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Influence of different fermented fruit wastes phytobiotic as feed additive on zootechnical performance, bacteriological analysis, digestive enzymes, and immune response of *Litopenaeus vannamei*

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ABSTRACT

A 45-day feeding trial using solid-state fermented orange peel (FOP) and prickly pear peel (FPP) by Brewer's yeast, *Saccharomyces cerevisiae* was conducted to evaluate water quality, microbiological analysis, digestive enzyme activities, gene expression, growth performance and survival of shrimp *Litopenaeus vannamei* as phytobiotic products feed additives. Three treatments were formulated: Control (C, only commercial diet); FOP (2.5% FOP + commercial diet) and FPP (2.5% FPP + commercial diet). 720 post-larvae shrimp (0.05± 0.09g) were divided in three experimental treatments, with three replicate tanks each and stocked with 80 PL. It was observed that weight gain, feed conversion ratio, specific growth rate and survival % differed significantly (P<0.05) between the FPP and the control. The total *vibrio* population in water and shrimp intestines declined significantly (P<0.05) in FOP and FPP; whilst, the heterotrophic was significantly enhanced (P<0.05) in FOP and FPP, compared to control. All digestive enzyme activities were influenced by an increase in the hepatopancreas, stomach and intestine of shrimp fed with FOP and FPP diets. Furthermore, both growth and immune-related gene expressions increased in FPP. The present study showed that the addition of 2.5% solid-state fermented orange or prickly pear peels by Brewer's yeast, *Saccharomyces cerevisiae* to the shrimp diet can enhance shrimp development, intestinal bacteria, digestive enzymes and the gene expression of shrimp *L. vannamei.*

INTRODUCTION

Shrimps are the most important crustaceans that contribute to the worldwide fishery sector and have already been identified as the most traded fish products **(Bondad-Reantaso *et al.*, 2012)**. However, Asia and South America make up the vast majority of shrimp production, whereas the United States, the European Union and Japan are the main customers. *Litopenaeus vannamei* is one of the most frequently grown shrimp species in the world because of its exceptional salinity adaptation, superb flavor and

quick development (**Chen *et al.*, 2018**). In 2018, *L. vannamei* produced over 4.9 million tonnes worldwide **(FAO, 2020)**.

The increased cost of aqua-feed has impacted the economic viability and profitability of fish and shrimp production, accounting for up to 60-70% of the entire operational costs. Conventional products are becoming expensive due to increased scarcity and demand **(Naylor *et al.*, 2021)**. As a result, alternative ingredients with considerable nutritional value and relatively inexpensive, such as plant by-products or wastes are proposed **(Dawood & Koshio, 2020)**. Plants by-products acquired from trustworthy food sources are preferred over animal by-products since they are devoid of fungal, bacterial and parasitic diseases that have an indirect impact on human health. Certain leftover foods have been investigated as potential by-products that could be used as non-traditional components and as functional feed additives, such as grape, pineapple and papaya wastes **(Kang *et al.*, 2010; Amrutha & Shyama, 2018; Rosas *et al.*, 2022)**, citrus peels **(Shabana *et al.*, 2019)** and prickly pear peels **(Ahmed *et al.*, 2020)**. These components demonstrated that it's feasible to incorporate them as a replacement for protein, lipids, carbohydrates, vitamins and minerals in aquatic animals' meals to reduce feed expenditures without compromising their quality or hindering growth. In addition, the role of natural feed is to enhance digestive enzymes **(Sankar *et al.*, 2011; Labrador *et al.*, 2016)** and immune stimulation (**Chuchird *et al.*, 2017; Choi *et al.*, 2020**), ensure water quality **(Amrutha & Shyama, 2018; Rosas *et al.*, 2022)** and control the pathogenic microbes **(Goba *et al.*, 2018)**, in addition to stimulating growth in fish and shrimp aquaculture. According to **FAO (2022)**, global fruit production reached 800 million tons, with about 35-45% of wastes which can be reprocessed in the animal and aqua-feed sectors due to substantial nutritive content and plenty of useful components, with an attempt to minimize the negative effects on the environment **(Rifna *et al.*, 2021)**.

Peels are a key by-product of the fruit processing sector, accounting for around 45-50% of total mass **(Rafiq *et al.*, 2018; Elkady *et al.*, 2020)**. Orange peels have immunomodulatory, anti-microbial, anti-inflammatory, antioxidative, immune booster and digestive tonic characteristics **(Grosso *et al.*, 2014; Rafiq *et al.*, 2018)**. Ascorbic acid, citric acid, flavonoids, minerals, carotenoids, limonoids, phenolic compounds, essential oils, flavonoids, alkaloids, dietary fibre, terpenes, resins, saponins and tannins are important bio-components as immunonutritional **(Rafiq *et al.*, 2018; Gandhi *et al.*, 2020)**. Flavonoids, in particular, have been thought to be capable of modulating the antioxidant response to diverse stressors by triggering antioxidant defenses **(Virgili & Marino, 2008; Gandhi *et al.*, 2020)**.

Prickly pear peels contain high concentrations of mucilage, pectin, minerals, flavor and pigment components, notably polyphenols and betalains. Many investigations have shown that the prickly pear is rich in vitamins, minerals, amino acids carbohydrates in addition to fat, with a high-nutritional-value plant. Prickly pears, with their high sugar content are a suitable material for yeast fermentation **(Tamine *et al.*, 2018; Diboune *et***

***al.,* 2019)**. Furthermore, phytotherapies are cost-effective, more eco-friendly than synthetic molecules and are less likely to elicit drug resistance due to the high diversity of plant extract molecules **(Olusola *et al.,* 2013; Dawood *et al.,* 2022)**. These substances demonstrated that they can be used as phytobiotic products and feed supplementation in the commercial feed of aquatic animals to minimize the expenses of the feeds while simultaneously increasing the healthiness, growth development and productivity of the cultured species.

Fruit wastes contain many anti-nutritional factors (ANTFs) which pose a possible negative impact as a feed additive, while the yeast fermentation process could be useful to reduce such impacts **(Makinde *et al.,* 2013; Najjar *et al.,* 2014; Okomoda *et al.,* 2020)**. The fermentation process boosts the nutritious value of the diet through the creation of vital amino acids and vitamins. **Kang *et al.* (2010)** used papaya processing waste (PPW) as substrates for solid-state fermentation by *Saccharomyces cerevisiae* and found that 45% of the PPW product was a crude protein with other nutrients such as fat, fiber, lignin, cellulose and minerals. Fruit waste contains dietary fibre, which converts into simpler carbohydrates such as xylose and galactose during fermentation **(Qureshi *et al.,* 2017)**. Due to its low cost and acceptable protein content with a suitable amino acid composition, *S. cerevisiae* has shown to be a very suitable candidate for single-cell proteins **(Amrutha & Shyama, 2018)**.

Consequently, the target of the present effort was to assess the phytobiotic products advantages of fermented fruit wastes as feed additives based on the quality of water, bacteriological analysis, gastrointestinal enzymatic activities, gene expression and zootechnical performance of shrimp (*L. vannamei).*

MATERIALS AND METHODS

1. Shrimp and culture conditions

Litopenaeus vannamei post-larvae were obtained from the commercial shrimp hatchery, Berket Ghalioun, Kafr Al-Sheikh, Egypt. Prior to the commencement of the feeding, shrimp were acclimated in an indoor fibreglass tank (6 m^2, 5 tons) for 7 days at temperature (28-29°C), pH (7.8-8), and salinity (30–32 ppt), and samples were fed twice daily (8.00 and 20.00hr) with a commercial feed (38% CP).

The experiment was conducted for 45 days in the laboratory of the National Institute of Oceanography and Fisheries (NIOF), Suez, Egypt. A total of 720 healthy shrimps (initial body weight, 0.05±0.09g) were randomly selected, kept in 9 indoor rounded fiberglass tanks (200 L in triplicates) and fed on a basal diet. Three treatments were formulated: Control (C, only commercial diet (38% crude protein and 9% crude lipid)); FOP (2.5% FOP + commercial diet) and FPP (2.5% FPP + commercial diet). The tanks were coated with black plastic sheets to restrict light penetration while simulating the natural habitat, reducing shrimp stress and preventing escapes. The shrimp samples were fed at 10% of their starting weight three times a day (8:00, 14:00, and 20:00hr) and

subsequently reduced to 5% at the end of the trial (45 days). The biweekly measured mean biomass was used to estimate and modify the daily feeding ratio for each treatment. Water quality parameters such as temperature, salinity, pH, ammonia (NH_3) and nitrite (NO_2) were periodically checked during the experiment. Water temperature and salinity were monitored using a multi-parameter every day between 09:00 and 10:00hr. However, ammonia (NH_3), nitrite (NO_2) and pH were biweekly measured by using colorimetric analysis.

2. Preparation of the fruit waste products and experimental diets

A total of 5kg from each orange and prickly pear peels wastes were collected from local markets. Immediately after collection, the fruit wastes were rinsed with distilled water and divided into small pieces (1x0.2cm). Fermented fruit wastes (FFW) were done according to the method of **Qureshi *et al.* (2017)** and **Malik and Sushil (2019)**. For a week, the fermentation process was carried out using fruit wastes with (1g kg^{-1} FW) of *Saccharomyces cerevisiae* inoculums (2.5 x 10^6 cfu/g in 20L containers) according to **AOAC (1995)**. The nutritive values of FFW before and after fermentation are shown in Table (1), while Table (2) summarizes the composition of the tested and basal diets.

Table 1. The nutritive values of fruit wastes before and after fermentation

Treatments	Orange peel		Prickly pear peel	
Parameter	**Before**	**After**	**Before**	**After**
Moisture	**74.88 ± 1.01**	**91.78 ± 2.29**	**86.60 ± 1.20**	**91.82 ± 0.61**
Protein	**6.02±0.09**	**16.44 ± 0.12**	**4.99 ± 0.39**	**13.72 ± 0.33**
Lipid	**0.98 ± 0.76**	**3.89 ± 0.10**	**4.56 ± 0.78**	**3.87 ± 0.11**
Ash	**2.77 ± 0.47**	**6.23 ± 0.01**	**8.38 ± 0.33**	**13.73 ± 0.11**
Fiber	**36.66 ±3.08**	**22.40 ± 0.10**	**14.12 ± 0.67**	**8.70 ± 0.10**
Carbohydrates	**53.57 ±3.16**	**51.05 ± 0.33**	**67.95 ± 0.75**	**59.98 ± 0.42**
Energy y (kJ/g diet)	**12.22**	**14.25**	**14.73**	**15.14**
Total	**100**	**100.01**	**100**	**100**

After a week, the fermented orange and prickly pear peels were dried to a constant weight at 70°C, powdered, sieved (35µm) and stored in an air-tight container. The powders (25g/kg basal diet) were dissolved in 50ml water and mixed thoroughly in a cooled slurry of diet in a domestic mixer. These pelleted diets were then dried for 48h in a 45°C air convection oven before being manually broken up to the appropriate size.

Table 2. Proximate composition (%) of feed ingredients

Formulation	Commercial diet	FOP	FPP
Moisture (%)	**7.96**	**91.78 ±2.29**	**91.82 ± 0.61**
Protein (%)	**38.04**	**16.44±0.12**	**13.72 ± 0.33**
Lipid (%)	**9.27**	**3.89±0.10**	**3.87 ± 0.11**
Ash (%)	**10.4**	**6.23±0.01**	**13.73 ± 0.11**
Fiber (%)	**5.9**	**22.40±0.10**	**8.70 ± 0.10**
Carbohydrate (%)[a]	**36.39**	**51.05 ± 0.33**	**59.98 ± 0.42**
Energy y (kJ/g diet)[b]	**18.93**	**14.25**	**15.14**
Total	**100**	**100.01**	**100**

[a] According to **Castell and Tiews (1980).**
[b] According to **Chatzifotis *et al.* (2010).**

3. Bacteriological analysis

Samples from water and shrimp intestine were twice monthly analyzed to estimate total viable bacteria (THB) and total *Vibrio* spp. population (TVC), using trypticase soy agar (TSA) and Thiosulphate Citrate Bile Salts Sucrose Agar (TCBS), respectively **(Liu *et al.*, 2010)**. Water samples from each trial tank were serially diluted (1:10) with sterile salt solution (1.0%NaCl) to obtain dilutions of 10^{-4} and 10^{-3} and then plated (0.1mL) in duplicate for THB and TVC analysis. Each incubated colony was counted between 30 and 300 cfu (bacterial colony-forming units) **(Ganesh *et al.*, 2010; Kumar *et al.*, 2014)**.

Shrimps from each tank were randomly picked, and the digestive tracts were aseptically removed and quantified. From each replication, 100mg of the intestinal tract was collected and preserved in the beaker. The contents were then homogenized in a mortar for about 2 minutes with sterile saline. To assess THB and TVC frequencies, the samples were serially diluted (10-fold) in sterile saline, and 100l of the supernatant was distributed over TSA agar and TCBS agar. Lastly, the plates were exposed to 37 and 28°C for 24h before being monitored. The ratio of TVC: THB was selected for microbial analysis during the experiment time as recorded in the study of **Sharawy *et al.* (2022a)**.

4. Digestive enzymes activities

At the end of the experiment, three shrimps were randomly sampled from each replicate for the measurement of the digestive enzyme activities in the hepatopancreas, stomach and intestine. The shrimp organs were separated and homogenized in clean distilled water before being weighed and homogenized separately with cooled buffer phosphate (pH 7, 0.65%, 1:10 w/v). The supernatant was used for enzyme tests after being centrifuged (3000g for 1 minute at 4°C). Samples were immediately transferred into sterile containers and refrigerated at -80°C until use. Protease activity was determined by the casein digestion method of **Drapeau (1976)**. Lipase activity was determined based on **Cherry and Crandall (1932)**. Amylase activity was measured by

the 3, 5-dinitrosalicylic acid (DNS) method **(Rick & Stegbauer, 1974)**. Cellulase activity was tested by the Dinitrosalicylic acid (DNS) method **(Marsden *et al.*, 1982)**.

5. RNA extraction

After the feeding period, three shrimp samples from each replication were chosen at a random behavior to be used in growth and immune gene expression assays. Growth hormone (*GH*), insulin-like growth factor 1 (*IGF1*), β-glucan binding protein (*β-BGP*), and prophenoloxidase (*Proph)* expression levels were measured in the hepatopancreas. The specimens were promptly immersed in liquid nitrogen and preserved at -80°C for RNA isolation using TRIzol (Easy-RED, INTRON, Korea) according to the manufacturer's instructions. Afterwards, the amount and concentration of RNA were determined using a spectrophotometer at 260 and 280nm. RNA ratios (A260:A280) larger than 1.8 were employed in subsequent investigations. Electrophoresis on a 1.5% agarose gel stained with ethidium bromide was used to assess the quality of the RNA. SuPrime Script RT Premix (2X) cDNA Synthesis Kit (GeNet BIO Inc., Daejeon, South Korea) was used for first-strand cDNA synthesis according to the manufacturer's procedure.

Relative mRNA expression

Primer 5.0 software has been employed to generate primers for every gene identified in the cDNA bank (Table 3). The quantitative real-time PCR reaction was performed with a total volume of 20µl, consisting of 10µl of SensiFAST™ Syber green with low rox kit (Bioline, United Kingdom), 0.8µl of each primer, 2µl of cDNA, 6.4µl of RNase free water, and the program was carried out by initial heating at 95ºC for 10min, followed by 40 cycles of 95ºC for 15sec and annealing temperature of 60ºC. The cycle threshold was determined for each sample, using the exponential growth phase and baseline signal from the fluorescent versus the cycle number blots. The analysis of the melting curve was conducted using PCR products after each run to confirm that a signal product was amplified. The expression of target genes was performed by the comparative threshold cycle (ct) and Fold change = $2^{\wedge-\Delta\Delta ct}$ of **Rao *et al.* (2013)**.

Table 3. The primers sequences used in qRT- PCR

Genes	Sequences	Amplicon size (bp)	Reference
Growth Hormone (*GH*) XM027360152	**F: AATTTGCGCTTGCACTACGG R: ATCCGGTTGAGGTGTAGCTG**	**100**	**Designed by NCBI tool**
Insulin-like Growth Factor 1 (*IGF-I*) KP420228	**F: GTGGGCAGGGACCAAATC R: TCAGTTACCACCAGCGATT**	**123**	**Designed by NCBI tool**
***β*-Glucan Binding Protein (*β-GBP*) AY249858**	**F: ACGAGAACGGACAAGAAGTG R: TTCAGCATAGAAGCCATCAGG**	**137**	**Wang *et al.*, 2008**
Prophenoloxidase (*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. Zootechnical indices

The growth indices such as weight gain (WG), specific growth rate (SGR), food conversion rate (FCR) and survival rate (S%) were determined using the formula of **Tekinay and Davis (2001)**.

- Weight gain (WG) = final weight- initial weight.
- Specific growth rate (%) = 100[(ln W2 – ln W1)/ (ln W1)]/ T; where, W1 and W2 are initial weight and final weight, and T is the number of days in the feeding period.
- Feed conversion ratio (FCR) = feed intake / weight gain.
- Survival (%) = final count – initial count*100.

7. Data analysis

The values of water quality parameters, digestive enzymes, presumptive THB, TVC and TVC/THB% in the water and shrimp, 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. The IBM SPSS 19.0 program was used for all analyses, and the findings were displayed as Means±SD.

RESULTS

1. Water quality

As noted in Table (4), the findings of parameters related to water quality did not show any significant variations ($P>0.05$) across the several parameters studied. The various treatments' water quality measurements stayed within the ranges permitted for shrimp culture throughout the trial.

Table 4. Water quality parameters of *L. vannamei* during 45 experimental days, fed on fermented orange and prickly pear wastes

Parameters	Treatments		
	C	FOP	FPP
Salinity ppt	**32.01 ± 0.12**	**32.08 ± 0.24**	**32.07 ± 0.27**
Temp ^{o}C	**28.06 ± 1.1**	**27.94 ± 1.14**	**27.87 ± 1.02**
pH	**7.81 ± 0.18**	**7.68 ± 0.12**	**7.66 ± 0.16**
NH_3 (mg/L)	**0.20 ± 0.06**	**0.17 ± 0.03**	**0.17 ± 0.03**
NO_2 (mg/L)	**0.22 ± 0.06**	**0.18 ± 0.08**	**0.16 ± 0.08**

Means±SD (n=3) represent the results for each group. Averages in the same row with superscript varied significantly.

2. Growth indices of shrimp

The FPP treatment had the greatest final body weight (FBW) with 3.37±0.06g and was significantly different from the C and FOP treatments ($P< 0.05$), whereas C presented the lowest growth value as presented in Table (5). WG and SGR (%/days), revealed comparable findings, with the FPP exhibiting statistically significant differences ($P< 0.05$) compared to the C, with the lowest SGR and WG. The FCR also indicated significant differences ($P< 0.05$), with the C having the greatest value, followed by the FOP, and the FPP having the lowest value. The survival % also varied significantly between treatments ($P< 0.05$), ranging from 82.08% to 85.83%.

Table 5. Zootechnical and nutritional indices of shrimp *L. vannamei* after 45 days, fed fermented orange and prickly pear wastes

Parameter	Treatments		
	C	FOP	FPP
FBW (g)	2.33 ±0.06^{c}	2.90 ± 0.04^{b}	3.37 ± 0.06^{a}
WG (g)	2.28 ± 0.06^{c}	2.85 ±0. 20^{b}	3.32 ±0.06^{a}
SGR (%/day)	8.50 ± 0.06^{c}	8.98 ± 0.03^{b}	9.32 ± 0.04^{a}
FCR	1.37 ± 0.06^{a}	1.28 ± 0.06^{ab}	1.24 ± 0.01^{b}
S%	82.08 ± 0.72^{b}	85.83 ±0.72^{a}	85.83 ± 0.72^{a}

Means±SD (n=3) represent the results for each group. Averages in the same row with superscript varied significantly.

3. Bacteriological analysis

The fermented fruit waste additives influenced the results of the THB and TVC of water and shrimp intestines, as seen in Fig. (1). The THB count in water and shrimp intestine showed the highest values in the FOP treatment, followed by FPP treatment compared to the C treatment. However, TVC count and TVC/THB% in water and shrimp intestine had the highest values in the C treatment ($P< 0.05$), compared to the fermented fruit waste additives treatments. In both the control and treatment tanks, the TVC was larger in the shrimp gut than in the water.

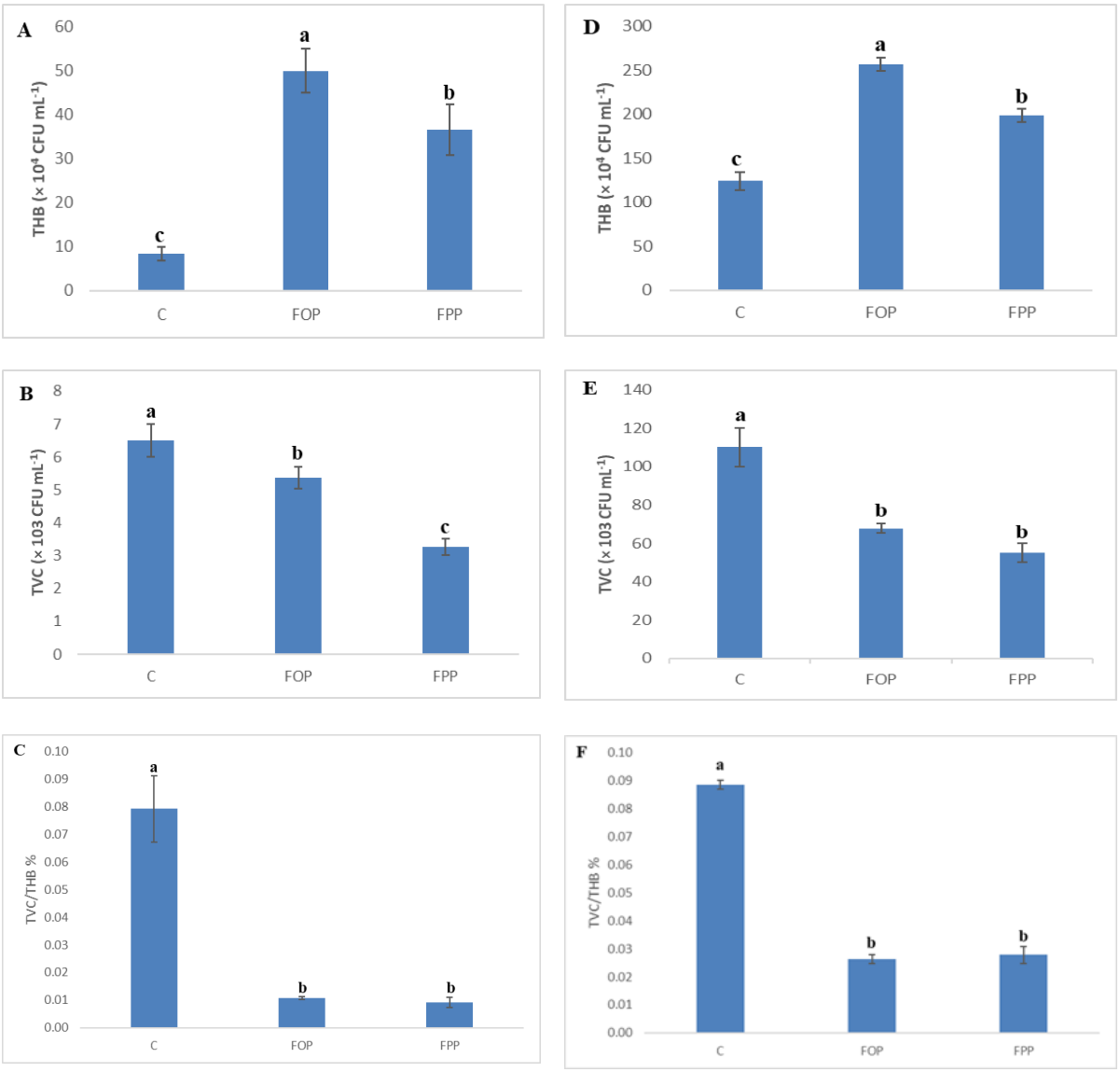

Fig. 1. Microbial count in water (A) THB, (B) TVC and (C) TVC/TH, and in shrimp intestine (D) THB, (E) TVC and (F) TVC/THB, of *L. vannamei* after 45 experimental days fed on fermented orange (FOP) and prickly pear wastes (FPP).

4. Activities of digestive enzymes

The activities of the digestive tract are shown in Fig. (2). In the hepatopancreas, the activities of digestive enzymes such as protease, lipase, amylase and cellulase were significantly increased (P<0.05) in the fermented fruit waste additives treatments compared to C. The protease activity exhibited the highest value in FPP. However, the lipase and amylase activities showed the highest value in FOP. The cellulase activity had significantly increased (P<0.05) in the C in all hepatopancreas, stomach and intestine, compared to the other treatments. While, the results were significantly similar in the stomach and intestine.

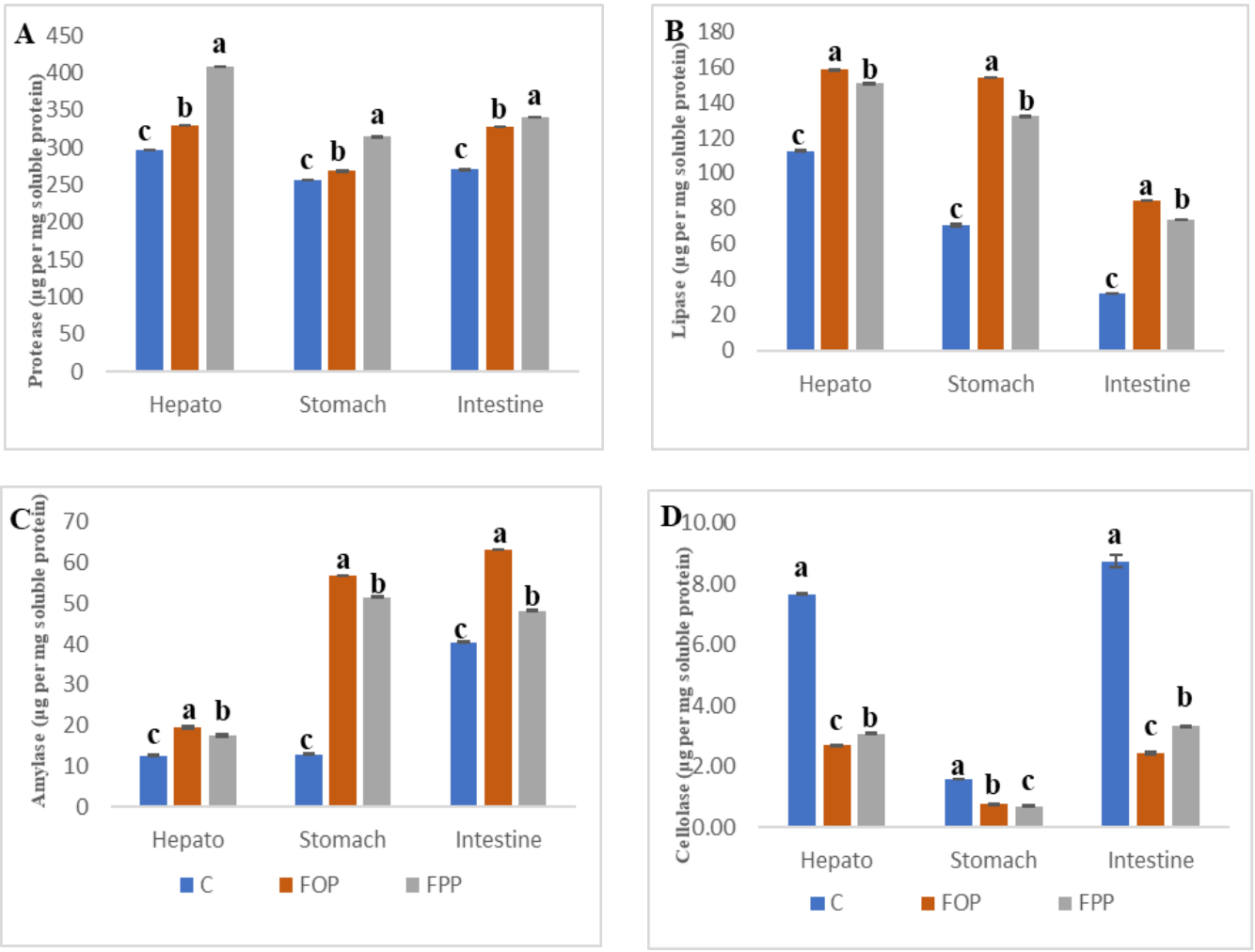

Fig. 2. Digestive tract activities of the digestive enzymes (A) protease, (B) lipase, (C) amylase, and (D) cellulase, of *L. vannamei* after 45 experimental days fed fed on fermented orange (FOP) and prickly pear wastes (FPP).

5. Expression of growth- and immune-related genes

The expression of growth and immune-related genes results are presented in Fig. (3). Compared to the C, growth and immune-related genes in hepatopancreas tissue enhanced significantly ($P<0.05$) in the fermented fruit waste additives treatments. The FPP was significantly higher ($P<0.05$) in *GH*, *IGF-I*, *β-BGP* and *Proph* genes expression, followed by FOP compared to the C, as presented in Fig. (3).

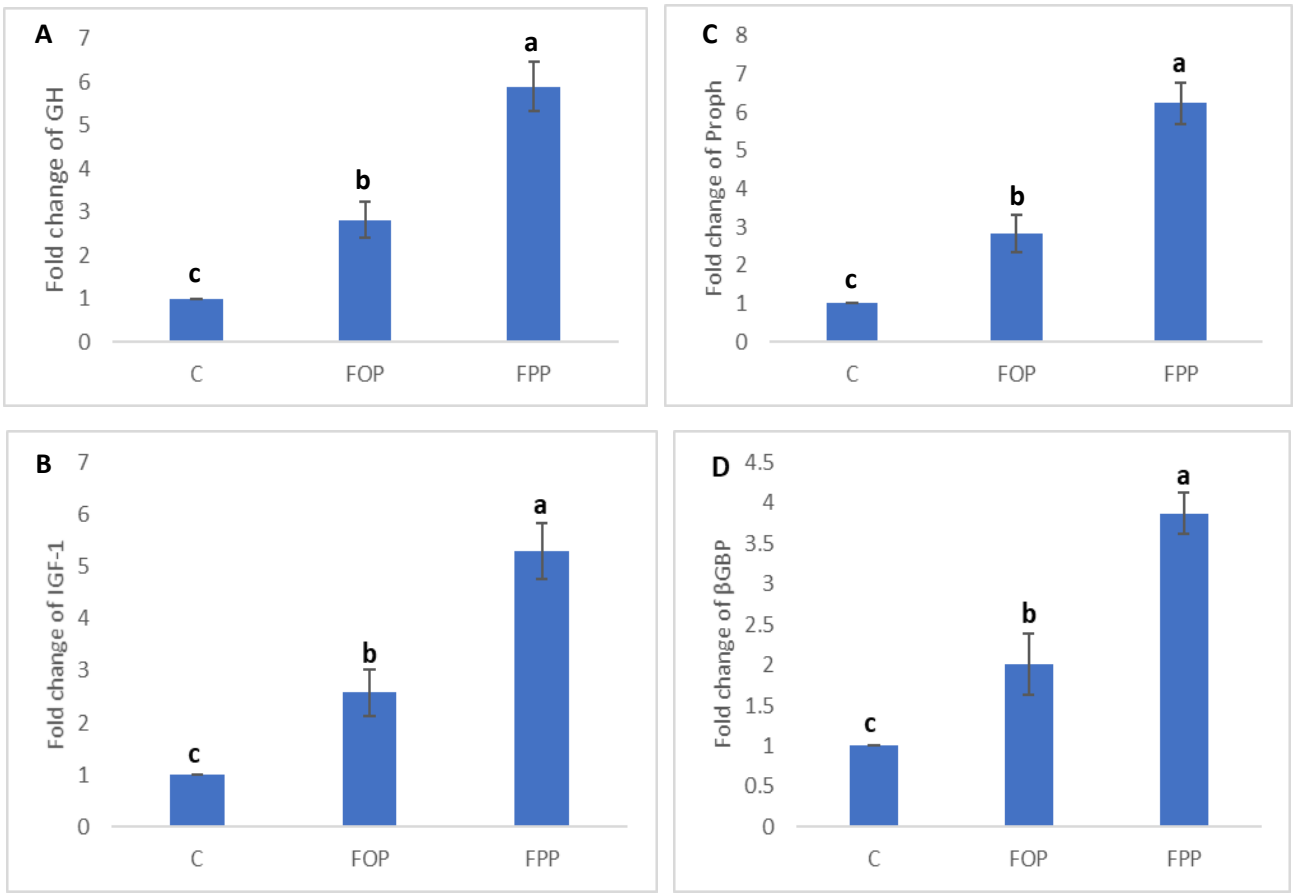

Fig. 3. Expression of (A) *GH*, (B) *IGF-I*, (C) *β-BGP* and (D) *Proph* genes in the hepatopancreas tissue of *L. vannamei* after 45 experimental days fed on fermented orange (FOP) and prickly pear wastes (FPP).

DISCUSSION

The current study showed that phytobiotics from fermented fruit wastes used as feed additives influenced the water quality, productivity and health of *L. vannamei* PL. The results suggested that water quality was not influenced when phytobiotics from fermented fruit wastes were added to the diets. **Chuchird *et al.* (2017)** showed that, shrimp (*L. vannamei)* development and health were positively affected by the phytobiotic feed additives derived from the yeast cell walls and grape pomace. **Rosas *et al.* (2022)** observed that the addition of grape promace in the shrimp diets did not affect water quality. Additionally, water characteristics in shrimp culture were maintained within an optimal range **(Van Wyk & Scarpa, 1999; Boyd & Clay, 2002)**.

After 45 days, shrimp *L. vannamei* fed with fermented fruit wastes as phytobiotics feed additives showed increases in FBW, WG, SGR, FCR and S%. These results showed that prickly pear peel's mode of action may be responsible for improved feed efficiency, including the preservation of a balanced microbial population and an improvement in food digestibility. The present results are compatible with those of **Ahmed *et al.* (2020)**

who observed that, both WG, SGR and S% of shrimp were significantly increased when diets were supplemented with PPP up to 20%. These findings corroborated the striking improvement in the immune-reaction in diets supplemented with PPP. However, the results of this study confirmed previous findings indicating that plant extracts may be able to promote shrimp growth **(Sankar *et al.*, 2011; Moh *et al.*, 2021)** and different fish species **(Amrutha & Shyama, 2018; Ahmed *et al.*, 2020)**.

Despite the variability in FCR during the initial culture stage, the data showed a reduction in FCR with FPP feed additions. This finding supports previous studies that detected reduced FCR in shrimp and freshwater prawns with various supplements **(Sankar *et al.*, 2011; Saravana *et al.*, 2013; Labrador *et al.*, 2016; Moh *et al.*, 2021; Rosas *et al.*, 2022)**. The drop in FCR meant that the shrimp needed less food to be transformed into meat. This strengthened the animal body system's efficiency in turning food into flesh and reducing waste in the system **(Fry *et al.*, 2018; Moh *et al.*, 2021)**.

Shrimp productivity is significantly impacted by their survival capacity, particularly net yield after the production cycle. Survival of shrimp fed with phytobiotics feed additives increased notably with phytobiotics from fermented fruit wastes compared to the control, but considerable advantages were seen in terms of individual growth performance. Similar trends regarding the high survival % were also obtained in shrimp and prawns provided with herbal-supplemented meals compared to the control diet **(Poongodi *et al.*, 2012; Labrador *et al.*, 2016)**. The contents of fruit wastes, such as immunostimulants, anti-stress compounds, antimicrobial agents and antioxidants are responsible for the enhanced S% in the experimental treatments. The study of **Goncalves and Santos (2015)** was conducted on the effect of phytosanitary additives on shrimp growth performance using small amounts of commercial phytogenic products as a feed additive in the diet which increased shrimp weight, feed conversion ratio, and growth rate compared to shrimp that did not use dietary supplements. In another study, fermented papaya processing waste (PPW) increased protein digestion, feed conversion ratio, and growth in *L. vannamei* shrimp larvae **(Kang *et al.*, 2010)**.

The most common harmful bacteria in aquaculture, *Vibrio* spp. was dramatically reduced in the present investigation by the phytobiotic derived from fermented fruit wastes in both the pond and in shrimp intestines **(Deng *et al.*, 2013; Goda *et al.*, 2018)**. Several critical physiological operations of the cultured species, such as nutrition, digestion, basal metabolism, immune response and development may be affected by changes in gut microbial morphology and composition **(Gorokhova *et al.*, 2015; Li *et al.*, 2018)**. It is worthy to mention that, gastrointestinal microflora is intimately related to physiological mechanisms and has an important impact on the development of *L. vannamei*, which is a crucial component for sustaining the intestinal environment's stability **(Abid *et al.*, 2013)**. In this study, the effects of Brewer's yeast (*S. cerevisiae*) as a probiotic were examined in fermented fruit wastes containing β-glucan, nucleic acids and

mannan oligosaccharides, among many other components. As a consequence, the THB in both the water tank and the shrimp intestine significantly improved (FOP followed by FPP compared to control). **Burgents *et al.* (2004)** observed that, *S. cerevisiae* supplementation improved *L. vannamei* survivability upon exposure to *Vibrio* spp. Moreover, **Boonanuntanasarn *et al.* (2016)** elucidated that, dietary β-glucan reduced *Vibrio* species, but the effect was not statistically significant. Co-supplementation with *B. subtilis* and β-glucan resulted in significantly more lactic acid bacteria and fewer *Vibrio* spp., implying that combined prebiotics and probiotics could exclude pathogens. These findings are symmetrical with previous research on probiotics.

The present study demonstrated that fruit wastes increased the microbial intestine through the fermentation process and reduced the ANTFs, which improved digestive enzymes in shrimp. The action of fermentation against the ANFs in this study is in line with the findings of **Makinde *et al.* (2013)**, **Najjar *et al.* (2014)** and **Okomoda *et al.* (2020)**. Fruit by-product and wastes contain significant amounts of cellulose and hemicellulose, as well as the digestion-soluble carbohydrates fructose, glucose and sucrose **(Choi *et al.*, 2015)**. Fruit waste is an effective biomass supplier for the synthesis of different by-products due to its nutrient content and plentiful availability. On the other hand, citrus peel has high levels of crude materials such as limonene, pectin, d-galacturonic acid and ethanol **(Rivas-Cantu *et al.*, 2013)**. In this context, **Qureshi *et al.* (2017)** investigated the effect of solid-state fermentation using fruit waste as an energy source for a week, and the authors found the highest activities of lipase and pectinase in orange peel. The lipase and cellulase activity in the hepatopancreas, stomach and intestine of *L. vannamei* shrimp fed FOP considerably improved the mode of action of the dietary fermentation process. Citrus-supplemented meals have considerably higher levels of digestive enzymes, indicating that citrus-supplementation fosters the excretion of such enzymes, which in turn increases nutrient digestion, accompanied mostly with the growth fish **(Shabana *et al.*, 2019)**. As a result, orange peels have been explored as a functional additive, demonstrating improved growth and reproductive performance among aquatic species.

The production of digestive enzymes including lipase, protease, amylase and cellulase demonstrated the plant's role in growth. Based on the results, the increase in digestive enzymes from fermented fruit wastes may be responsible for better growth indices of shrimp (*L. vannamei*). Earlier research demonstrated that using plant extracts enhanced development by improving the digestive tract **(Shabana *et al.*, 2019; Moh *et al.*, 2021)**. Protease activities showed increases in hepatopancreas, stomach and intestine for shrimp *L. vannamei* fed FPP. Additionally, **Ahmed *et al.* (2020)** reported that, fish fed diets with PPP supplements had higher levels of all digestive enzymes and grew faster than controls. Because of improvements in intestinal secretions and resistance to opportunistic native bacteria, digestive enzyme levels were favorably linked with growth

promotion **(Dimitroglou *et al.*, 2009; Ahmed *et al.*, 2020)**, which was proved in the present study.

Growth is considered a polygenic and environmentally controlled trait, with the most important genes being IGF-I and growth hormone **(Triantaphyllopoulos *et al.*, 2020)**. In the current study, phytobiotic feed additives of the shrimp diet with fermented fruit wastes, particularly the FPP resulted in a significant increase in *GH* and *IGF-I*, which matches with the prior outcomes related to FBW, SGR and FCR. Shrimp growth supports this since the expression offers a holistic view of growth. In this study, fermented fruit wastes were incorporated to enhance the expression of the *GH* and *IGF-I* genes, comparable findings were obtained in shrimp *L. vannamei* **(Sharawy *et al.*, 2022a, b)**. Furthermore, **El-Bab *et al.* (2022)** postulated that, the supplementations of the sea bream (*Sparus aurata*) diets with *S. cerevisiae* increased *IGF-I* significantly, mainly in the 4g/ kg diet.

Phytobiotic additions are among the most important natural treatments for improving aquatic animals' general immune defense systems **(Giannenas *et al.*, 2012; Peterson *et al.*, 2014)**. Notably, shrimp infections are one of the major constraints to achieving sustainable growth in the shrimp aquaculture sector worldwide; hence, research on shrimp immune-related expression levels has attracted considerable attention. The current study's findings were based solely conducted on the expression of specific hepatopancreas genes. Additionally, earlier research has shown that the hepatopancreas is an essential organ in the immune response of penaeid shrimp **(Pan *et al.*, 2005)**. In this study, the immune-related genes (*β-GBP* and *Proph*) were clearly shown to be expressed in phytobiotic from fermented fruit waste as feed additives; both *β-GBP* and *Proph* were significantly increased in FPP, followed by FOP compared to C treatment. **Kesselring *et al*. (2021)** investigated the health benefits of the commercial phytogenic feed supplement Digestarom using hemolymph, giving valuable information on shrimp health and immune condition. It is an essential component in nutritional, physiological and immunological mechanisms **(Nguyen *et al.*, 1998; Meena *et al.*, 2013; Nurhayati & Yuhana, 2015; Chuchird *et al.*, 2017; Choi *et al.*, 2020)**.

CONCLUSION

The present results showed that agricultural wastes, such as orange peel and prickly pear peel have the potential to serve as valuable feed additions for the long-term success of the shrimp aquaculture industry. Solid-state fermented prickly pear peel increased the growth and enhanced the well-being of *L. vannamei* PL substantially. The diet supplemented with FOP and FPP at the level of 2.5% may result in a decreased intestinal *Vibrio* count as well as increased digestive enzyme activity and immunological response.

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