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Enhancing growth, immunity, and gene expression in Nile Tilapia (*Oreochromis niloticus*) through dietary supplementation with avocado (*Persea americana*) seed powder

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ABSTRACT

This study evaluated the effects of dietary supplementation with different doses (0, 10, 20, 40, and 80 g kg^{-1}) of powdered avocado seed (AS) on the growth performance, immunological response, and gene expression of Nile tilapia (*Oreochromis niloticus*) reared in a biofloc system over 8 weeks. A total of 300 Nile tilapia fingerlings (average weight 14.67 ± 0.07 g) were randomly assigned to five treatment groups, each with three replicates, and 20 fish per tank. The results demonstrated significant improvements ($p < 0.05$) in growth and immune response in AS-supplemented fish, particularly in those fed the 10 g kg^{-1} AS diet (AS10), which showed the most notable increases. In contrast, fish fed higher AS doses (AS20, AS40, and AS80) exhibited no statistically significant differences compared to the control group ($p > 0.05$). Additionally, the AS10 group exhibited a significant upregulation ($p < 0.05$) in the mRNA expression of key immune-related genes (*IL-1*, *IL-8*, and *LBP*) and antioxidant-related genes (*GST-α, GPX*, and *GSR*) in both liver and intestinal tissues, indicating enhanced immune and antioxidant responses. The highest expression levels were found in the AS10 group. These findings suggest that the inclusion of 10 g kg^{-1} powdered avocado seed in the diet substantially enhances growth, immune function, and gene expression in Nile tilapia reared in a biofloc system. The results highlight avocado seed as a promising feed additive for improving the sustainability of Nile tilapia aquaculture.

1. Introduction

Aquaculture is rapidly becoming the primary source of aquatic food for humans and is expected to play a crucial role in global food security in the coming years (FAO, 2024). The industry's growth has been driven by the selection of economically viable fish species and the introduction of new techniques, along with systematic advancements in existing technologies (Rowan, 2023). Nile tilapia (*Oreochromis niloticus*), the third most farmed freshwater fish globally in 2018 with a production of 6.3 million tons, is widely cultivated and consumed worldwide (Barría et al., 2023). Its rapid growth rate, high disease resistance, short culture period, and desirable flavor have made it increasingly popular (Deck et al., 2023). With advancements in seed production techniques and specialized nutrition research, Nile tilapia has become the dominant farmed species in many countries, particularly in intensive farming systems (Debnath et al., 2023). However, the species is sensitive to stress and exhibits reduced growth performance when reared at high densities (Goda et al., 2024). Additionally, poor water quality increases susceptibility to infectious diseases, reduces productivity, and can lead to high mortality rates, resulting in significant economic losses (Yaparatne et al., 2024). Over the past decade, veterinary drugs, chemicals, and antibiotics have been extensively used in aquaculture to manage diseases

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(Suyamud et al., 2024). However, the overuse of these products raises serious concerns, including the development of antibiotic resistance, immune suppression, environmental degradation, and harmful residues in the food supply (Devadas et al., 2024; Okaiyeto et al., 2024). In response, there has been a growing focus on the safety and efficacy of natural feed additives to promote growth and disease resistance in aquaculture (Bondad-Reantaso et al., 2023; Xia et al., 2024; Yang et al., 2024). Beyond enhancing fish growth and health, feed additives must be readily available, scalable, and cost-effective (Zlaugotne et al., 2022). In this context, avocado seed, a by-product of the fruit industry, presents a promising and sustainable option for feed supplementation.

Avocado (*Persea americana*) is cultivated globally, with demand driven by the growing need for fruit and related products (Bangar et al., 2022). The global avocado processing market is projected to grow from $1.70 billion in 2018 to $2.70 billion by 2024 (Ramos-Aguilar et al., 2021). With annual avocado production expected to reach around four million metric tons, avocado seeds, which constitute 13–18 % of the fruit's weight, are typically discarded as waste (Tesfaye et al., 2022). This underutilization poses environmental challenges (Nyakang'i et al., 2023). Effective management of this byproduct could yield both economic and environmental benefits (Del Castillo-Llamosas et al., 2021). Avocado seeds are rich in bioactive compounds, including polysaccharides, proteins, lipids, minerals, vitamins, phenolics, flavonoids, and tannins (Bangar et al., 2022). These compounds are known for their health benefits, such as anti-hyperglycemic, anti-cancer, anti-inflammatory, and antioxidant properties, making avocado seeds valuable for the food, pharmaceutical, and cosmetic industries (Dabas et al., 2019; Lara-Márquez et al., 2020; Soledad et al., 2021; Tremocoldi et al., 2018; Villarreal-Lara et al., 2019). Exploring avocado seeds as a source of natural bioactive compounds could lead to the development of innovative products that offer added value and serve as a safe alternative to synthetic chemicals (Pedro et al., 2024). Additionally, valorizing avocado seed waste has positive implications for both environmental sustainability and the avocado processing industry (Santos et al., 2024). In aquaculture, research has shown that combining avocado and pumpkin seeds in fish diets can improve the growth performance of Nile tilapia (Wasilah et al., 2021).

In recent years, aquaculture has increasingly adopted biofloc technology (BFT) in combination with agricultural byproducts. BFT is recognized as a sustainable approach that can enhances water quality, reduces feed costs, and supports increased production (Haraz et al., 2023; Meitei et al., 2022). Studies have demonstrated that aquatic species reared in biofloc systems show improved growth, enhanced immune responses, and increased disease resistance (Khanjani and Sharifinia, 2020; Mugwanya et al., 2021). Given these benefits, this study aimed to evaluate the effects of a diet supplemented with powdered avocado seed on the growth, immune activity, and gene expression of Nile tilapia reared in a biofloc system.

2. Materials and methods

2.1. Preparation of dragon peel powder

Avocados were purchased from a local market in Chiang Mai. Upon arrival, the seeds were extracted and thoroughly washed with clean water. The seeds were then dried in a hot air oven and ground into a fine powder. The mineral composition and bioactive compounds of the avocado seed powder were subsequently analyzed (Tables 1 and 2).

2.2. Experimental fish

Healthy Nile tilapia fingerlings were sourced from a local hatchery in Chiang Mai, Thailand, and acclimated to the rearing conditions by feeding them a commercial diet twice daily for two weeks before the introduction of the experimental diets. The fish were housed in 500-liter fiberglass tanks for the duration of the experiment. All experimental procedures were conducted in accordance with international guidelines for the use of animals and were approved by the Faculty of Agriculture, Chiang Mai University Committee (AGIACUC001/2565).

2.3. Diet preparation and experimental design

Dietary treatments with varying levels of AS were prepared based on the standard diet formulation as outlined by Le Xuan et al. (2024), and the chemical composition was determined following AOAC guidelines (Cunniff and Association of Official Analytical, 1995). The ingredients and their proportions are detailed in Table 3. After thoroughly mixing the dry ingredients, a pelletizer was used to combine the oil, water, and other components into 2-mm diameter pellets. The prepared feeds were then packed and stored in a refrigerator at 4 °C until use.

After a two-week acclimation period, 300 fish (14.67 ± 0.07 g) were randomly assigned to one of five experimental treatment groups: AS0 (0 g kg^{-1}), AS10 (10 g kg^{-1}), AS20 (20 g kg^{-1}), AS40 (40 g kg^{-1}), and AS80 (80 g kg^{-1}). Each treatment was conducted in triplicate, with 20 fish per tank. The experimental trial lasted for 8 weeks, with fish samples collected at 4 and 8 weeks for growth and immunological parameter analyses. Gene expression analysis of immune-related markers was performed at the end of the 8-week feeding period. After collecting skin mucus and blood samples, the fish were promptly returned to their tanks. The fish were fed their respective diets twice daily at a rate of 4 % of their body weight.

Fish was fed to the diets twice a day at a rate of 4 % body weight.

2.4. Biofloc and water management

Floc water was prepared in each tank three weeks prior to the start of the trial. To 150-liter experimental tanks, 400 g of salt, 5 g of molasses, 5 g of dolomite, and 2 g of fish feed were added. The carbon-to-nitrogen (C:N) ratio was maintained at 15:1 by adding molasses (40 % carbon) as a carbon source two hours after feeding (Avnimelech and Kochba, 2009). The C:N ratio was calculated based on the remaining nitrogen levels in each tank, as well as the carbon and nitrogen content of the feed (Cardona et al., 2016).

The water's dissolved oxygen, pH, temperature, and ammonium levels were measured twice daily at 8:30 AM and 4:30 PM using HI96733 and HI98196 meters. The volume of floc in each tank was estimated using an Imhoff cone, following the method described by Avnimelech and Kochba (2009). During the feeding trial, water quality parameters were consistently stable. Ammonium levels averaged 0.19 ± 0.01 mg/L, temperature was maintained at 27.5 ± 0.58°C, pH remained at 7.91 ± 0.72, and dissolved oxygen levels were 5.64 ± 0.05 mg/L. To sustain these conditions, only 5 % of the water was changed weekly.

Table 1
Mineral composition of avocado seed used in the experiment.

N (%)	0.93 ± 0.01
P (%)	0.21 ± 0.01
K (%)	1.61 ± 0.06
Ca (%)	0.02 ± 0.00
Mg (%)	0.06 ± 0.01

Table 2
Bioactive compounds of avocado seed powder used in the experiment.

Test items	Results	Units	Methods
DPPH (IC_{50})	1.28 ± 0.41	mg/mL	Lin *et al.* (2020)
$ABTS^{+}$	23.09 ± 3.22	mg TE/g	Lin *et al.* (2020)
FRAP	14.40 ± 0.26	mg TE/g	Lin *et al.* (2020)
Total flavonoid content	6.58 ± 0.69	mg CE/g	Juan and Chou (2010)
Total phenolic content	9.84 ± 0.11	mg GAE/g	Juan and Chou (2010)

Table 3
Avocado seed and chemical composition (g kg^{-1}) of the basal diets.

	AS0	AS10	AS20	AS40	AS80
Fish meal	150	150	150	150	150
Corn meal	200	199	198	196	192
Soybean meal	390	391	392	394	398
Wheat flour	70	70	70	70	70
Rice bran	150	145	140	125	90
Avocado seeds	**0**	**10**	**20**	**40**	**80**
Binder	20	15	10	5	0
Soybean oil	5	5	5	5	5
Premix[a]	10	10	10	10	10
Vitamin C 98 %	5	5	5	5	5
Composition of the experimental diets (g kg^{-1})					
Dry matter	917.10	917.14	917.10	917.18	916.98
Crude protein	30.28	30.35	30.40	30.44	30.48
Crude lipid	70.35	73.66	73.80	74.21	74.42
Ash	100.36	100.58	100.72	100.86	100.94
Fiber	60.20	59.63	59.46	59.41	59.32
Gross energy (Cal/g)	4026	4019	4015	4012	4008

[a] Vitamin and trace mineral mix supplemented as follows (IU kg^{-1} or g kg^{-1} diet): retinyl acetate 1085,000 IU; cholecalciferol 217,000 IU; D, L-a-tocopherol acetate 0.5 g; thiamin nitrate 0.5 g; pyridoxine hydrochloride 0.5 g; niacin 3 g; folic 0.05 g; cyanocobalamin 10 g; Ca pantothenate 1 g kg-1; inositol 0.5 g; zinc 1 g; copper 0.25 g; manganese 1.32 g; iodine 0.05 g; sodium 7.85 g.

2.5. Growth performance analysis

Fish weight was recorded individually after four and eight weeks of experimental feeding. The weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), and survival rate (SR) were calculated using the following formulas (Shi et al., 2024): WG (g) = FW – IW; SGR (%) = 100 x (ln FW - ln IW)/experimental days; FCR = feed given (dried weight)/WG (wet weight); and SR (%) = (final fish number/initial fish number) x 100.

2.6. Immunity analysis

2.6.1. Collection of skin mucus and serum samples

To collect skin mucus samples, three fish were randomly selected from each experimental tank. The fish were briefly anesthetized with clove oil (5 mL/L) and then placed in plastic bags containing 10 mL of 50 mM NaCl solution. The solution was obtained by gently rubbing the fish for 1 minute, after which it was transferred into new tubes. The skin mucus samples were then stored at 4°C until use.

Blood samples were collected following a previously described method (Van Doan et al., 2022) with minor modifications. Briefly, 1 mL of blood was drawn from the caudal vein of each fish using a 1-mL syringe and immediately transferred into sterilized tubes without anticoagulant. The blood samples were maintained at room temperature for one hour and then incubated at 4°C for four hours. The samples were centrifuged at 4°C for 15 minutes at 10,000 rpm. Serum was then collected using a micropipette and stored at –80°C until further analysis.

2.6.2. Lysozyme activity

Lysozyme activity in undiluted serum and mucus was assessed using the method originally developed by Parry Jr et al., (1965), with slight modifications as outlined by Van Doan et al. (2021). In brief, 25 µL of undiluted serum and 100 µL of skin mucus from each fish were added in triplicate to 96-well plates, followed by the addition of *Micrococcus lysodeikticus* to each well. Absorbance readings were taken every 30 seconds for 10 minutes using a microplate reader (Synergy H1, Bio-Tek, USA) at a wavelength of 540 nm. A standard curve was generated by correlating the decrease in optical density (OD) with the concentration of hen egg-white lysozyme, ranging from 0 to 20 µg mL^{-1} (Sigma Aldrich, USA).

2.6.3. Peroxidase activity

The methodology originally developed by Quade and Roth (1997) was slightly modified to measure peroxidase levels in serum and skin mucus. In summary, 5 µL of either serum or skin mucus was added to each well of triplicate 96-well flat-bottom plates, followed by 45 µL of Hank's balanced salt solution (HBSS) without Ca^{2+} or Mg^{2+}. Then, 100 µL of a solution containing 40 mL of distilled water, 10 mL of 30 % H_2O_2 (Sigma Aldrich), and one tablet of 3,3′,5,5′-tetramethylbenzidine (TMB; Sigma Aldrich) was added to each well. To stop the colorimetric reaction, 50 µL of 2 M H2SO4 was added. The optical density was then measured at 450 nm using a microplate reader. Control wells without serum or skin mucus served as references. One unit of peroxidase activity was defined as the amount needed to produce a change in absorbance of 1, with activity expressed in units (U) per milligram of serum or mucus.

2.7. qPCR for immune gene expressions

2.7.1. Tissue sampling, total RNA isolation, and cDNA synthesis

To investigate the relative transcript levels of immune-related genes (IL-1, IL-8, and LBP) and antioxidant-related genes (GSTα, GPX, and GSR), liver and intestine tissues (n=5) were sampled after eight weeks of the feeding trial. Tissue samples (25–50 mg) were collected and stored in sterilized tubes containing 500 µL of Trizol (Invitrogen, USA) at –80°C for further analysis. Total RNA was extracted using the PurLink™ RNA Mini Kit (Invitrogen, USA) following the manufacturer's instructions. The quantity and quality of RNA were assessed using a NanoDrop™ 2000 spectrophotometer (Thermo Scientific, Wilmington, USA). Complementary DNA (cDNA) was synthesized from 500 ng of total RNA using the iScript™ cDNA kit (BIO-RAD, USA) according to the manufacturer's protocol. The primers used for RT-qPCR are listed in Table 4.

2.7.2. Quantitative real-time PCR

For the qPCR assay, the reaction mixture included 1 µL of cDNA (100 ng), 0.4 µL of each primer (10 µM), 10 µL of 2× Universal SYBR Green (BIO-RAD, USA), and DNase-free water. RT-qPCR was conducted using a CFX Connect™ real-time PCR machine (BIO-RAD, USA) under the conditions previously described by Le Xuan et al. (2024). The $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) was used to analyze the RT-qPCR data. The relative mRNA transcript levels of the target genes were quantified using Cq values, with 18S rRNA serving as the internal reference gene.

2.8. Statistical analysis

The statistical analyses were performed using the SAS computer program (SAS and Version, 2003). ANOVA and Duncan's Multiple

Table 4
Primer used for quantitative real-time PCR.

Target genes	Primer sequence (5′-3)	Tm (°C)	Product size (bp)	Accession No.
18S rRNA	GTGCATGGCCGTTCTTAGTT CTCAATCTCGTGTGGCTGAA	60	150	XR_003216134
IL1	GTCTGTCAAGGATAAGCGCTG ACTCTGGAGCTGGATGTTGA	59	200	XM_019365844
IL8	CTGTGAAGGCATGGGTGTG GATCACTTTCTTCACCCAGGG	59	196	NM_001279704
LBP	ACCAGAAACTGCGAGAAGGA GATTGGTGGTCGGAGGTTTG	59	200	XM_013271147
GSTα	ACTGCACACTCATGGGAACA TTAAAAGCCAGCGGATTGAC	60	190	NM_001279635
GPX	GGTGGATGTGAATGGAAAGG CTTGTAAGGTTCCCCGTCAG	60	190	NM_001279711
GSR	CTGCACCAAAGAACTGCAAA CCAGAGAAGGCAGTCCACTC	60	172	XM_005467348

F: forward primer; R: reverse primer; bp: base pair

Range Test were used to determine the significance of differences among groups. The statistical significance threshold was set at $p < 0.05$ was considered as statistically significant. Data normality was evaluated using the Kolmogorov-Smirnov test.

3. Results

3.1. Growth performance

The growth parameters of the experimental fish are presented in Fig. 1. It is evident that fish fed AS-containing diets exhibited significantly better growth characteristics ($p < 0.05$) compared to the control group after both four and eight weeks of the trial. The highest weight gain (WG) and specific growth rate (SGR) were observed in fish fed the AS10 diet (10 g kg^{-1}) ($p < 0.05$), while lower growth parameters were recorded in the AS20, AS40, and AS80 diet groups. No statistically significant differences were found among these groups ($p > 0.05$). Additionally, fish in the AS10 group had the lowest feed conversion ratio (FCR) compared to other dietary treatments ($p < 0.05$). In contrast, fish in the control group (0 g kg^{-1}AS) exhibited the highest FCR. The survival rate of fish across all tested diets remained consistent ($p > 0.05$).

3.2. Immunological response

3.2.1. Skin mucosal analysis

The activities of skin mucus lysozyme (SMLA) and skin mucus peroxidase (SMPA) are shown in Fig. 2. At both time points during the feeding trial, diets supplemented with varying levels of AS significantly enhanced the immunological parameters of skin mucus ($p < 0.05$). The AS10 treatment group exhibited the highest SMLA and SMPA activities, followed by the AS20, AS40, and AS80 groups ($p < 0.05$).

3.2.2. Serum assay analysis

The lysozyme activity in serum (SL) varied significantly across treatments, as shown in Fig. 3. At both time points (four and eight weeks of the feeding trial), fish fed the AS-supplemented diets exhibited significantly higher SL values ($p < 0.05$) compared to those on the control diet. The AS10 treatment group showed the highest SL values, followed by the AS20, AS40, and AS80 groups ($p < 0.05$). Similarly, the highest serum peroxidase activity (SP) at both time points was observed in fish fed the AS10 diet ($p < 0.05$), followed by the AS20, AS40, and AS80 groups, while the control group had the lowest levels. No statistically significant differences were found among the AS20, AS40, and

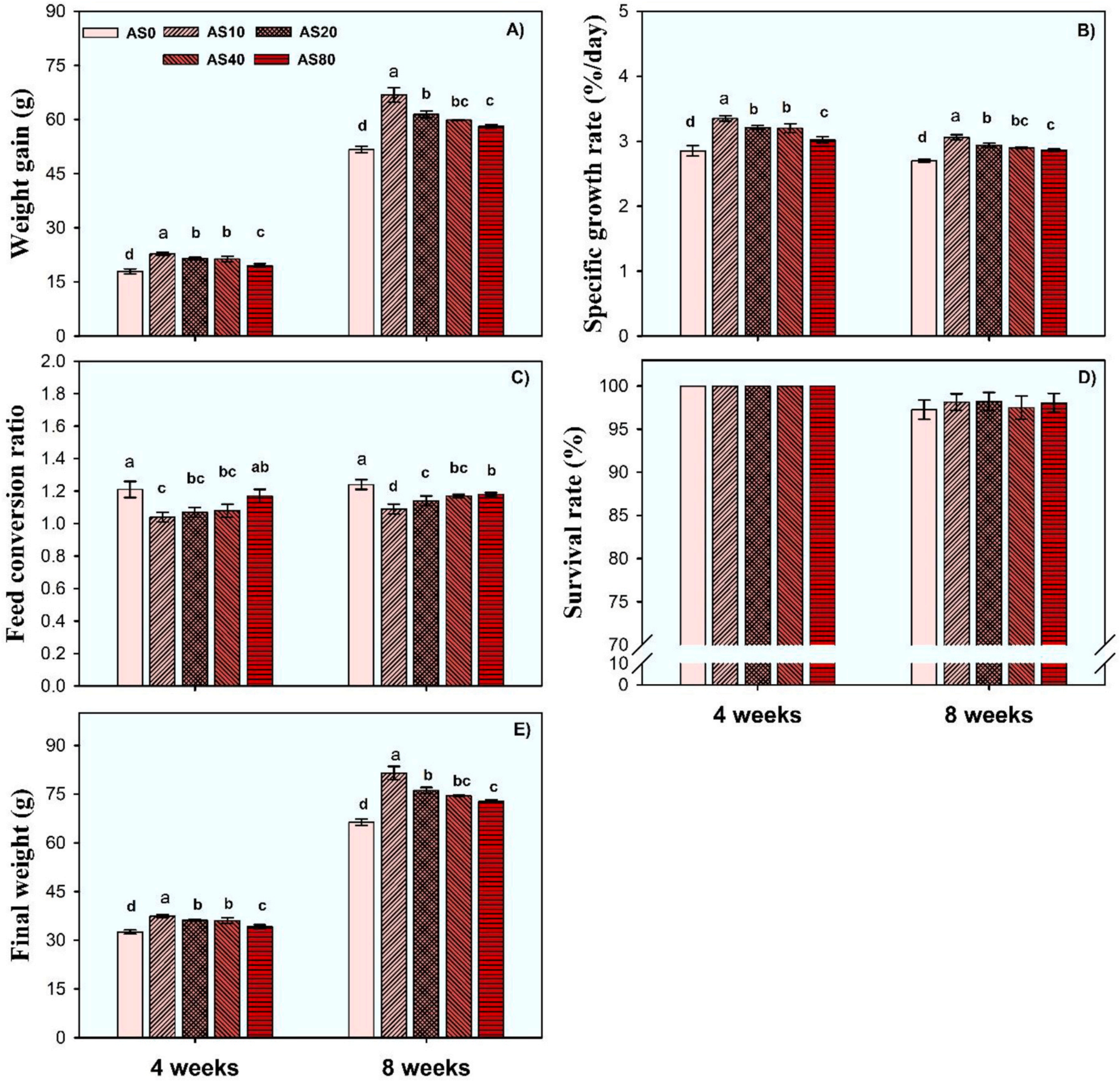

Fig. 1. Weight gain (**A**), specific growth rate (**B**), feed conversion rate (**C**), survival rate (**D**), and final weight (**E**) of Nile tilapia after four and eight weeks of feeding diets containing powdered avocado fruit seed 0 (AS0, control), 10 (AS10), 20 (AS20), 40 (AS40), and 80 (AS80) g kg^{-1}. Each column is the mean of three replicates and the data represent as mean ± SE. Different superscript letters indicate significant differences among groups ($p < 0.05$).

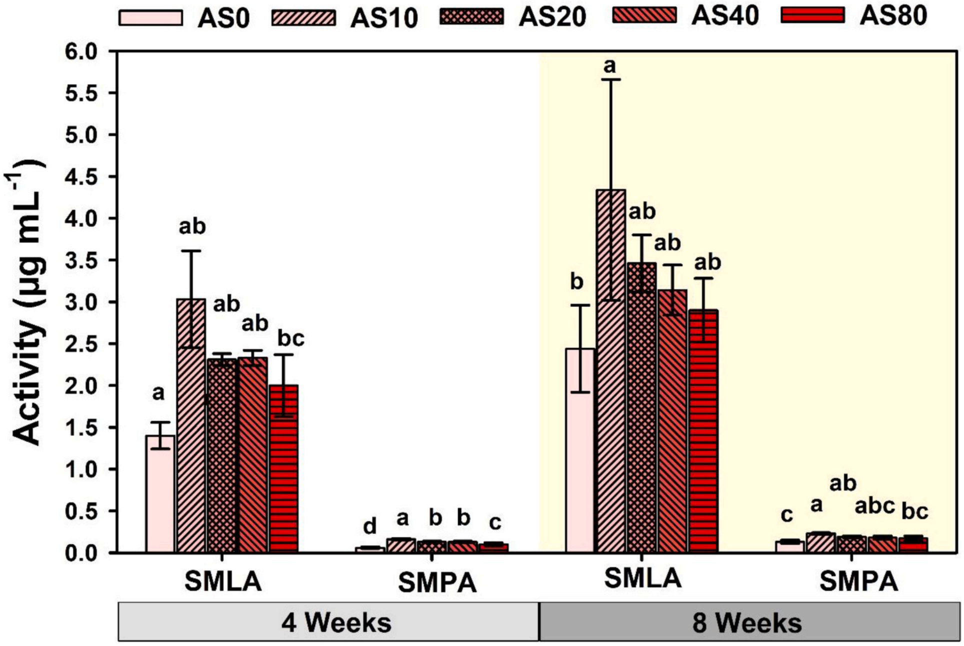

Fig. 2. Lysozyme (SMLA) and peroxidase activity (SMPA) in skin mucus of Nile tilapia after four and eight weeks of feeding with powdered avocado fruit seed at 0 (AS0, control), 10 (AS10), 20 (AS20), 40 (AS40), and 80 (AS80) g kg^{-1}. All data are means of three replicates and the data represent as mean ± SE. Different superscript letters indicate significant differences among groups ($p < 0.05$).

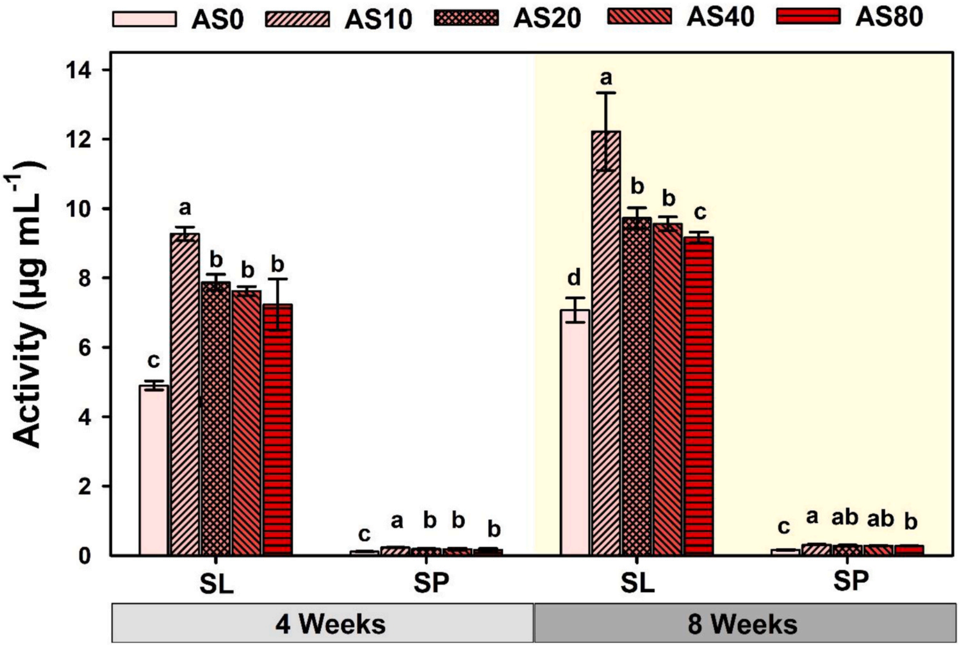

Fig. 3. Lysozyme (SL) and peroxidase activity (SP) in serum of Nile tilapia after four and eight weeks of feeding with powdered avocado fruit seed 0 (AS0, control), 10 (AS10), 20 (AS20), 40 (AS40), and 80 (AS80) g kg^{-1}. All data are means of three replicates and the data represent as mean ± SE. Different superscript letters indicate significant differences among groups ($p < 0.05$).

AS80 dietary groups ($p > 0.05$).

3.3. *Gene expression analysis using qPCR*

The relative transcript levels of immune-antioxidant related genes in liver tissue are presented in Fig. 4. The expression of immune-related genes (IL-1, IL-8, and LBP - lipopolysaccharide-binding protein) and antioxidant-related genes (GSTα, GPX, and GSR) were significantly upregulated in the AS-treated groups compared to the control group. With the exception of GSR, the highest expression levels were observed in the AS10 treatment ($p < 0.05$), followed by the AS20, AS40, and AS80 groups, with no significant differences among these latter treatments ($p > 0.05$). The relative transcript levels of immune-antioxidant related genes in intestinal tissue are shown in Fig. 5. Except for IL-1, where no significant difference was detected between the treatment groups, the other genes indicated that the AS10 diet led to the highest expression levels ($p < 0.05$). The expression levels of IL-8, LBP, GPX, GSR, and GSTα did not significantly differ among the AS20, AS40, and AS80 groups ($p >$

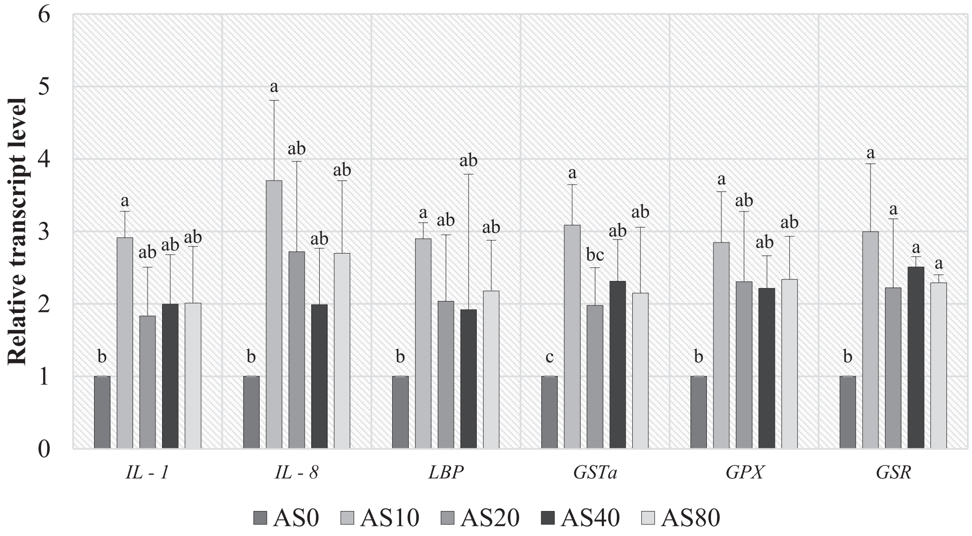

Fig. 4. Effect of powdered avocado fruit seed on related-immune and antioxidant gene expressions in the liver (n = 5); *IL-1*, *IL-8*, *LBP*, *GSTα*, *GPX*, and *GSR* of Nile tilapia after feeding with experimental diets: AS0 (0 - control), AS10 (10 g kg^{-1} AS), AS20 (20 g kg^{-1} AS), AS40 (40 g kg^{-1} AS), and AS80 (80 g kg^{-1} AS). Significant differences between groups are denoted by different superscript letters ($p < 0.05$). Data presented are expressed as mean ± SE.

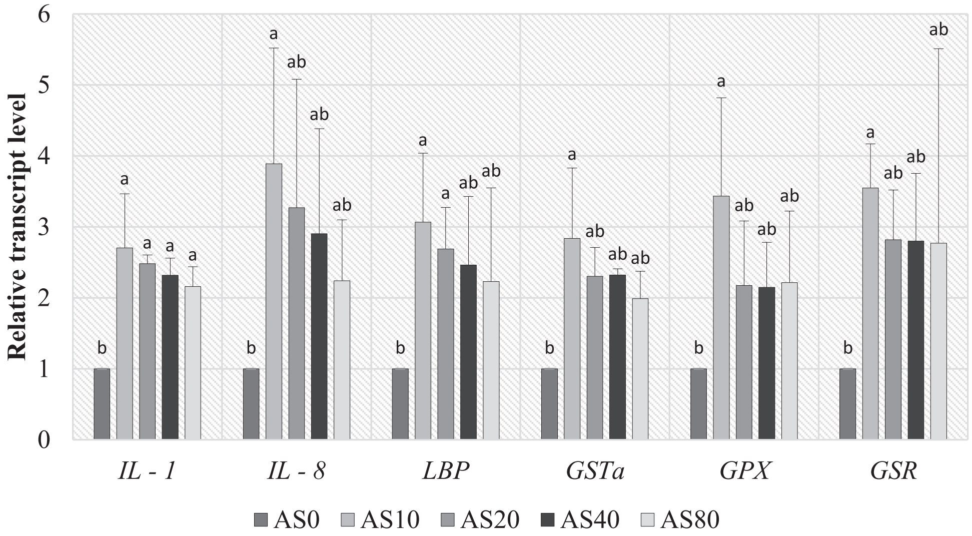

Fig. 5. Effect of powdered avocado fruit seed on related-immune and antioxidant gene expressions in the intestine (n = 5); *IL-1*, *IL-8*, *LBP*, *GSTα*, *GPX*, and *GSR* of Nile tilapia after feeding with experimental diets: AS0 (0 - control), AS10 (10 g kg^{-1} AS), AS20 (20 g kg^{-1} AS), AS40 (40 g kg^{-1} AS), and AS80 (80 g kg^{-1} AS). Significant differences between groups are denoted by different superscript letters ($p < 0.05$). Data presented are expressed as mean ± SE.

0.05).

4. Discussion

Addressing challenges such as disease outbreaks, improving survival rates of farmed species, and reducing the cost of aquafeed production are critical for the long-term sustainability of the aquaculture industry (Aly and Fathi, 2024; Islam et al., 2024). In recent decades, significant research has focused on identifying new, sustainable, and cost-effective aquafeed ingredients that can effectively replace traditional components while providing both environmental and economic benefits (Eroldoğan et al., 2023; Fantatto et al., 2024; Sampathkumar et al., 2023). This study was conducted to evaluate the impact of supplementing diets with avocado seed (AS) on the growth, immunological activity, and expression of immune-antioxidant-related genes in Nile tilapia. The findings contribute to the ongoing efforts to explore alternative feed resources that could enhance aquaculture productivity while promoting sustainability.

Growth performance is a crucial parameter for aquaculture practices and fish farmers. In the present study, we observed that fish in the AS treatment groups exhibited significantly better specific growth rate (SGR), weight gain (WG), and final weight (FW) after four and eight weeks of feeding, with the highest improvements seen in the group receiving 10 g/kg of AS in their diet. These findings align with previous research by Safrida et al. (2021), who reported enhanced growth in Gourami (*Osphronemus gouramy*) when a diet containing a combination of avocado seed (20 g) and pumpkin peel meal (60 g) was used. Similarly, Wasilah et al. (2021) found positive effects on Nile tilapia growth when these ingredients were included in the diet. Recent studies have also shown that dietary supplementation with various seeds can significantly enhance the growth performance of different fish species. For example, rainbow trout (*Oncorhynchus mykiss*) benefited from diets enriched with medicinal plant seeds (Rashidian et al., 2023), while Nile tilapia (*Oreochromis niloticus*) showed improved growth when fed makiang and garden cress seeds (*Oreochromis niloticus*) (El-Houseiny et al., 2024; Le Xuan et al., 2024). Similarly, European sea bass

(*Dicentrarchus labrax*) experienced growth benefits when their diets were supplemented with herbal seeds (Ashry et al., 2024).

The bioactive components in avocado seeds may contribute to these improvements in growth and feed conversion ratio. Avocado seeds are rich in fatty acids, including palmitic, linoleic, and oleic acids (Gonçalves et al., 2024), as well as protein (Chuacharoen et al., 2024), which are known to promote fish growth (Gonçalves et al., 2021; Nayak et al., 2020; Safrida et al., 2021). Additionally, avocado seeds contain high levels of essential minerals such as phosphorus, calcium, potassium, iron, sodium, zinc, copper, cobalt, and lead, along with vitamins A, B1, B2, B3, C, and E (Bangar et al., 2022), all of which have been shown to enhance fish growth (El-Sayed et al., 2023; Li et al., 2023; Saleh et al., 2022).

Avocado seeds are also rich in phospholipids (Nyakang'i et al., 2023), which have been found to stimulate the development of tight junction proteins like ZO-1, claudin-4, and claudin-5, and to enhance the activity of digestive enzymes such as lipase, trypsin, and alkaline phosphatase, ultimately improving fish growth performance (Tian et al., 2018; Wang et al., 2017). Moreover, a recent study by Permal et al. (2020) highlighted the potential of natural compounds found in avocado seeds, such as phytosterols, triterpenes, flavonol dimers, and oligomeric proanthocyanidins in the development of functional foods designed to promote metabolism and growth. Additionally, the phytochemicals in avocado seed powder may increase the acidic conditions in the pancreas, duodenum, and gallbladder, thereby enhancing protease activity and aiding in the breakdown of proteins into amino acids (Delles et al., 2014). These amino acids are then absorbed and converted into myofibrillar, sarcoplasmic, and connective tissue proteins, contributing to overall growth (Akbarian et al., 2016).

Mucosal surfaces serve as the first line of defense between the internal and external environments of fish (Li et al., 2024). These surfaces provide both non-specific and specific immune functions, helping fish defend against external particles, stressors, and naturally occurring pathogens (Hussain and Sachan, 2023). In the current study, the administration of AS significantly increased lysozyme and peroxidase activities in the skin mucus after four and eight weeks, compared to the control. Recent studies have shown that various fruit by-products and immunostimulants, when added to the diet of Nile tilapia and other fish species, can enhance skin immunity. For example, makiang seed (Le Xuan et al., 2024), green algae (Govindharajan et al., 2024), and *Debaryomyces hansenii* (Sanahuja et al., 2023) have all been shown to provide such benefits. Lysozyme and peroxidase activities play crucial roles in pathogen breakdown and oxidative stress management (Song et al., 2021). In this study, AS supplementation at a dose of 10 g/kg significantly improved the lysozyme and peroxidase activities in the serum of Nile tilapia compared to the control and other treatment groups. To the best of our knowledge, there is no prior information on the effects of AS on serum lysozyme and peroxidase activities in Nile tilapia. However, previous studies have reported positive effects of fruit by-products or their extracts on the immunological activity of fish serum, including peroxidase and lysozyme activities (Abd El-Naby et al., 2023; Abdel Rahman et al., 2023; Ashry et al., 2024; Gupta et al., 2023). Interleukin-1 (IL-1) is an important inflammatory mediator that induces the production of a wide range of nonstructural, function-related genes during infection (Boraschi, 2022). It plays a critical role in the host's defense against pathogens and tissue injury by promoting phagocytosis, macrophage proliferation, lysozyme production, and leukocyte migration (Dinarello, 2006). *IL*-8, another pro-inflammatory cytokine, works closely with IL-1 by increasing its release during inflammation (Fast et al., 2007). Our findings indicate that IL-1 and IL-8 expressions in the liver and intestinal tissues of Nile tilapia were significantly upregulated in the groups supplemented with AS diets, with the highest mRNA expression observed in the AS10 group (10 g/kg AS). These results are consistent with previous studies on Nile tilapia (Linh et al., 2024; Xuan et al., 2024), rainbow trout (Aghili et al., 2024), and olive flounder (*Paralichthys olivaceus*) (Choi et al., 2023).

Lipopolysaccharide-binding protein (LBP) is a soluble acute-phase protein essential for lipopolysaccharide signaling and non-specific immune responses (Ding and Jin, 2014). LBP plays a crucial role in the immune response to gram-negative bacteria, contributing to both innate and adaptive immunity in fish (Fu et al., 2014; Zhou et al., 2019). Our study showed that a diet supplemented with 10 g kg^{-1} AS resulted in higher expression of LBP in the liver and intestines of Nile tilapia, consistent with findings in other fish species such as Nile tilapia (Linh et al., 2024; Xuan et al., 2024), blunt snout bream (*Megalobrama amblycephala*) (Lee et al., 2017), and largemouth bass (*Micropterus salmoides*) (Byadgi et al., 2016). Glutathione peroxidase (GPX), glutathione S-transferase alpha (GSTα), and glutathione reductase (GSR) are key enzymes in the fish antioxidant defense system (Zheng et al., 2016). The regulation of these antioxidant enzymes is considered a reliable measure of fish antioxidant capacity in the absence of biological interference (Herath et al., 2017). In this study, fish in the AS-supplemented groups showed significant upregulation of the antioxidant genes GSTα, GPX, and GSR in both liver and intestinal tissues, with the highest expression levels observed at the 10 g kg^{-1} AS dose. The observed enhancement of immune and gene expression may be attributed to the bioactive compounds found in avocado seed, which is a rich source of polyphenols (Bangar et al., 2022). Polyphenol-rich diets have been shown to influence epigenetic processes that regulate the expression of genes involved in immune function, such as DNA methylation, histone modification, and microRNA-mediated post-transcriptional suppression (Ding et al., 2018). Each type of polyphenol can activate and interact with specific immune cell receptors, triggering intracellular signaling pathways that ultimately regulate the host's immune response (Kozarski et al., 2023). Moreover, studies on mice have shown that the ethyl acetate fraction of avocado seed extract is effective in reducing oxidative stress and increasing superoxide dismutase enzyme activity (Athaydes et al., 2019). Additionally, avocado seeds are a rich source of fatty acids, which are known to enhance immune function (Báez-Magaña et al., 2019; Gutiérrez et al., 2019; Jalili et al., 2019; Mendivil, 2020). Avocado seeds also contain high levels of vitamins A, C, and E, which are known to boost the immune system (Bangar et al., 2022).

Biofloc technology is critically important in intensive aquaculture, offering the dual benefits of maintaining water quality while reducing the reliance on commercial feed for farmed fish (Ende et al., 2024; Khanjani et al., 2024). Recent studies have shown that aquatic animals raised in biofloc systems experience significant improvements in growth performance and health, particularly when feed additives are incorporated (Hersi et al., 2023; Qiu et al., 2023). In our study, Nile tilapia fed avocado seed diets within a biofloc system demonstrated noticeable enhancements in both development and health. Avocado seeds are rich in carbohydrates (Tesfaye et al., 2018), and the introduction of these carbohydrates into a biofloc system provides a vital energy source for microbes, enabling them to convert ammonium or nitrate into microbial biomass (Khanjani et al., 2023; Pekkoh et al., 2022). This process reduces the need for water exchange by lowering ammonia and nitrite levels (Chen et al., 2020; Dong et al., 2021; Tinh et al., 2021). The resulting microbial biomass not only improves feed utilization efficiency but also serves as a natural source of nutrition for the growing fish (Addo et al., 2021; Panigrahi et al., 2021). Moreover, avocado seeds are a rich source of carbon (Leite et al., 2018). It is well established that adding a carbon source to a biofloc system can enhance its nutritional content, which is believed to contribute to faster growth rates in fish (El-Hawarry et al., 2021).

5. Conclusion

In summary, this study found that dietary supplementation with 10 g kg^{-1} of avocado seed (AS) had beneficial effects on Nile tilapia cultured in a biofloc system, enhancing growth efficiency, immunological response, and immune gene expression. These results suggest that incorporating powdered avocado seed into basal diets could serve as a valuable functional feed additive for Nile tilapia.

CRediT authorship contribution statement

Chinh Le Xuan: Writing – original draft, Investigation. **Supreya Wannavijit:** Investigation, Formal analysis. **Nguyen Vu Linh:** Writing – review & editing, Formal analysis, Data curation. **Yuthana Phimolsiripol:** Formal analysis. **Yupa Chromkaew:** Formal analysis. **Hien Van Doan:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Vimbai Irene Machimbirike:** Writing – review & editing. **Anisa Rilla Lubis:** Writing – review & editing. **Piyatida Outama:** Investigation.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Data Availability

Data will be made available on request.

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