


Modulation of growth, immunity, and immune-antioxidant gene expression in Nile tilapia, *Oreochromis niloticus*, culture under biofloc system by dragon fruit, *Hylocereus undatus*, peel powder

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Abstract

This study examines the use of dragon fruit peel (DFP) powder as a dietary supplement on growth performance, immune responses, and gene expression of Nile tilapia, *Oreochromis niloticus*, cultured within biofloc systems. A total of 300 Nile tilapia fingerlings (14.64 ± 0.09 g) were subjected to five dietary treatments, with DFP added to a basal diet at doses of 0, 20, 40, 80, and 160 g kg⁻¹ DFP. Growth and immunological responses were assessed after 4 and 8 weeks of feeding, and the transcriptional level of immune and antioxidant-related genes was measured after 8 weeks. Fish fed diets containing DFP exhibited significantly greater weight gain, faster growth, and enhanced levels of key indicators of immunity than control fish ($p < 0.05$). A diet containing 40 g DFP kg⁻¹ produced the best result in terms of growth, enhanced immune response indicators in skin mucus and blood serum, and the

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upregulation of *IL-1*, *IL-8*, *LBP*, *GPx*, *GST-α*, and *GSR* expression ($p < 0.05$). Based on the quadratic regression analysis, the optimum concentration of DFP was 80 g kg^{-1} . These findings collectively suggest that powdered DFP may serve as a promising feed supplement for Nile tilapia raised in biofloc systems.

KEYWORDS

biofloc technology, fruit peel, innate immunity, mRNA expression, Nile tilapia

1 | INTRODUCTION

Nile tilapia, *Oreochromis niloticus*, is cultivated in more than 100 countries and accounts for 8% of the global finfish aquaculture production (Geletu & Zhao, 2023; Verdegem et al., 2023). It is a popular and intensively farmed species because of its rapid growth rate and commercial acceptability (Charo-Karisa, 2024). Intensification frequently results in deteriorating water quality, which in turn makes fish more susceptible to infectious diseases. This leads to high mortality rates among farmed fish and significant financial losses (Magouz et al., 2024). Antibiotics and other chemicals are widely used by farmers as a straightforward and cost-effective method for disease prevention and treatment (Bardhan et al., 2024; S.-W. Chen et al., 2019; Ding & He, 2010; Harikrishnan & Balasundaram, 2005; Rico et al., 2014). However, the overuse of antibiotics and chemicals contributes to the emergence of antibiotic-resistant bacteria and has adverse effects on environmental ecosystems and human health (Cherian et al., 2023; Magna et al., 2024). On the other hand, feed, which represents 50%–80% of total production costs, is another critical factor affecting the efficiency of intensive tilapia farming (Boyd et al., 2020; Gule & Geremew, 2022). To lower the costs of aquafeed, plant-based feed ingredients are increasingly utilized in aquatic diets (Eroldoğan et al., 2023). In addition to herbs that effectively prevent and treat fish diseases, numerous fruit by-products have been identified as potential dietary supplements for aquaculture species (Ahmadifar et al., 2021; Harikrishnan et al., 2009, 2010, 2011; Hazreen-Nita et al., 2022; Majeed et al., 2024).

Dragon fruit, *Hylocereus undatus*, known for its coagulant properties, belongs to the Cactaceae family (Rodriguez et al., 2016). Most dragon fruit undergoes processing before being sold in supermarkets and fruit processing facilities, resulting in a significant waste product known as dragon fruit peels (DFPs; Chumroenvidhayakul et al., 2023). Additionally, it is not cost-effective or environmentally friendly to just throw them out (Chen et al., 2022). Peel is the most common by-product of consuming or processing dragon fruit, accounting for 30%–35% of the total fruit weight (Li et al., 2022), and is rich in phytonutrients (Arivalagan et al., 2021). The DFP is well-documented to contain high amount of phenolic compounds, fatty acids, and flavonoids (Jimenez-Garcia et al., 2022). It is also a rich source of pectin (Liu et al., 2023), which is known for its potential prebiotic properties (Guo et al., 2023). These bioactive compounds have the ability to alter human's blood lipid profiles and exhibit anti-inflammatory, anti-angiogenic, antibacterial, antiproliferative, and cytotoxic properties (Le, 2022). Thus, utilizing powdered DFP appears to be a promising option for reducing environmental impacts and preventing waste and microbiological contamination (Chumroenvidhayakul et al., 2023; Jiang et al., 2021; Rifna et al., 2021). Approximately 100 g of DFP can be produced from 1 kg of fresh peel after being sun-dried to a water content of 14%, providing a potentially valuable feed additive for land-based domestic animals and aquatic animals (Matra et al., 2021).

Biofloc technology (BFT) is a culture system in where the water environment contains a mixture of microorganisms and other organic materials (Khanjani, Mozanzadeh, et al., 2024). These components form a coagulated floc that can serve as prebiotics and probiotics, helping to maintain water quality by recycling nutrients (Khanjani, Sharifinia, & Emerenciano, 2024). Moreover, biofloc can also be used as a feed supplement for fish (Khanjani & Sharifinia, 2020).

Additionally, the presence of feed supplements in the biofloc may lead to the development of microorganisms that can combat pathogenic bacteria in both the farm water and the intestine of fish (Khanjani & Sharifinia, 2020; Kishawy et al., 2020; Mohammadi et al., 2021). Integrating DFP, an agricultural by-product, into this system aligns with the principles of BFT by providing an alternative, sustainable feed ingredient. This not only reduces waste and makes use of underutilized resources but also offers potential nutritional and health benefits to the fish, possibly enhancing their growth and disease resistance. The use of BFT in aquaculture is a step toward more sustainable and environmentally friendly practices, addressing concerns about waste management and resource efficiency. Thus, this study aimed to examine the effects of powdered DFP on growth, immunological parameters, and the expression of key immune-antioxidant genes in Nile tilapia cultured in a biofloc system. To the best of our knowledge, this has not been investigated previously.

2 | MATERIALS AND METHODS

2.1 | Preparation of dragon peel powder

Dragon fruit was purchased from a local market at Chiang Mai city, Chiang Mai, Thailand. Upon arrival, the peels were taken and thoroughly washed with clean water. The peels were thenceforth dried in hot air oven and ground into fine powder. Proximate analysis and bioactive compound of DFP were then determined (Tables 1 and 2).

2.2 | Experimental diets

Five test diets were prepared: a basal control diet (DFP0) suitable for Nile tilapia (Xuan et al., 2022), and four diets consisting of the basal diet supplemented with powdered DFP at four levels—20 g kg⁻¹ (DFP20), 40 g kg⁻¹ (DFP40), 80 g kg⁻¹ (DFP80), and 160 g kg⁻¹ (DFP160) (Table 3). The inclusion levels were based on a previous study of Prastiya and Yusuf (2021). Powdered DFP was prepared by oven-drying DFP at a temperature of 60°C for 48 h.

TABLE 1 Proximate composition of dragon fruit peel powder used in the experiment.

Dry matter (DM)	94.91 ± 2.45
Ash	17.93 ± 0.84
Crude fiber (CF)	33.84 ± 1.01
Crude protein (CP)	8.78 ± 0.55
Ether extract (EE)	0.87 ± 0.05
Nitrogen free extract (NFE)	33.49 ± 0.65

TABLE 2 Bioactive compounds of dragon fruit peel powder used in the experiment.

Test items	Results	Units	Methods
DPPH (IC ₅₀)	7.66 ± 0.06	mg mL ⁻¹	Lin et al.
ABTS ⁺	17.54 ± 4.44	mg TE g ⁻¹	Lin et al.
FRAP	14.94 ± 0.21	mg TE g ⁻¹	Lin et al.
Total flavonoid content	0.69 ± 0.13	mg CE g ⁻¹	Juan and Chou
Total phenolic content	2.86 ± 0.20	mg GAE g ⁻¹	Juan and Chou

TABLE 3 Proximate composition (g kg⁻¹) of experimental diets.

Ingredients	Experimental diets (g kg ⁻¹)				
	DFP0	DFP20	DFP40	DFP80	DFP160
Soybean meal	390	393	396	403	418
Corn meal	200	182	164	122	32
Fish meal	150	150	150	150	150
Rice bran	150	150	150	150	150
Wheat flour	70	70	70	70	70
Dragon fruit peel	0	20	40	80	160
Binder	20	15	10	5	0
Premix ^a	10	10	10	10	10
Vitamin C 98%	5	5	5	5	5
Soybean oil	5	5	5	5	5
Proximate composition of the experimental diets (g kg ⁻¹)					
Ash	100.80 ± 2.54	101.26 ± 3.55	101.59 ± 3.21	101.89 ± 4.32	102.05 ± 3.76
Dry matter	920.55 ± 4.65	920.60 ± 6.32	920.60 ± 7.21	920.58 ± 5.55	920.62 ± 7.13
Gross energy (cal g ⁻¹)	4038 ± 12.51	4029 ± 14.43	4025 ± 15.22	4018 ± 13.87	4012 ± 16.32
Crude lipid	70.10 ± 2.44	68.25 ± 3.08	68.10 ± 3.32	67.88 ± 2.87	67.50 ± 3.19
Fiber	60.25 ± 2.11	61.33 ± 1.97	61.50 ± 2.35	61.78 ± 2.09	61.96 ± 2.21
Crude protein	30.30 ± 0.97	30.42 ± 0.85	30.58 ± 1.10	30.73 ± 1.05	30.77 ± 1.32

^aVitamin and trace mineral mix supplemented. All dietary constituents were analyzed using standard methods.

The dried peel was then ground into fine powder using a mill and stored at 4°C until used in the diet. To prepare the dough, the feedstuffs were blended, then oil and water were added. The dough was then pelleted (2 mm in diameter) in a pelleting machine (Extruder—Siam Farm Services Co, Ltd.). Pellets were dried at 50°C to attain 10% moisture and stored in sealed polyethylene bags at 4°C until used.

2.3 | Experimental design

Nile tilapia fingerlings were obtained from a tilapia hatchery (Chiang Mai Aquatic Development Farm Co., Ltd). Prior to starting the experiments, fish were acclimatized in 500-L glass tanks and fed a commercial diet (CP 9950, Thailand) for 14 days. At the end of the acclimation period, 10 fish were sampled and their general health was assessed by examining their physical morphology and organs. Then, Nile tilapia fingerlings (14.64 ± 0.09 g) were allocated randomly to each of the 15 glass tanks (150 L capacity) at a stocking density of 20 fish tank⁻¹. All five dietary treatments, each with three replicates, were arranged in a completely randomized design. Fish were fed two times per day (8:30 a.m. and 4:30 p.m.) for 8 weeks. The experiments complied with Assessment and Accreditation of Laboratory Animal Care International (AAALAC) guidelines on the use of animals, approved by the Chiang Mai University Committee (RAGIACU002/2565).

2.4 | Management of water quality

Water quality was examined twice daily. Total ammonia-nitrogen (TAN, mg L⁻¹) was analyzed using an Ammonia Portable Photometer (HI96733, Hana Instruments, Romania), which was maintained at 0.16 ± 0.1 mg L⁻¹.

Temperature ($^{\circ}\text{C}$), pH, and dissolved oxygen (DO , mg L^{-1}) were monitored using Hana Multiparameter Meter (HI98196, Hana Instruments). The volume of floc was measured using an Imhoff cone (Avnimelech, 2009). Water temperature, pH, and DO levels were maintained at $27.83 \pm 0.85^{\circ}\text{C}$, 7.78 ± 0.58 , and $4.87 \pm 0.05 \text{ mg L}^{-1}$, respectively.

2.5 | Biofloc water preparation

Flocs waters were prepared 3 weeks before the feeding trial by adding 400 g of salt, 5 g of dolomite, 2 g of wheat flour, and 5 g of molasses to each of the 150-L experimental tanks. One gram of probiotics (PondPlus—Bayer) was added to each tank every 2 weeks. The C:N ratio was maintained at 15:1 through the addition of molasses (40% C) as a carbon source, applied 2 h after feeding (Avnimelech, 2009). The C:N ratio was calculated based on the leftover nitrogen levels in each tank and the carbon and nitrogen content of the diet (Cardona et al., 2016).

2.6 | Growth parameters

All fish in each tank were weighed collectively at the beginning of the experiment and subsequently at 4-week intervals throughout the experiment. Survival and growth parameters of the fish are calculated as follows:

$$\text{Weight gain (WG, g)} = \text{Final weight (FW)} - \text{initial weight (IW)}$$

$$\text{Specific growth rate (SGR\%)} = 100 \times (\ln \text{FW} - \ln \text{IW}) / \text{total duration of experiment}$$

$$\text{Feed conversion ratio (FCR)} = \text{total feed given} / \text{WG}$$

$$\text{Survival rate (SR, \%)} = 100 \times (\text{final fish number} / \text{initial fish number}).$$

2.7 | Innate immunological assays

2.7.1 | Skin mucus and serum collection

Skin mucus samples were collected from each treatment group ($n = 3$) after 4 and 8 weeks of feeding. Fish were first anesthetized with clove oil at a concentration of 5 mL L^{-1} , then placed in plastic bags containing 10 mL of 50 mM NaCl (Merck, Germany), and gently rubbed for 2 min. The mixture was transferred to 15-mL sterile tube and centrifuged at $1500 \times g$ at 4°C for 10 min. One milliliter of supernatant (mucus) was stored in a 1.5-mL Eppendorf tube at -80°C for future analysis. For blood serum collection ($n = 3$), approximately 1 mL of blood serum was drawn from the caudal vein of each fish with a 1 mL syringe (without adding coagulant). Blood samples were incubated at room temperature for 1 h and stored for 4 h at 4°C . Subsequently, the samples were then centrifuged at $1500 \times g$ for 5 min at 4°C , and the extracted serum was stored in 1.5-mL Eppendorf tubes at -80°C for further evaluation.

2.7.2 | Lysozyme assays

The method of Parry et al. (1965) was used to measure the levels of serum and mucus lysozyme following modifications provided in a previous work (Outama et al., 2022). Briefly, 100 μL of skin mucus and 25 μL of undiluted serum from each fish were loaded in triplicate into 96-well plates. Each well received 175 μL of *Micrococcus lysodeikticus*

(0.3 mg mL⁻¹ in 0.1 M citrate phosphate buffer, pH 5.8). The solution was quickly mixed, and changes in turbidity were monitored using a microplate reader at 540 nm and 25°C, recording every 30 s for 5 min. The standard curve was produced by plotting the optical density (OD) values against the concentration of hen egg-white lysozyme, ranging from 0 to 20 µg mL⁻¹ (Sigma Aldrich, USA). The results were expressed as microgram per milliliter, and the curve was used to calculate the equivalent unit of activity for the sample.

2.7.3 | Peroxidase assays

The Quade and Roth (1997) method was slightly modified to detect peroxidase levels in serum and skin mucus. Briefly, triplicate 96-well flat-bottom plates were loaded with 5 µL of serum or skin mucus, followed by the addition of 45 µL of Hank's balanced salt solution (HBSS) without Ca²⁺ or Mg²⁺. Subsequently, 100 µL of solution comprising 40 mL of distilled water, 10 mL of H₂O₂ (30%—Sigma Aldrich), and 1 pill of 3,3', 5,5'-tetramethylbenzidine (TMB; Sigma Aldrich) were added to each well. After adding 50 µL of 2 M H₂SO₄ to stop the color-change reaction, the OD was measured at 450 nm using a microplate reader. As a control, standard samples without serum or skin mucus were used. One unit was defined as the amount needed to produce an absorbance change of 1, and the activity was measured in units (U) per milligram of serum or mucus peroxidase.

2.7.4 | cDNA synthesis and immune-related gene expression

The mRNA transcript levels of six target genes were analyzed after 8 weeks of feeding. Six fish from each dietary treatment were first anesthetized ($n = 6$). Then, approximately 50 mg of liver and intestinal tissues were collected from each fish for the extraction of total RNA. This was done using TRIzol reagent (Invitrogen, USA) and the RNA extraction kit (Invitrogen, PureLink™ RNA Mini Kit), following the manufacturer's instructions. The quality and quantity of the total RNA were assessed using a NanoDrop™ One spectrophotometer, measuring the absorbance ratios at 260/280 nm and 260/230 nm. Then, 1 µg of total RNA was used to synthesize cDNA using the iScript™ reverse transcription supermix (Bio-Rad, USA) for quantitative RT-PCR (qRT-PCR).

The mixture consisted of 1 µL of cDNA (100 ng), 1 µL of each primer (4 µM), 5 µL of 2× iTaq Universal SYBR Green supermix (Bio-Rad), and distilled water to a total volume of 10 µL. The qRT-PCR was conducted in a CFX Connect™ real-time system (Bio-Rad). The qRT-PCR analysis was conducted under following conditions: an initial denaturation at 95°C for 30 s, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. This was succeeded by a melt curve consisting of 95°C for 15 s, 60°C for 60 s, and 95°C for 15 s. The qRT-PCR results were evaluated using the 2^{-ΔΔCt} method and a standard curve was plotted (Livak & Schmittgen, 2001). The mRNA transcript levels of the studied genes were quantified using C_q values and normalized to 18S rRNA. The relative expression level of the control diet (DFPO) was set at 1. The primers used for qRT-PCR are listed in Table 4.

2.8 | Statistical analysis

The Kolmogorov–Smirnov test was used to assess the normality of the data. To evaluate whether different levels of substitution had significant impacts on the parameters, a one-way analysis of variance (ANOVA) followed by Tukey's multiple range test was used. Additionally, a trend analysis using orthogonal polynomial contrasts was conducted to assess whether the impact was linear or quadratic (Rosales et al., 2017). SAS statistical software (Cary, NC, USA) was used for all the analyses (Wakai et al., 2009). Statistical significance was set at $p < 0.05$.

TABLE 4 Primers used for quantitative real-time polymerase chain reaction in this study.

Target genes	Sequence (5'-3')	T_m (°C)	Product size (bp)	Accession No.
18S ribosomal RNA	GTGCATGGCCGTTCTTAGTT CTCAATCTCGTGTGGCTGAA	60	150	XR_003216134
Interleukin 1	GTCTGTCAAGGATAAGCGCTG ACTCTGGAGCTGGATGTTGA	59	200	XM_019365844
Interleukin 8	CTGTGAAGGCATGGGTGTG GATCACTTTCTTACCCAGGG	59	196	NM_001279704
Lipopolysaccharide binding protein	ACCAGAACTCGGAGAAGGA GATTGGTGGTCGGAGGTTTG	59	200	XM_013271147
Glutathione S-transferase α	ACTGCACACTCATGGGAACA TTAAAAGCCAGCGATTGAC	60	190	NM_001279635
Glutathione peroxidase	GGTGGATGTGAATGGAAAGG CTTGTAAAGTTCCCGTCAG	60	190	NM_001279711
Glutathione reductase	CTGCACCAAAGAACTGAAA CCAGAGAAGGCAGTCCACTC	60	172	XM_005467348

3 | RESULTS

3.1 | Growth performance analysis

Overall, fish fed diets containing powdered DFP exhibited significantly higher weight gain ($p < 0.05$) and marginally higher specific growth rates than those fed the control diet (without DFP), except for diet DFP160 ($p > 0.05$, Table 5). Fish fed the diet containing 40 g kg⁻¹ DFP achieved the highest weight gain, which was 1.5-fold greater than that of the control group, after 8 weeks of feeding. This was followed by those fed the diet containing 80 g kg⁻¹ DFP ($p < 0.05$, Table 5). Similar observations in specific growth rate were recorded after 4 and 8 weeks of feeding (Figure 1b). After 8 weeks of feeding, average feed conversion rates ranged from 1.14 in treatment DFP40 to 1.24 in treatment DFPO (without DFP). While this difference was statistically significant ($p < 0.05$), it was minor and is unlikely to have substantial practical significance (Table 5). Based on quadratic regression analysis of FW, SGR, WG, and FCR (Figure 1), the optimum level of DFP was determined to be 80 g kg⁻¹ diet. Survival rates exceeded 95% for all dietary treatments, with no statistically significant differences between treatments (Table 5).

3.2 | Immunological activity

3.2.1 | Lysozyme and peroxidase activity in skin mucus

Lysozyme activity in the skin mucus was 1.2-fold higher in fish fed DFP20 diet and 1.7-fold higher in fish fed the DFP40 diet compared with control fish not fed DFP ($p < 0.05$, Table 6). Similarly, the peroxidase activity in the skin mucus was significantly elevated in fish fed diets containing DFP, with increases of 1.2-fold in treatments DFP20, DFP80, and DFP160, and 1.6-fold in treatment DFP40 ($p < 0.05$, Table 6). Based on quadratic regression analysis of skin mucus lysozyme activity and skin mucus peroxidase activity (Figure 2), the optimum level of DFP was determined to be 80 g kg⁻¹ diet.

TABLE 5 Growth performances and feed utilization of Nile tilapia after 4 and 8 weeks feeding with dragon fruit peel diets.

	DFP0	DFP20	DFP40	DFP80	DFP160
IW (g)	14.63 ± 0.13 ^a	14.60 ± 0.13 ^a	14.67 ± 0.10 ^a	14.73 ± 0.03 ^a	14.60 ± 0.10 ^a
FW (g)					
4 weeks	30.70 ± 0.27 ^c	31.33 ± 0.39 ^b	34.10 ± 0.27 ^a	33.20 ± 0.33 ^{ab}	32.67 ± 0.88 ^{ab}
8 weeks	55.40 ± 0.81 ^d	61.67 ± 1.51 ^c	74.63 ± 0.58 ^a	65.35 ± 0.61 ^b	60.73 ± 1.53 ^c
WG (g)					
4 weeks	19.30 ± 0.93 ^c	22.63 ± 1.29 ^b	26.53 ± 0.90 ^a	24.21 ± 0.33 ^b	23.32 ± 0.99 ^b
8 weeks	40.70 ± 0.73 ^d	47.07 ± 1.38 ^c	59.93 ± 0.50 ^a	50.65 ± 0.56 ^b	46.07 ± 1.47 ^c
SGR (% day ⁻¹)					
4 weeks	2.65 ± 0.06 ^c	2.73 ± 0.03 ^c	3.01 ± 0.05 ^a	2.90 ± 0.04 ^{ab}	2.88 ± 0.05 ^b
8 weeks	2.65 ± 0.00 ^d	2.76 ± 0.01 ^c	2.96 ± 0.01 ^a	2.80 ± 0.01 ^b	2.77 ± 0.03 ^{bc}
FCR					
4 weeks	1.22 ± 0.03 ^a	1.18 ± 0.04 ^a	1.08 ± 0.03 ^b	1.11 ± 0.02 ^b	1.12 ± 0.02 ^b
8 weeks	1.24 ± 0.01 ^a	1.20 ± 0.01 ^c	1.14 ± 0.01 ^d	1.21 ± 0.01 ^{bc}	1.21 ± 0.01 ^b
SR (%)					
4 weeks	100	100	100	100	100
8 weeks	96.68 ± 1.22	95.74 ± 1.08	97.20 ± 1.57	97.13 ± 1.08	98.01 ± 1.36

Note: Different letters in the same row indicate significant differences ($p < 0.05$).

Abbreviations: FCR, feed conversion ratio; FW, final weight; IW, initial weight; SGR, specific growth rate; SR, survival rate; WG, weight gain.

3.2.2 | Serum lysozyme and peroxidase activities

Enhancement of lysozyme and peroxidase activity in blood serum by DFP mirrored the enhancements observed in skin mucus. Blood serum lysozyme activity was 1.6-fold higher and peroxidase activity was 1.8-fold higher in treatment DFP40 compared with treatment DFP0 ($p < 0.05$, Table 7). No significant differences in lysozyme and peroxidase activity were observed in the blood serum among treatments DFP20, DFP80, and DFP160. However, all these treatments exhibited significantly higher activity compared with the control and significantly lower activity than treatment DFP40. Based on quadratic regression analysis of SL and SP (Figure 2), the optimum level of DFP was determined to be 80 g kg⁻¹ diet.

3.3 | Immune and antioxidant-related gene expression

Figure 3 The mRNA transcripts of six genes involved in immune-antioxidant responses, interleukin-1 (*IL-1*), interleukin-8 (*IL-8*), lipopolysaccharide-binding protein (*LBP*), glutathione S-transferase- α (*GST- α*), glutathione peroxidase (*GPx*), and glutathione reductase (*GSR*) in liver and intestinal tissues of Nile tilapia.

In liver tissues, transcript levels for all six genes were higher in fish fed diets enriched with DFP. Specifically, increases ranged from approximately 2.4- to 3-fold for *IL-1*, 1.8- to 3.1-fold for *IL-8*, 1.8- to 2.9-fold for *LBP*, 1.7- to 2.8-fold for *GST- α* , 1.9- to 3.5-fold for *GPx*, and 1.7- to 3.5-fold for *GSR* ($p < 0.05$, Figure 3a). The highest transcript levels for all investigated genes, ranging from 2.5- to 3.5-fold, were observed in the DFP40 dietary treatment ($p < 0.05$). There were no statistically significant differences in *IL-1* and *IL-8* expression between DFP20 and DFP160 diets compared with the control treatment (DFP0) ($p > 0.05$).

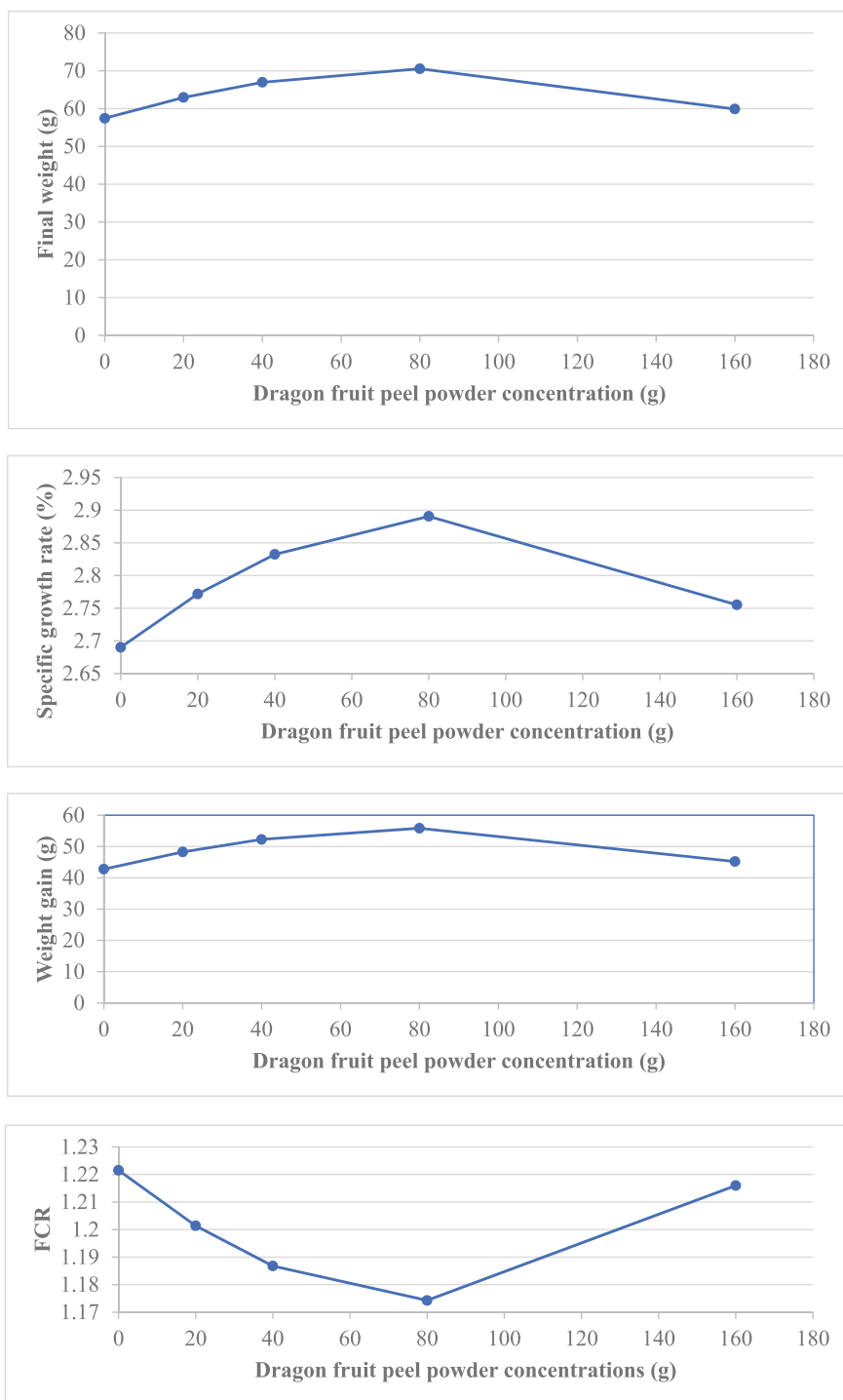


FIGURE 1 Weight gain (a), specific growth rate (b), feed conversion rate (c), and survival rate (d) of Nile tilapia after 4 and 8 weeks of feeding diets containing powdered dragon fruit peel at rates of 0 (DFP0, control), 20 (DFP20), 40 (DFP40), 80 (DFP80), and 160 (DFP160) g kg⁻¹. Each column is the mean of three replicates and the data represent as mean ± SE.

TABLE 6 Skin mucus lysozyme and peroxidase activities of Nile tilapia after 4 and 8 weeks feeding with dragon fruit peel diets.

	DFPO	DFF20	DFF40	DFF80	DFF160
4 weeks					
SMLA	1.44 ± 0.14 ^c	2.16 ± 0.21 ^b	2.45 ± 0.09 ^a	2.23 ± 0.07 ^{ab}	2.28 ± 0.19 ^{ab}
SMPA	0.05 ± 0.01 ^c	0.10 ± 0.03 ^b	0.14 ± 0.03 ^a	0.14 ± 0.02 ^a	0.10 ± 0.02 ^b
8 weeks					
SMLA	2.43 ± 0.19 ^d	2.89 ± 0.09 ^c	4.07 ± 0.30 ^a	3.62 ± 0.26 ^b	3.54 ± 0.21 ^b
SMPA	0.14 ± 0.01 ^c	0.17 ± 0.02 ^b	0.22 ± 0.01 ^a	0.17 ± 0.01 ^b	0.17 ± 0.01 ^b

Note: Different letters in the same row indicate significant differences ($p < 0.05$).

Abbreviations: SMLA, skin mucus lysozyme activity ($\mu\text{g mL}^{-1}$); SMPA, skin mucus peroxidase activity ($\mu\text{g mL}^{-1}$).

Transcript levels for all six genes in intestinal tissues mirrored those in liver tissues, with treatment DFF40 again showing the most significant enhancement compared with the control (DFPO) ($p < 0.05$, Figure 3b). This enhancement ranged approximately from 2.2- to 3.6-fold for *IL-1*, 1.9 to 2.4-fold for *IL-8*, 2.0- to 3.2-fold for *LBP*, 1.7- to 3.3-fold for *GST- α* , 1.9- to 3.3-fold for *GPx*, and 2.0- to 3.2-fold for *GSR* ($p < 0.05$, Figure 3b). However, the transcript levels of *IL-1*, *IL-8*, and *GST- α* in dietary treatments DFF20 and DFF160 were not significantly different from those in the control DFPO dietary treatment group ($p > 0.05$).

4 | DISCUSSION

Numerous publications have suggested that incorporating fruit peel into aquafeeds in appropriate quantities can enhance the growth, immune response, and overall health status of fish (M. A. Dawood, Habotta, et al., 2022). Given the increasing concern over diseases in commercial aquaculture, incorporating fruit peel into feed represents a significant, environmentally friendly, and sustainable approach to meeting the therapeutic needs of cultured fish (Gupta et al., 2023). Additionally, this approach aligns with contemporary food industry regulations that aims to effectively utilize waste from fruit remnants (Leong & Chang, 2022). In the present study, DFP powder was used as a functional feed additive to enhance growth, lysozyme and peroxidase activities in blood serum and skin mucus, and mRNA transcription levels of key genes associated with immune-antioxidant responses.

Growth enhancement is considered as a crucial characteristic in aquaculture, closely linked to the productivity of culture systems and the financial success of farmers (Gonzalez Parrao et al., 2021; Verdegem et al., 2023). The results of this study indicated that fish fed diets including DFP showed significantly improved growth performance and feed utilization. Similar to the current study, previous research achieved significant increases in the growth parameters with supplementation of rambutan, lemon, pineapple, and mango peels in *O. niloticus* (Harikrishnan et al., 2020; Van Doan et al., 2021) and juvenile bagrid catfish *Mystus nemurus* (See et al., 2024). These findings can be attributed to the phenolic compounds primarily concentrated in the DFPs. In the past, phenolic compounds have been recommended to improve growth performance in fish (Ahmadi et al., 2022; Ahmadifar et al., 2021). Moreover, the increase in weight gain and growth rates could be attributed to the high nutritional value (Madane et al., 2020) and relatively high fiber content of DFP (Jamilah et al., 2011; Madane et al., 2020; Tongkham et al., 2017). Reports indicate that DFPs contain a high concentration of dietary fibers, ranging from 59 to 90 g/100 g of dry matter, with the soluble fraction contributing roughly 55%–82% (Jamilah et al., 2011; Zhuang et al., 2012). Soluble dietary fiber, such as pectin, raffinose, stachyose, and galacto-oligosaccharides (Le, 2022), has potential prebiotic characteristics and has been reported to be beneficial for managing blood cholesterol and glucose levels, as well as having anti-inflammatory and anti-carcinogenic effects (Bhatt & Gupta, 2022; de Oliveira et al., 2022; Shah et al., 2020).

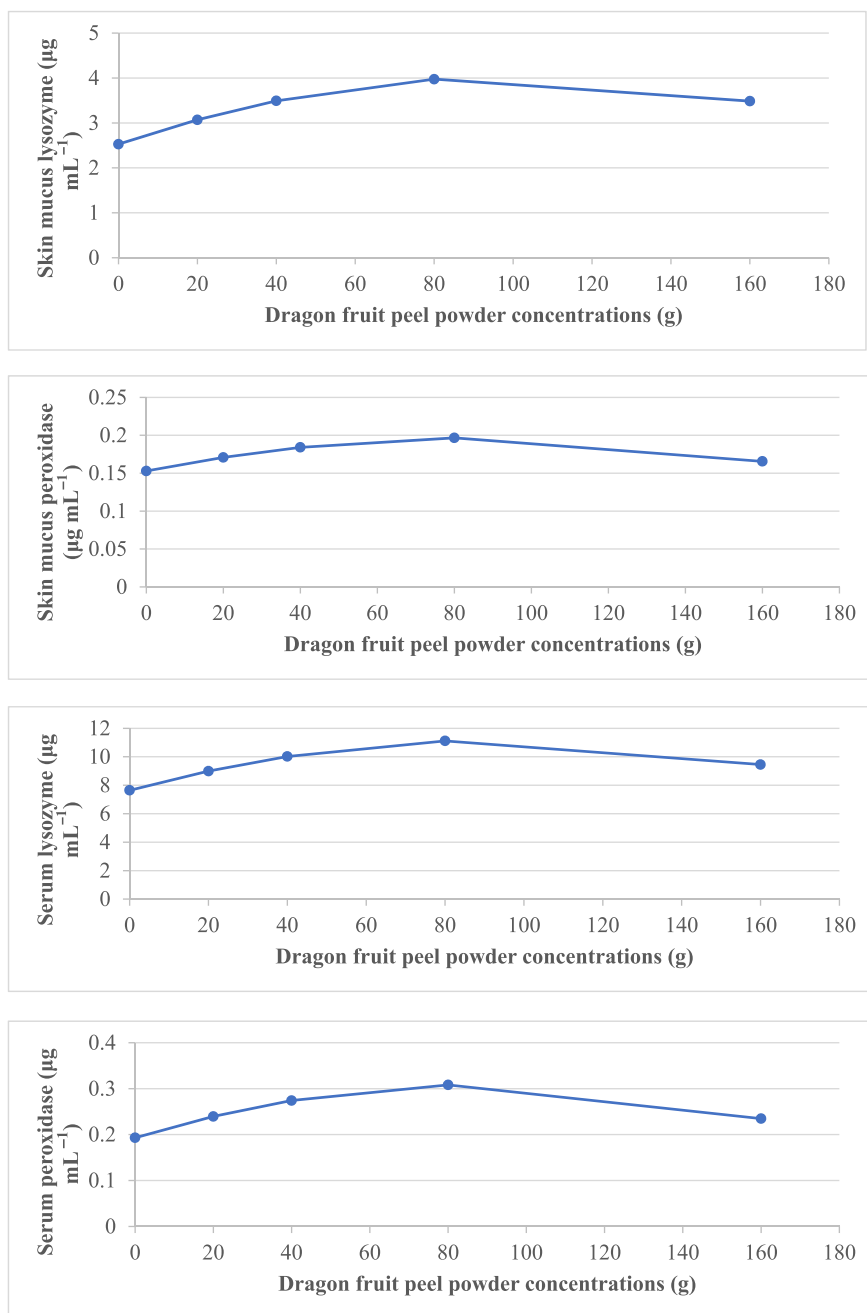


FIGURE 2 Lysozyme and peroxidase activity in skin mucus (a, c) and blood serum (b, d) of Nile tilapia after 4 and 8 weeks of feeding with powdered dragon fruit peel at rates of 0 (DFP0, control), 20 (DFP20), 40 (DFP40), 80 (DFP80), and 160 (DFP160) g kg⁻¹. All data are means of three replicates and the data represent as mean \pm SE.

Additionally, betacyanins from DFPs have been shown to positively affect the human serum lipid profile by reducing the levels of triglycerides, total cholesterol, low-density lipoprotein cholesterol, aspartate aminotransferase, and alanine aminotransferase in the blood (Le, 2022). Interestingly, we observed that weight gain and specific growth rates progressively declined at DFP levels above 40 g kg⁻¹. This decline may be attributed to the antinutritional effects of

TABLE 7 Serum lysozyme and peroxidase activities of Nile tilapia after 4 and 8 weeks feeding with dragon fruit peel diets.

	DFP0	DFP20	DFP40	DFP80	DFP160
4 weeks					
SL	4.86 ± 1.07 ^c	7.08 ± 0.30 ^b	8.50 ± 0.13 ^a	7.72 ± 0.12 ^{ab}	7.66 ± 0.11 ^{ab}
SP	0.12 ± 0.01 ^c	0.16 ± 0.01 ^{bc}	0.20 ± 0.02 ^a	0.16 ± 0.01 ^{ab}	0.16 ± 0.01 ^{bc}
8 weeks					
SL	7.00 ± 0.31 ^d	9.38 ± 0.41 ^c	11.07 ± 0.12 ^a	10.16 ± 0.44 ^b	9.62 ± 0.68 ^{bc}
SP	0.17 ± 0.02 ^c	0.26 ± 0.02 ^b	0.30 ± 0.02 ^a	0.28 ± 0.02 ^{ab}	0.24 ± 0.03 ^b

Note: Different letters in the same row indicate significant differences ($p < 0.05$).

Abbreviations: SL, serum lysozyme activity ($\mu\text{g mL}^{-1}$); SP, serum peroxidase activity ($\mu\text{g mL}^{-1}$).

tannins (Z. Chen et al., 2021), which can impair the digestion and absorption of proteins and lipids at higher concentrations (Omnes et al., 2017).

In fish, immune responses in skin mucus and blood serum serve as the primary line of defense against chemical, physical, and biological threats (Esteban, 2024). Fish fed diets supplemented with DFP exhibited significantly higher levels of lysozyme and peroxidase in both skin mucus and serum, particularly with the 40 g kg^{-1} DFP diet, compared with those on the DFP-free control diet. This indicates an enhanced immunological response in fish. One possible explanation is that dragon fruit by-products are rich in phenolic compounds, such as glycosides, flavanones, flavonoids, and xanthenes (Manihuruk et al., 2017; Nurliyana et al., 2010), as well as vitamins C and E (Arivalagan et al., 2021). Generally, phenolic compounds act as antioxidants through various mechanisms, including scavenging of free radicals, quenching of reactive oxygen species (ROS), inhibiting of oxidative enzymes, and interacting with bio-membranes. These actions collectively stimulate the immune system in fish (M. A. Dawood & Koshio, 2018; Hamre, 2011). Additionally, DFP contains terpenes, which are phytochemicals (Wahdaningsih et al., 2020) known for their beneficial role in enhancing mucosal immunity in fish species, such as Nile tilapia and rainbow trout (Firmino et al., 2021). Interestingly, similar to finding in Rohu, *Labeo rohita*, fed banana peel flour (Giri et al., 2016) and Nile tilapia fed watermelon rind and orange peel (Doan et al., 2020; Van Doan et al., 2019), we observed no enhancement of immunological properties in the skin mucus and blood serum of fish fed diets containing higher levels of DFP (DFP80 and DFP160). This lack of enhancement may be attributed to increased movement of feed through the gut, resulting in reduced absorption, as previously suggested (El-Barbary, 2016; Grundy et al., 2016).

Interleukin-1 is a pro-inflammatory cytokine that regulates the immune response by modulating cell proliferation, differentiation, and apoptosis, among other biological functions (Ethuin et al., 2001). It also controls the production of pro-inflammatory cytokines, such as *IFN-* and *IL-8*, by monocytes/macrophages, which help defend fish against infection (M. A. O. Dawood et al., 2020). We found that fish fed diets supplemented with DFP powder exhibited higher expression of *IL-1* and *IL-8* compared with those fed the control diet without DFP powder. This was particularly evident in fish fed the DFP40 diet. In general, dragon fruit by-products contain a high concentration of various constituents, most notably pectin, a family of galacturonic acid-rich polysaccharides with potential immunomodulatory functions that regulate the inflammatory response (Hassaan et al., 2021). This result may be attributable to the biological effects of betalains and betacyanins present in the DFP (Khoo et al., 2022; Le, 2022). These compounds have been shown to exhibit anti-inflammatory activity (Gao et al., 2021; Khoo et al., 2022; Moreno-Ley et al., 2021; Wang et al., 2022). LBP is a protein involved in lipopolysaccharide signaling and innate immunity (Ding & Jin, 2014). The gene *LBP* plays a significant role in the immune response to Gram-negative bacterial infections during the acute phase response (Weiss, 2003). *LBP* also promotes both nonspecific and specific immune responses in fish (Lazado & Caipang, 2014). The transcription level of *LBP* in Nile tilapia was significantly enhanced in all DFP dietary

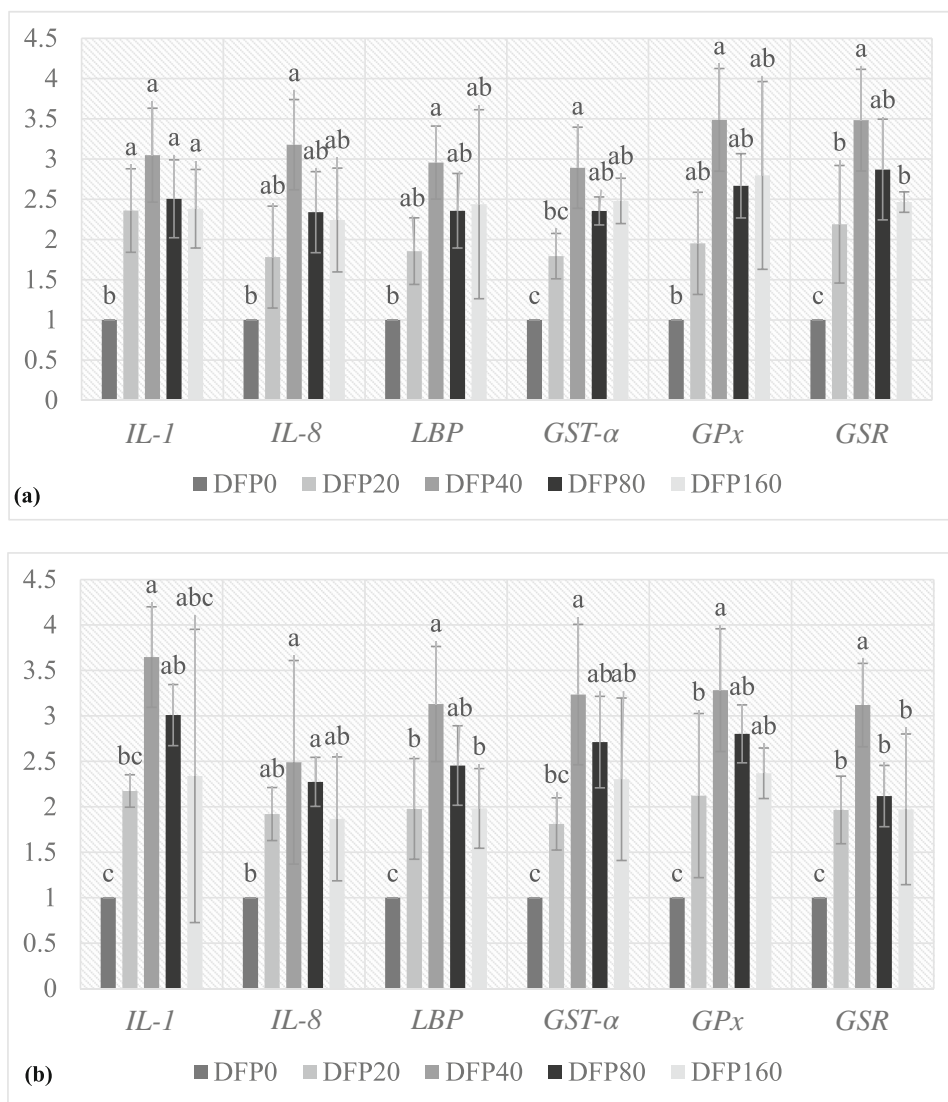


FIGURE 3 Relative gene expressions of immune-related (*IL-1*, *IL-8*, and *LBP*) and antioxidant-related (*GPx*, *GST-α*, and *GSR*) genes in (a) liver tissue and (b) intestinal tissue of Nile tilapia ($n = 6$) after feeding with diets containing 0 (DFP0, control), 20 (DFP20), 40 (DFP40), 80 (DFP80), and 160 (DFP160) g kg⁻¹. All data are means of three replicates and the data represent as mean \pm SE. Different superscript letters indicate significant differences between groups ($p < 0.05$).

treatments, with the most notable increase observed in the DFP40 dietary treatment. Our findings are consistent with studies reported for Nile tilapia (Le Xuan et al., 2024).

In fish, the immune and the antioxidant defense systems are inextricably connected (El Basuini et al., 2020). ROS are continuously produced in a living system, even without xenobiotic metabolism (Dickinson & Chang, 2011). Oxidative stress is caused by an imbalance between generation and elimination of ROS (Lushchak, 2011). GSR and GPx are key components in the elimination of excess ROS and the maintenance of cell homeostasis (Budak et al., 2014; Drozd et al., 2016). Additionally, GST is involved in the sequestration of free radicals (Sousa et al., 2020). These enzymes become more active in the presence of certain dietary supplements (El-Barbary, 2016). We found that the

expression of all three antioxidant genes (*GPx*, *GST-α*, and *GSR*) was considerably higher in fish fed dietary DFP compared with those in the control group, with the highest levels observed in the 40 g kg⁻¹ DFP treatment group. The active constituents in powdered DFP responsible for the enhancement of antioxidant gene expression are likely betalains, betacyanins, and phenolic compounds (Le, 2022). The betalains from DFPs exhibit activity comparable with quercetin, a well-known strong anti-inflammatory compound (Le, 2022). The powerful antioxidant properties of the betalains found in DFPs are attributed to the ability of a free phenolic group to transfer hydrogen atoms within the catechol moiety of betacyanins, as well as the presence of a cyclic amine group in the betalamic acid moiety (Le, 2022). These structures are also present in the well-known powerful antioxidant ethoxyquin (Esatbeyoglu et al., 2015). It has been established that phenolic substances predominantly contribute to their powerful antioxidant activities (Le, 2022). DFPs exhibit a high DPPH[·] radical scavenging capacity compared with butylated hydroxyanisole (Nurliyana et al., 2010) and ascorbic acid (Fathordoobady et al., 2016). Several other studies have demonstrated an increase in antioxidant enzyme gene expression in fish after administering plant-based additives as part of the diet (A. Dawood, Zuberi, & Shi, 2022; Xie et al., 2021), which is consistent with our findings.

Our study was conducted in a biofloc system, which simplifies the management of water, feed, and wastes (Emerenciano et al., 2013), and provides a suitable environment for reducing farming expenses using plant-based feed supplements for aquatic animals. DFP contains high levels of pectin, betanin, phyllocatin, hylcoerenin, and betacyanin, which have prebiotic properties and could contribute to maintaining a healthy microbial community in biofloc systems. This has been observed in previous studies where Nile tilapia were fed various fruit by-products in biofloc system (Du et al., 2024; Matra et al., 2021). However, questions remain regarding the extent to which the enhancement in growth and immune responses observed with the addition of powdered DFP to the diet of Nile tilapia can be attributed to a direct physiological effect, and how much can be attributed to the role of DFP as a prebiotic in biofloc system. This warrants further investigation.

5 | CONCLUSION

Our results clearly show that after 8 weeks of feeding a diet supplemented with DFP in a biofloc system under controlled laboratory conditions, the immunological characteristics of skin mucus, blood serum, liver tissue, and intestinal tissue were significantly enhanced. The 80 g kg⁻¹ DFP diet may be used as a promising feed additive for Nile tilapia raised in biofloc systems.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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REFERENCES

- Ahmadi, A., Bagheri, D., Hoseinifar, S. H., Morshedi, V., & Paolucci, M. (2022). Beneficial role of polyphenols as feed additives on growth performances, immune response and antioxidant status of Lates Calcarifer (Bloch, 1790) juveniles. *Aquaculture*, 552, 737955.
- Ahmadifar, E., Yousefi, M., Karimi, M., Fadaei Raieni, R., Dadar, M., Yilmaz, S., Dawood, M. A. O., & Abdel-Latif, H. M. R. (2021). Benefits of dietary polyphenols and polyphenol-rich additives to aquatic animal health: An overview. *Reviews in Fisheries Science & Aquaculture*, 29, 478–511.
- Arivalagan, M., Karunakaran, G., Roy, T., Dinsha, M., Sindhu, B., Shilpashree, V., Satisha, G., & Shivashankara, K. (2021). Biochemical and nutritional characterization of dragon fruit (*Hylocereus* species). *Food Chemistry*, 353, 129426.
- Avnimelech, Y. (2009). *Biofloc technology: A practical guide book*. World Aquaculture Society.
- Bardhan, A., Abraham, T. J., Sar, T. K., Rajisha, R., Panda, S. K., & Patil, P. K. (2024). Pharmacokinetics and residues of florfenicol in Nile tilapia (*Oreochromis niloticus*) post-oral gavage. *Environmental Toxicology and Pharmacology*, 108, 104471.
- Bhatt, S., & Gupta, M. (2022). Dietary fiber from fruit waste as a potential source of metabolites in maintenance of gut milieu during ulcerative colitis: A comprehensive review. *Food Research International*, 164, 112329.
- Boyd, C. E., D'Abramo, L. R., Glencross, B. D., Huyben, D. C., Juarez, L. M., Lockwood, G. S., McNevin, A. A., Tacon, A. G. J., Teletchea, F., Tomasso, J. R., Jr., Tucker, C. S., & Valenti, W. C. (2020). Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. *Journal of the World Aquaculture Society*, 51, 578–633.
- Budak, H., Kocpinar, E. F., Gonul, N., Ceylan, H., Erol, H. S., & Erdogan, O. (2014). Stimulation of gene expression and activity of antioxidant related enzyme in Sprague Dawley rat kidney induced by long-term iron toxicity. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 166, 44–50.
- Cardona, E., Lorgeoux, B., Chim, L., Goguenheim, J., Le Delliou, H., & Cahu, C. (2016). Biofloc contribution to antioxidant defence status, lipid nutrition and reproductive performance of broodstock of the shrimp *Litopenaeus stylirostris*: Consequences for the quality of eggs and larvae. *Aquaculture*, 452, 252–262.
- Charo-Karisa, H. (2024). Tilapia. In M. Dikeman (Ed.), *Encyclopedia of meat sciences* (3rd ed., pp. 29–39). Elsevier.
- Chen, S.-W., Liu, C.-H., & Hu, S.-Y. (2019). Dietary administration of probiotic *Paenibacillus ehimensis* NPUST1 with bacteriocin-like activity improves growth performance and immunity against *Aeromonas hydrophila* and *Streptococcus iniae* in Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology*, 84, 695–703.
- Chen, Z., Zhong, B., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. R. (2021). Identification of phenolic compounds in Australian grown dragon fruits by LC-ESI-QTOF-MS/MS and determination of their antioxidant potential. *Arabian Journal of Chemistry*, 14, 103151.
- Chen, D., Bai, R., Yong, H., Zong, S., Jin, C. & Liu, J. (2022). Improving the digestive stability and prebiotic effect of carboxymethyl chitosan by grafting with gallic acid: In vitro gastrointestinal digestion and colonic fermentation evaluation. *International journal of biological macromolecules*, 214, 685–696.
- Cherian, T., Ragavendran, C., Vijayan, S., Kurien, S., & Peijnenburg, W. J. G. M. (2023). A review on the fate, human health and environmental impacts, as well as regulation of antibiotics used in aquaculture. *Environmental Advances*, 13, 100411.
- Chumroenvithayakul, S., Thilavech, T., Abeywardena, M., & Adisakwattana, S. (2023). Dragon fruit peel waste (*Hylocereus undatus*) as a potential ingredient for reducing lipid peroxidation, dietary advanced glycation end products, and starch digestibility in cookies. *Antioxidants*, 12, 1–14.

- Dawood, A., Zuberi, A., & Shi, W. (2022). Plant-based β -mannanase supplemented diet modulates the gut microbiota and up-regulates the expression of immunity and digestion-related genes in *Cyprinus carpio*. *Journal of Applied Animal Research*, 50, 21–30.
- Dawood, M. A., Habotta, O. A., Elsabagh, M., Azra, M. N., Van Doan, H., Kari, Z. A., & Sewilam, H. (2022). Fruit processing by-products in the aquafeed industry: A feasible strategy for aquaculture sustainability. *Reviews in Aquaculture*, 14, 1945–1965.
- Dawood, M. A., & Koshio, S. (2018). Vitamin C supplementation to optimize growth, health and stress resistance in aquatic animals. *Reviews in Aquaculture*, 10, 334–350.
- Dawood, M. A. O., Moustafa, E. M., Gewaily, M. S., Abdo, S. E., AbdEl-kader, M. F., SaadAllah, M. S., & Hamouda, A. H. (2020). Ameliorative effects of *Lactobacillus plantarum* L-137 on Nile tilapia (*Oreochromis niloticus*) exposed to deltamethrin toxicity in rearing water. *Aquatic Toxicology*, 219, 105377.
- de Oliveira, F. L., Arruda, T. Y. P., Morzelle, M. C., Pereira, A. P. A., & Casarotti, S. N. (2022). Fruit by-products as potential prebiotics and promising functional ingredients to produce fermented milk. *Food Research International*, 161, 111841.
- Dickinson, B. C., & Chang, C. J. (2011). Chemistry and biology of reactive oxygen species in signaling or stress responses. *Nature Chemical Biology*, 7, 504–511.
- Ding, C., & He, J. (2010). Effect of antibiotics in the environment on microbial populations. *Applied Microbiology and Biotechnology*, 87, 925–941.
- Ding, P.-H., & Jin, L. J. (2014). The role of lipopolysaccharide-binding protein in innate immunity: A revisit and its relevance to oral/periodontal health. *Journal of Periodontal Research*, 49, 1–9.
- Doan, H. V., Lumsangkul, C., Hoseinifar, S. H., Hung, T. Q., Stejskal, V., Ringø, E., Dawood, M. A. O., & Esteban, M. Á. (2020). Administration of watermelon rind powder to Nile tilapia (*Oreochromis niloticus*) culture under biofloc system: Effect on growth performance, innate immune response, and disease resistance. *Aquaculture*, 528, 735574.
- Drozd, E., Krzysztos-Russjan, J., Marczevska, J., Drozd, J., Bubko, I., Bielak, M., Lubelska, K., Wiktorska, K., Chilmonec, Z., Anuszewska, E., & Gruber-Bzura, B. (2016). Up-regulation of glutathione-related genes, enzyme activities and transport proteins in human cervical cancer cells treated with doxorubicin. *Biomedicine & Pharmacotherapy*, 83, 397–406.
- Du, H., Olawuyi, I. F., Said, N. S., & Lee, W.-Y. (2024). Comparative analysis of physicochemical and functional properties of pectin from extracted dragon fruit waste by different techniques. *Polymers*, 16, 1097.
- El Basuini, M. F., Teiba, I. I., Zaki, M. A. A., Alabssawy, A. N., El-Hais, A. M., Gabr, A. A., Dawood, M. A. O., Zaineldin, A. I., Mzengereza, K., Shadrack, R. S., & Dossou, S. (2020). Assessing the effectiveness of CoQ10 dietary supplementation on growth performance, digestive enzymes, blood health, immune response, and oxidative-related genes expression of Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology*, 98, 420–428.
- El-Barbary, M. I. (2016). Detoxification and antioxidant effects of garlic and curcumin in *Oreochromis niloticus* injected with aflatoxin B1 with reference to gene expression of glutathione peroxidase (GPx) by RT-PCR. *Fish Physiology and Biochemistry*, 42, 617–629.
- Emerenciano, M., Ballester, E. L. C., Cavalli, R. O., & Wasielesky, W. (2012). Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). *Aquaculture Research*, 43, 447–457.
- Eroldoğan, O. T., Glencross, B., Novoveska, L., Gaudêncio, S. P., Rinkevich, B., Varese, G. C., de Fátima Carvalho, M., Tasdemir, D., Safarik, I., Nielsen, S. L., Rebours, C., Lada, L. B., Robbens, J., Strode, E., Haznedaroglu, B. Z., Kotta, J., Evliyaoğlu, E., Oliveira, J., Girão, M., ... Rotter, A. (2023). From the sea to aquafeed: A perspective overview. *Reviews in Aquaculture*, 15, 1028–1057.
- Esatbeyoglu, T., Wagner, A. E., Schini-Kerth, V. B., & Rimbach, G. (2015). Betanin—A food colorant with biological activity. *Molecular Nutrition & Food Research*, 59, 36–47.
- Esteban, M. Á. (2024). A review of soluble factors and receptors involved in fish skin immunity: The tip of the iceberg. *Fish & Shellfish Immunology*, 145, 109311.
- Ethuin, F., Delarche, C., Benslama, S., Gougerot-Pocidallo, M.-A., Jacob, L., & Chollet-Martin, S. (2001). Interleukin-12 increases interleukin 8 production and release by human polymorphonuclear neutrophils. *Journal of Leukocyte Biology*, 70, 439–446.
- Fathoridoobady, F., Mirhosseini, H., Selamat, J., & Abd Manap, M. Y. (2016). Effect of solvent type and ratio on betacyanins and antioxidant activity of extracts from *Hylocereus polyrhizus* flesh and peel by supercritical fluid extraction and solvent extraction. *Food Chemistry*, 202, 70–80.
- Firmino, J. P., Galindo-Villegas, J., Reyes-López, F. E., & Gisbert, E. (2021). Phytochemical bioactive compounds shape fish mucosal immunity. *Frontiers in Immunology*, 12, 1–20.
- Gao, Y., Liang, X., Tian, Z., Ma, Y., & Sun, C. (2021). Betalain exerts cardioprotective and anti-inflammatory effects against the experimental model of heart failure. *Human & Experimental Toxicology*, 40, S16–S28.
- Geletu, T. T., & Zhao, J. (2023). Genetic resources of Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) in its native range and aquaculture. *Hydrobiologia*, 850, 2425–2445.

- Giri, S. S., Jun, J. W., Sukumaran, V., & 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, 1–11.
- Gonzalez Parrao, C., Shisler, S., Moratti, M., Yavuz, C., Acharya, A., Eysers, J., & Snilstveit, B. (2021). Aquaculture for improving productivity, income, nutrition and women's empowerment in low- and middle-income countries: A systematic review and meta-analysis. *Campbell Systematic Reviews*, 17, e1195.
- Grundy, M. M.-L., Edwards, C. H., Mackie, A. R., Gidley, M. J., Butterworth, P. J., & Ellis, P. R. (2016). Re-evaluation of the mechanisms of dietary fibre and implications for macronutrient bioaccessibility, digestion and postprandial metabolism. *British Journal of Nutrition*, 116, 816–833.
- Gule, T. T., & Geremew, A. (2022). Dietary strategies for better utilization of aquafeeds in Tilapia farming. *Aquaculture Nutrition*, 2022, 1–11.
- Gupta, S. K., Gupta, A., Sarkar, B., Gupta, R., Kumar, M., Kumari, A., & Foyсал, M. J. (2023). Pomegranate (*Punica granatum*) peel extract supplementation in diet influences growth performance, haemato-immunological responses and cytokine expression in pathogen-aggravated *Labeo rohita* fingerlings. *Aquaculture*, 562, 738823.
- Guo, X., Zhang, X., Ying, X., Ma, A., Li, Z., Liu, H. & Guo, Q. (2023). Fermentation properties and prebiotic potential of different pectins and their corresponding enzymatic hydrolysates. *Food Hydrocolloids*, 143, 108878.
- Hamre, K. (2011). Metabolism, interactions, requirements and functions of vitamin E in fish. *Aquaculture Nutrition*, 17, 98–115.
- Harikrishnan, R., & Balasundaram, C. (2005). Modern trends in *Aeromonas hydrophila* disease management with fish. *Reviews in Fisheries Science*, 13, 281–320.
- Harikrishnan, R., Balasundaram, C., & Heo, M.-S. (2010). Herbal supplementation diets on hematology and innate immunity in goldfish against *Aeromonas hydrophila*. *Fish & Shellfish Immunology*, 28, 354–361.
- Harikrishnan, R., Balasundaram, C., & Heo, M.-S. (2011). Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. *Aquaculture*, 317, 1–15.
- Harikrishnan, R., Balasundaram, C., Kim, M.-C., Kim, J.-S., Han, Y.-J., & Heo, M.-S. (2009). Innate immune response and disease resistance in *Carassius auratus* by triherbal solvent extracts. *Fish & Shellfish Immunology*, 27, 508–515.
- Harikrishnan, R., Thamizharasan, S., Devi, G., Van Doan, H., Ajith Kumar, T. T., Hoseinifar, S. H., & Balasundaram, C. (2020). Dried lemon peel enriched diet improves antioxidant activity, immune response and modulates immuno-antioxidant genes in *Labeo rohita* against *Aeromonas sorbia*. *Fish & Shellfish Immunology*, 106, 675–684.
- Hassaan, M. S., Mohammady, E. Y., Soaudy, M. R., Elashry, M. A., Moustafa, M. M. A., Wassel, M. A., El-Garhy, H. A. S., El-Haroun, E. R., & Elsaied, H. E. (2021). Synergistic effects of *Bacillus pumilus* and exogenous protease on Nile tilapia (*Oreochromis niloticus*) growth, gut microbes, immune response and gene expression fed plant protein diet. *Animal Feed Science and Technology*, 275, 114892.
- Hazreen-Nita, M. K., Abdul Kari, Z., Mat, K., Rusli, N. D., Mohamad Sukri, S. A., Che Harun, H., Lee, S. W., Rahman, M. M., Norazmi-Lokman, N. H., Nur-Nazifah, M., Firdaus-Nawi, M., & Dawood, M. A. O. (2022). Olive oil by-products in aquafeeds: Opportunities and challenges. *Aquaculture Reports*, 22, 100998.
- Jamilah, B., Shu, C., Kharidah, M., Dzulkily, M., & Noranizan, A. (2011). Physico-chemical characteristics of red pitaya (*Hylocereus polyrhizus*) peel. *International Food Research Journal*, 18, 279–286.
- Jiang, H., Zhang, W., Li, X., Shu, C., Jiang, W., & Cao, J. (2021). Nutrition, phytochemical profile, bioactivities and applications in food industry of pitaya (*Hylocereus* spp.) peels: A comprehensive review. *Trends in Food Science & Technology*, 116, 199–217.
- Jimenez-Garcia, S. N., Garcia-Mier, L., Ramirez-Gomez, X. S., Aguirre-Becerra, H., Escobar-Ortiz, A., Contreras-Medina, L.M., Garcia-Trejo, J. F., & Feregrino-Perez, A. A. (2022). Pitahaya Peel: A By-Product with Great Phytochemical Potential, Biological Activity, and Functional Application. *Molecules*, 27, 5339.
- Khanjani, M. H., Mozanadeh, M. T., Sharifinia, M., & Emerenciano, M. G. C. (2024). Broodstock and seed production in bio-floc technology (BFT): An updated review focused on fish and penaeid shrimp. *Aquaculture*, 579, 740278.
- Khanjani, M. H., & Sharifinia, M. (2020). Biofloc technology as a promising tool to improve aquaculture production. *Reviews in Aquaculture*, 12, 1836–1850.
- Khanjani, M. H., Sharifinia, M., & Emerenciano, M. G. C. (2024). Biofloc technology (BFT) in aquaculture: What goes right, what goes wrong? A scientific-based snapshot. *Aquaculture Nutrition*, 2024, 7496572.
- Khoo, H. E., He, X., Tang, Y., Li, Z., Li, C., Zeng, Y., Tang, J., & Sun, J. (2022). Betacyanins and anthocyanins in pulp and peel of red pitaya (*Hylocereus polyrhizus* cv. Jindu), inhibition of oxidative stress, lipid reducing, and cytotoxic effects. *Frontiers in Nutrition*, 9, 1–11.
- Kishawy, A. T., Sewid, A. H., Nada, H. S., Kamel, M. A., El-Mandrawy, S. A., Abdelhakim, T. M., El-Murr, A. E. I., Nahhas, N. E., Hozzein, W. N., & Ibrahim, D. (2020). Mannan oligosaccharides as a carbon source in biofloc boost dietary plant protein and water quality, growth, immunity and *Aeromonas hydrophila* resistance in Nile tilapia (*Oreochromis niloticus*). *Animals*, 10, 1724.

- Lazado, C. C., & Caipang, C. M. A. (2014). Probiotics–pathogen interactions elicit differential regulation of cutaneous immune responses in epidermal cells of Atlantic cod *Gadus morhua*. *Fish & Shellfish Immunology*, *36*, 113–119.
- Le, N. L. (2022). Functional compounds in dragon fruit peels and their potential health benefits: A review. *International Journal of Food Science & Technology*, *57*, 2571–2580.
- Le Xuan, C., Linh, N. V., Wannavijit, S., Outama, P., Fontana, C. M., Meepowpan, P., & Van Doan, H. (2024). Influences of making *Syzygium nervosum* seed powder on growth performance, immunological response, antioxidant and immune related gene expression in juvenile Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *588*, 740943.
- Leong, Y. K., & Chang, J.-S. (2022). Valorization of fruit wastes for circular bioeconomy: Current advances, challenges, and opportunities. *Bioresource Technology*, *359*, 127459.
- Li, X., Zhang, Z.-H., Qiao, J., Qu, W., Wang, M.-S., Gao, X., Zhang, C., Brennan, C. S., & Qi, X. (2022). Improvement of betalains stability extracted from red dragon fruit peel by ultrasound-assisted microencapsulation with maltodextrin. *Ultrasonics Sonochemistry*, *82*, 105897.
- Liu, Y., Chen, H., Chen, S., Zhang, Y., Zhang, J., Zhu, X., Li, W., Liu, J., Jiang, Y. & Li, D. (2023). Pectin-rich dragon fruit peel extracts: An environmentally friendly emulsifier of natural origin. *Food Chemistry*, *429*, 136955.
- Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2– $\Delta\Delta$ CT method. *Methods*, *25*, 402–408.
- Lushchak, V. I. (2011). Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology*, *101*, 13–30.
- Madane, P., Das, A. K., Nanda, P., Bandyopadhyay, S., Jagtap, P., Shewalkar, A., & Maity, B. (2020). Dragon fruit (*Hylocereus undatus*) peel as antioxidant dietary fibre on quality and lipid oxidation of chicken nuggets. *Journal of Food Science and Technology*, *57*, 1449–1461.
- Magna, E. K., Ofosu-Koranteng, F., Asmah, R., Mensah, E. T., Appiah, E. K., Fatsi, P. S., Adu-Nti, F., Kpodo, Z. C., & Lente, I. (2024). Preliminary investigation on the occurrence and health risk assessment of antibiotics in cultured tilapia reared at a commercial outlet in Tema, Ghana. *Heliyon*, *10*, e28193.
- Magouz, F. I., Moustafa, E. M., Abo-Remela, E. M., Halawa, M. R., Barakaat, P. M., & Omar, A. A. (2024). Summer mortality syndrome bacterial pathogens in farmed Nile tilapia (*Oreochromis niloticus*). *Open Veterinary Journal*, *14*, 53–69.
- Majeed, T., Shabir, I., Srivastava, S., Maqbool, N., Dar, A. H., Jan, K., Pandey, V. K., Shams, R., Bashir, I., Dash, K. K., Hanan, E., & Wani, N. R. (2024). Valorization of food wastes by implementation of subcritical water extraction: A comprehensive review. *Trends in Food Science & Technology*, *143*, 104316.
- Manihuruk, F. M., Suryati, T., & Arief, I. I. (2017). Effectiveness of the red dragon fruit (*Hylocereus polyrhizus*) peel extract as the colorant, antioxidant, and antimicrobial on beef sausage. *Media Peternakan*, *40*, 47–54.
- Matra, M., Totakul, P., Viennasay, B., Phesatcha, B., & Wanapat, M. (2021). Dragon fruit (*Hylocereus undatus*) peel pellet as a rumen enhancer in Holstein crossbred bulls. *Animal Bioscience*, *34*, 594–602.
- Mohammadi, G., Rafiee, G., Tavabe, K. R., Abdel-Latif, H. M., & Dawood, M. A. (2021). The enrichment of diet with beneficial bacteria (single-or multi-strain) in biofloc system enhanced the water quality, growth performance, immune responses, and disease resistance of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *539*, 736640.
- Moreno-Ley, C. M., Osorio-Revilla, G., Hernández-Martínez, D. M., Ramos-Monroy, O. A., & Gallardo-Velázquez, T. (2021). Anti-inflammatory activity of betalains: A comprehensive review. *Human Nutrition & Metabolism*, *25*, 200126.
- Nurliyana, R., Syed Zahir, I., Mustapha Suleiman, K., Aisyah, M., & Kamarul Rahim, K. (2010). Antioxidant study of pulps and peels of dragon fruits: A comparative study. *International Food Research Journal*, *17*, 367–375.
- Omnes, M.-H., Le Goasduff, J., Le Delliou, H., Le Bayon, N., Quazuguel, P., & Robin, J. H. (2017). Effects of dietary tannin on growth, feed utilization and digestibility, and carcass composition in juvenile European seabass (*Dicentrarchus labrax* L.). *Aquaculture Reports*, *6*, 21–27.
- Outama, P., Le Xuan, C., Wannavijit, S., Lumsangkul, C., Linh, N. V., Montha, N., Tongsir, S., Chitmanat, C., & Van Doan, H. (2022). Modulation of growth, immune response, and immune-antioxidant related gene expression of Nile tilapia (*Oreochromis niloticus*) reared under biofloc system using mango peel powder. *Fish & Shellfish Immunology*, *131*, 1136–1143.
- Parry, R. M., Jr., Chandan, R. C., & Shahani, K. M. (1965). A rapid and sensitive assay of muramidase. *Proceedings of the Society for Experimental Biology and Medicine*, *119*, 384–386.
- Prastiya, R., & Yusuf, A. (2021). Red dragon fruit peel (*Hylocereus polyrhizus*) as feed biosupplement on performance of fattening rabbits. *IOP Conference Series: Earth and Environmental Science*, *743*, 012060.
- Quade, M. J., & Roth, J. A. (1997). A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. *Veterinary Immunology and Immunopathology*, *58*, 239–248.
- Rico, A., Oliveira, R., McDonough, S., Matser, A., Khatikarn, J., Satapornvanit, K., Nogueira, A. J., Soares, A. M., Domingues, I., & Van den Brink, P. J. (2014). Use, fate and ecological risks of antibiotics applied in tilapia cage farming in Thailand. *Environmental Pollution*, *191*, 8–16.
- Rifna, E. J., Misra, N. N., & Dwivedi, M. (2021). Recent advances in extraction technologies for recovery of bioactive compounds derived from fruit and vegetable waste peels: A review. *Critical Reviews in Food Science and Nutrition*, *63*, 719–752.

- Rodriguez, E. B., Vidallon, M. L. P., Mendoza, D. J. R., & Reyes, C. T. (2016). Health-promoting bioactivities of betalains from red dragon fruit (*Hylocereus polyrhizus* (Weber) Britton and Rose) peels as affected by carbohydrate encapsulation. *Journal of the Science of Food and Agriculture*, 96, 4679–4689.
- Rosales, M., Castillo, S., Pohlenz, C., & Gatlin, D. M., III. (2017). Evaluation of dried yeast and threonine fermentation biomass as partial fish meal replacements in the diet of red drum *Sciaenops ocellatus*. *Animal Feed Science and Technology*, 232, 190–197.
- See, M. S., Musa, N., Liew, H. J., Harun, N. O., & Rahmah, S. (2024). Sweet orange peel waste as a feed additive in growth promoting and protective effect against *Aeromonas hydrophila* of juvenile bagrid catfish *Mystus nemurus*. *Journal of Environmental Management*, 351, 119677.
- Shah, B. R., Li, B., Al Sabbah, H., Xu, W., & Mráz, J. (2020). Effects of prebiotic dietary fibers and probiotics on human health: With special focus on recent advancement in their encapsulated formulations. *Trends in Food Science & Technology*, 102, 178–192.
- Sousa, A. A., Lopes, D. L. A., Emerenciano, M. G. C., Nora, L., Souza, C. F., Baldissera, M. D., Baldisserotto, B., Alba, D. F., & Da Silva, A. S. (2020). Phosphatidylcholine in diets of juvenile Nile tilapia in a biofloc technology system: Effects on performance, energy metabolism and the antioxidant system. *Aquaculture*, 515, 734574.
- Tongkham, N., Juntasalay, B., Lasunon, P., & Sengkhampan, N. (2017). Dragon fruit peel pectin: Microwave-assisted extraction and fuzzy assessment. *Agriculture and Natural Resources*, 51, 262–267.
- Van Doan, H., Hoseinifar, S. H., Harikrishnan, R., Khamlor, T., Punyatong, M., Tapingkae, W., Yousefi, M., Palma, J., & El-Haroun, E. (2021). Impacts of pineapple peel powder on growth performance, innate immunity, disease resistance, and relative immune gene expression of Nile tilapia, *Oreochromis niloticus*. *Fish & Shellfish Immunology*, 114, 311–319.
- Van Doan, H., Hoseinifar, S. H., Naraballoh, W., Jaturasitha, S., Tongsir, S., Chitmanat, C., & Ringø, E. (2019). Dietary inclusion of Orange peels derived pectin and *Lactobacillus plantarum* for Nile tilapia (*Oreochromis niloticus*) cultured under indoor biofloc systems. *Aquaculture*, 508, 98–105.
- Verdegem, M., Buschmann, A. H., Latt, U. W., Dalsgaard, A. J. T., & Lovatelli, A. (2023). The contribution of aquaculture systems to global aquaculture production. *Journal of the World Aquaculture Society*, 54, 206–250.
- Wahdaningsih, S., Wahyuono, S., Riyanto, S., & Murwanti, R. (2020). Terpenoid-lupeol of red dragon fruit (*Hylocereus polyrhizus*) and its immunomodulatory activity. *Pakistan Journal of Pharmaceutical Sciences*, 33, 505–510.
- Wakai, K., Hamajima, N., Okada, R., Naito, M., Morita, E., Hishida, A., Kawai, S., Nishio, K., Yin, G., & Asai, Y. (2009). SAS/STAT 9.1 user's guide SAS/STAT 9.1 user's guide, 2004. *Journal of Epidemiology*, 19, 72–80.
- Wang, Y., Fernando, G. S. N., Sergeeva, N. N., Vagkidis, N., Chechik, V., Do, T., Marshall, L. J., & Boesch, C. (2022). Uptake and immunomodulatory properties of Betanin, Vulgaxanthin I and Indicaxanthin towards Caco-2 intestinal cells. *Antioxidants*, 11, 1627.
- Weiss, J. (2003). Bactericidal/permeability-increasing protein (BPI) and lipopolysaccharide-binding protein (LBP): Structure, function and regulation in host defence against Gram-negative bacteria. *Biochemical Society Transactions*, 31, 785–790.
- Xie, D., Dai, Q., Xu, C., & Li, Y. (2021). Dietary tributyrin modifies intestinal function by altering morphology, gene expression and microbiota profile in common carp (*Cyprinus carpio*) fed all-plant diets. *Aquaculture Nutrition*, 27, 439–453.
- Xuan, C. L., Wannavijit, S., Outama, P., Lumsangkul, C., Tongsir, S., Chitmanat, C., & Doan, H. V. (2022). Dietary inclusion of rambutan (*Nephelium lappaceum* L.) seed to Nile tilapia (*Oreochromis niloticus*) reared in biofloc system: Impacts on growth, immunity, and immune-antioxidant gene expression. *Fish & Shellfish Immunology*, 122, 215–224.
- Zhuang, Y., Zhang, Y., & Sun, L. (2012). Characteristics of fibre-rich powder and antioxidant activity of pitaya (*Hylocereus undatus*) peels. *International Journal of Food Science & Technology*, 47, 1279–1285.

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