Wang, S.; Aurora, A.B.; Johnson, B.A.; Qi, X., McAnally, J.; Hill, J.A.; Richardson, J.A.; Bassel-Duby, R. and Olson, E.N., (2008). The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. *Developmental cell*, *15*(2): pp.261-271.

Xia, E. Q.; Deng, G. F.; Guo, Y. J. and Li, H. B. (2010). Biological activities of polyphenols from grapes. International journal of molecular sciences, 11(2): 622-646.

Yang, S. P.; Wu, Z. H. and Jian, J. C. (2013). Effect of marine red yeast *Rhodosporidium paludigenum* on antioxidant-related gene expression in *Litopenaeus vannamei.* Israeli Journal of Aquaculture Bamidgeh, 65(817): 1–6.

Zamani, A. ; Khajavi, M.; Moafi, A. and Gisbert, E. (2021). Utilization of grape seed oil as a dietary lipid source in rainbow trout (*Oncorhynchus mykiss*) diets. *Journal of Applied Aquaculture*, *33*(2): 126-149.

Zhang, Y.; Bian, Y.; Cui, Q.; Yuan, C.; Lin, Y.; Li, J. and Meng, P. (2021). Effect of dietary complex Chinese herbal medicine on growth performance, digestive enzyme activities in tissues and expression of genes involved in the digestive enzymes and antioxidant enzymes and bacterial challenge in *Litopenaeus vannamei*. *Aquaculture Research*, *52*(12): 6741-6750.