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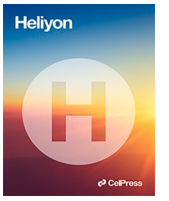

Research article

Evaluation of longan (*Dimocarpus longan*) peel powder as fruit by-product additive in Nile tilapia (*Oreochromis niloticus*) feed: Effects on growth, immunity, and immune-antioxidant gene expressions

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ABSTRACT

The study, herein, investigated the effects of the inclusion of longan peel (LP) powder in the diet of Nile Tilapia (*Oreochromis niloticus*), focusing on comparative evaluations of growth performance, immunity, and immune-antioxidant related gene expressions. For this purpose, a total of 300 healthy fish (average initial weight: 13.70 ± 0.06 g) were randomly distributed into five experimental treatment groups: 0 g kg^{-1} (LP0), 10 g kg^{-1} (LP10), 20 g kg^{-1} (LP20), 40 g kg^{-1} (LP40), and 80 g kg^{-1} (LP80), all in triplicate, for 60 days. The results indicated that dietary supplementation with LP exhibited a significant influence ($P < 0.05$) in weight gain (WG), specific growth rate (SGR) and provided a better feed conversion ratio (FCR) in contrast to the control group (0 g kg^{-1} LP). Moreover, skin mucus and serum immune parameters (lysozyme and peroxidase activity) were significantly higher ($P < 0.05$) in fish fed with different LP concentrations at both 4 and 8 weeks. Similarly, analysis of mRNA transcripts of immune (*IL-1, IL-8,* and *LBP*) and antioxidant (*GSTa, GPX,* and *GSR*) gene expressions showed a significant upregulation ($P < 0.05$) in LP-fed diet groups compared to the control group. Based on the polynomial regression analysis the inclusion of LP at 46–49 g kg^{-1} can be used effectively in Nile tilapia diets for improving the growth, immunity, and immune-antioxidant gene expressions. All in all, our results prove that LP is a very promising feed supplement for the Nile tilapia in the context of aquaculture.

Abbreviations: CRD, completely randomized design; FCR, feed conversion ratio; GPX, glutathione peroxidase; GSR, glutathione reductase; GSTa, glutathione S-transferase; IL-1, interleukin-1; IL-8, interleukin-2; LBP, lipopolysaccharide-binding protein; LP, Longan peels; RT-qPCR, Real Time quantitative PCR; SGR, specific growth rate; SL, serum lysozyme; SMLA, skin mucus lysozyme activity; SMPA, skin mucus peroxidase activity; SP, serum peroxidase; TMT, thousand metric ton.

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1. Introduction

Nile tilapia (*O. niloticus*) is one of the most significant aquaculture species due to its rapid growth, high survival rate, adaptability to captive reproduction, excellent flesh quality, and strong market value [1]. In 2018, approximately 6.882 million metric tons of Nile tilapia were produced across more than 120 countries, with production projected to reach 7.3 million tons by 2030 [2]. However, the sustainability of tilapia farming is severely challenged by diseases primarily bacterial infections, which cause substantial economic losses globally [3]. To ensure sustainable production, effective strategies to improve fish health and reduce disease outbreaks are essential [4]. One promising approach is the supplementation of aquafeeds with functional additives, which has gained popularity in recent years as a cost-effective alternative for improving fish health and performance [5]. Feed additives are utilized to enhance immunity, growth, and feed utilization, while reducing pathogen infections [6]. These additives can be classified based on their composition and function, with their selection depending on efficacy, cost, and commercial availability [7,8]. Among these, fruit by-products stand out as a particularly promising category [9]. The agro-industrial sector generates large quantity by-products and plant waste from processing of fruits and vegetables for food, pharmaceuticals, and cosmetics [10]. Globally, about one-third of fruits are lost during processing, creating substantial waste [11]. However, these by-products often contain valuable nutrients and bioactive compounds, making them suitable for recycling into livestock and aquafeed industries [12]. The growing aquaculture industry, which faces rising production costs and intense competition for traditional feed ingredients like fishmeal, soybean meal, and cornmeal, urgently needs alternative raw materials to reduce feed costs and enhance sustainability [13,14]. Agricultural waste utilization aligns with circular economy principles, promoting food safety, quality, and low environmental impact while reducing feed costs [15,16].

Longan (*Dimocarpus longan*), a subtropical and tropical fruit from the *Sapindaceae* family, is extensively cultivated in China and Southeast Asia, including Thailand, Vietnam and the Philippines [17]. From 2015 to 2017, annual longan production in Asia averaged 3445.4 thousand metric tons (TMT), with China contributing 1919.4 TMT (55.7 %), followed by Thailand at 980.3 TMT (28.4 %) and Vietnam at 517.1 TMT (15.0 %) [18,19]. Longan is consumed fresh, juiced, or dried, but its peel and seeds, accounting for 12.4–19.6 % of the fruit's weight, are often discarded as waste [17,20]. Studies have shown that longan peel is rich in bioactive compounds, including flavonoids, phenolic acids, and polysaccharides, with demonstrated antioxidant, anticancer, anti-inflammatory, anti--hyperglycemic, and antimicrobial properties [21,22]. Despite these promising attributes, the use of longan peel as an aquafeed supplement remains unexplored. This study aims to evaluate the potential of longan peel as a feed additive for Nile tilapia reared in a biofloc system, focusing on its effects on growth, innate immunity, and gene expression. By addressing a significant research gap, this study seeks to advance sustainable aquaculture practices through innovative feed solutions.

2. Materials and methods

2.1. Preparation of longan peels

The longan fruits were purchased from a locally available fruit market in Mueang Chiang Mai, Chiang Mai Province, Thailand. The fruits were gently washed under running tap water and the peels were carefully removed. The peels were then dried in a hot air oven at 60 °C for two days and subsequently ground into a fine longan peel (LP) powder. The LP powder was stored at 4 °C until further use. The proximate composition and bioactive compounds of long peel were analyzed and presented in Tables 1 and 2. The chemical composition of the experimental diets and longan peel was determined as follows: moisture content was measured using a moisture meter at 105 °C. Dry matter, ash, protein, and lipids were analyzed following AOAC methods [23], while energy content was determined using a bomb calorimeter. Antioxidant activities, including DPPH (IC50), ABTS+, and FRAP, were assessed using the method described in Ref. [24]. Total flavonoid and total phenolic contents were measured using the method outlined in Ref. [25].

2.2. Experimental diets

A basal diet, already well-optimized in previous studies, was used as a control for the feeding experiment [26]. The LP testing included four different treatments with inclusion level of 10 (LP10), 20 (LP20), 40 (LP40) and 80 (LP80) g kg^{-1}, respectively. These inclusion levels were selected based on previous studies by Ref. [26]. The specific composition and proportions of the ingredients used in the trial diets are provided in Table 3.

Table 1
Proximate composition of longan peel powder used in the experiment.

Dry matter (DM)	89.96
Ash	5.57
Crude fibre (CF)	30.00
Crude protein (CP)	10.34
Ether extract (EE)	0.75
Nitrogen free extract (NFE)	43.29

2.3. Tilapia husbandry and diet administration

Fish were obtained from a local hatchery in Chiang Mai Province, Thailand. They were acclimatized in a large household cage and fed commercial (CP 9951) for the first two weeks, followed by a basal diet for an additional two weeks. Prior to the feeding trial, the health status of 20 fish was assessed by examining their bodies, gills, and internal organs. Subsequently, 300 fish (13.70 ± 0.02 g) were randomly distributed at a density of 20 fish per tank across 15 aerated tanks (150 L each). Each dietary treatment was conducted in triplicate following a completely randomized design (CRD). Fish were fed twice daily, at 8.30 a.m. and 16.30 p.m., with a daily amount equivalent to 4 % of their body weight.

2.4. Biofloc (BF) water preparation and management

Three weeks prior to the start of the treatment, tanks were prepared as biofloc (BF) inoculant sources. To create floc water, each tank was supplemented with 2 g of wheat flour, 400 g of salt, 5 g of dolomite, and 5 g of molasses. The carbon-to-nitrogen (C: N) ratio was maintained at 15:1 throughout the experiment by adding molasses (containing 40 % carbon) as a carbon source, following the guidelines of previous studies [27]. Molasses was introduced once daily, 2 h after feeding. The C:N ratio was calculated based on the residual nitrogen levels in each tank and the nitrogen content contributed by the diet.

Water temperature, pH, and dissolved oxygen (DO) were all measured using the HI98196 Meter (Hanna Instruments, Inc. Romania). Ammonium ion (NH_4^+) levels in the water were determined with the HI96733 High Range Ammonia Meter (Hanna Instruments, Inc. Romania). The water parameters were maintained within the following ranges: temperature at 27 ± 0.55 °C, pH at 7.9 ± 0.05, and DO at 4.75 ± 0.10 mg L^{-1}. The NH_3 level was maintained at 0.01 ± 0.005 mg L^{-1}. At the end of the experiment, gene expression analysis was performed, and the fish growth and immunological activity were sampled and examined at 4 and 8 weeks.

2.5. Innate immunological assays

2.5.1. Sample collection

Skin mucus from Nile tilapia was collected following the method described in previous study [26]. Three fish were randomly selected from each tank and anesthetized using clove oil. Skin mucus was gently extracted by massaging the fish in a 10-ml-NaCl plastic bag. and immediately transferred into sterile tubes. The samples were immediately transferred into sterile tubes and centrifuged at 1500×*g* at 4 °C for 10 min. One milliliter of supernatant was carefully collected and stored in a freezer for future assays.

The serum sample was collected according to previous studies [28]. One milliliter of blood was drawn from the caudal vein of each fish using a 1 mL syringe and transferred in anticoagulant-free 1.5 mL Eppendorf tubes. The blood samples were left to coagulate at room temperature for 1 h, after which the serum was separated using a micro-pipette. The serum samples were stored at −80 °C until further analysis.

2.5.2. Lysozyme and peroxidase activity

Lysozyme activity was measured according to the procedure [29]. Briefly, 100 μL of skin mucus and 25 μL of undiluted serum from each fish were loaded in triplicate into a 96-well plate. Each well was then supplemented with 175 μL of Micrococcus lysodeikticus suspension (0.3 mg mL^{-1} in 0.1 M citrate phosphate buffer, pH 5.8). The mixture was immediately stirred, and changes in turbidity were monitored at 540 nm and 25 °C using a microplate reader, with measurements taken every 30 s for 5 min. A standard curve was generated by plotting the OD values against the concentrations of hen egg-white lysozyme (0–20 μg mL^{-1}; Sigma Aldrich, USA). The results were expressed as μg mL^{-1} and used to calculate the equivalent lysozyme activity for the samples.

Peroxidase activity was investigated on Nile tilapia skin mucus and serum according to the previous method [30]. Briefly, triplicate 96-well flat-bottom plates were prepared by loading each well with 5 μL of serum or skin mucus, followed by the addition of 45 μL of Hank's Balanced Salt Solution (HBSS) without Ca^{2+} or Mg^{2+}. Next, 100 μL of a reaction solution, comprising 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. The reaction was stopped by adding 50 μL of 2M H_2SO_4, and the optical density (OD) was measured at 450 nm using a microplate reader. Control wells containing no serum or skin mucus were used as blanks. One unit (U) of peroxidase activity was defined as the amount required to produce an absorbance change of 1 at 450 nm. The activity was expressed in units (U) per milligram of serum or mucus.

Table 2
Bioactive compounds of longan peel powder used in the experiment.

Test items	Results	Units	Methods
DPPH (IC_{50})	1.26 ± 0.08	mg/mL	Lin et al. (2020)
$ABTS^+$	13.25 ± 0.20	mg TE/g	Lin et al. (2020)
FRAP	18.80 ± 0.21	mg TE/g	Lin et al. (2020)
Total flavonoid content	3.24 ± 0.50	mg CE/g	Juan and Chou (2010)
Total phenolic content	3.07 ± 0.19	mg GAE/g	Juan and Chou (2010)

Table 3
Longan peels and chemical composition (g kg^{-a}) of the basal *diets*.

	LP0	LP10	LP20	LP40	LP80
Fish meal	150	150	150	150	150
Corn meal	200	200	200	200	200
Soybean meal	390	389	389	388	389
Wheat flour	70	70	70	70	70
Rice bran	150	147	138	127	88
Longan peels	0	10	20	40	80
Binder	20	14	13	5	3
Soybean oil	5	5	5	5	5
Premix[a]	10	10	10	10	10
Vitamin C[b]	5	5	5	5	5
Proximate composition of the experimental diets (g kg^{-a})					
Dry matter	916.15	914.50	914.68	915.77	916.33
Crude protein	303.23	302.71	302.75	302.53	302.34
Crude lipid	50.28	49.89	49.51	49.10	49.92
Ash	78.28	79.68	78.13	79.20	79.46
Fiber	50.32	59.84	60.12	61.46	62.20
GE (Cal/g)[c]	4045	4098	4102	4089	4094

[a] Vitamin and trace mineral mix supplemented as follows (IU kg^{-1} or g kg^{-1} diet): retinyl acetate 1,085,000 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.
[b] Vitamin C98 % 5 g.
[c] GE = gross energy.

2.6. Relative immune and antioxidant gene expression analysis

2.6.1. Tissue sampling

At the end of the trial, three fish were randomly selected from each LP treatment group for liver and gut sampling to analyze immunological activities. The fish were dissected, and approximately 40 mg of liver and gut tissues were collected. The samples were placed into 1.5 containing 500 μL of Trizol reagent (Invitrogen #1IV1115596026). The tubes were then immediately frozen at −80 °C for RNA extraction at a later stage.

2.6.2. RNA extraction and cDNA conversion

Pellet pestles were used to homogenize liver and gut tissues for RNA extraction. The homogenized samples were incubated at room temperature for 5 min, followed by the addition of 100 μL of chloroform. After 2 min incubation at room temperature, the samples were centrifuged at 12,000 g for 15 min at 4 °C. The aqueous phase containing RNA was carefully collected and processed using the PureLinkTM RNA Mini Kit (Invitrogen), following the manufacturer's instructions. The concentration and purity of the extracted RNA were measured using a NanoDropTM 2000 spectrophotometer (Thermo Scientific, Wilmington, USA) at an absorbance ratio of 260/280 nm. Complementary DNA (cDNA) was synthesized from the extracted RNA using the iScriptTM cDNA Synthesis Kit (BIO-RAD, USA), according to the manufacturer's protocol.

Table 4
Primers utilized for qRT-PCR analysis to determine the Nile tilapia gene expression modulation.

Accession ID	Gene name	Primer name	Primer sequence	Tm (°C)	Product size (bp)
XM_019365844	Interleukin-1	IL1-F	GTCTGTCAAGGATAAGCGCTG	59	200
		IL1-R	ACTCTGGAGCTGGATGTTGA	58	
NM_001279704	Interleukin-8	IL8-F	CTGTGAAGGCATGGGTGTG	59	196
		IL8-R	GATCACTTTCTTCACCCAGGG	58	
XM_013271147	Lipopolysaccharide-binding protein	LBP-F	ACCAGAAACTGCGAGAAGGA	59	200
		LBP-R	GATTGGTGGTCGGAGGTTTG	59	
NM_001279635	Glutathione S-transferase	GSTa-F	ACTGCACACTCATGGGAACA	60	190
		GSTa-R	TTAAAAGCCAGCGGATTGAC	60	
NM_001279711	Glutathione peroxidase	GPX-F	GGTGGATGTGAATGGAAAGG	60	190
		GPX-R	CTTGTAAGGTTCCCCGTCAG	59	
XM_005467348	Glutathione reductase	GSR-F	CTGCACCAAAGAACTGCAAA	60	172
		GSR-R	CCAGAGAAGGCAGTCCACTC	60	
XR_003216134	18S rRNA (reference gene)	18S rRNA –F	GTGCATGGCCGTTCTTAGTT	60	150
		18S rRNA -R	CTCAATCTCGTGTGGCTGAA	60	

2.6.3. Quantitative Real-Time Polymerase chain reaction (qRT-PCR)

The qRT-PCR reaction was conducted using the CFX Connect™ Real-Time PCR System (BIO-RAD, 166 USA) with iTaq Universal SYBR Green supermix 2X (BIO-RAD, USA). The genes *IL-1, IL-8, LBP, GSTa, GPX,* and *GSR* were analyzed to evaluate gene expression modulation in Nile tilapia. The 18S rRNA gene of *Oreochromis niloticus* was used as reference gene (Table 4). For the assay, 100 ng of cDNA was used, and the qRT-PCR protocol was described in our previous studies [26]. Gene expression levels were quantified using the $2^{-\Delta\Delta Ct}$ method [31].

2.7. Growth and food efficiency parameters

Fish were carefully retrieved from each tank at the 4-week and 8-week marks of the feeding trial, and their weights were recorded. Growth parameters were subsequently analyzed using following formulas:

$$\textit{Weight gain}\ (WG;\ g) = W_2 - W_1$$

$$\textit{Specific growth rate}\ (\mathrm{SGR}) = 100 \times \ln\ [\![\textit{final weight}] - \ln\ [\![\textit{initial weight}]\!]\ ]\!]\ /T$$

$$\textit{Feed conversion ratio}\ (\mathrm{FCR}) = (\text{Total feed (g)})/(\text{Weight gain (g)})$$

$$\textit{Survival rate}\ (\mathrm{SR};\ \%) = 100 \times (\text{Number of fish survived})/(\text{Number of fish leased})$$

Where, W2 and W1 denote the fish weight at a given time and at the beginning of the experiment, respectively, while T represents experimental duration (days).

2.8. Statistical analysis

Statistical analysis of variance (ANOVA) was perfomed on all experimental data using SAS Enterprise Guide Software V.9.4 (SAS Institute, Cary, NC, USA) [32] and R package 'agricolae' version 1.3-7. Tukey's test was employed for multiple comparisons of least-squares means. The optimal LP level was determined through polynomial regression analysis [33]. Statistical significance was determined at a probability level of $P \leq 0.05$.

3. Results

3.1. Growth performance

The growth-related parameters of Nile tilapia in this experiment are summarized in Table 5. At both 4 and 8 weeks, fish fed a longan-rich diet exhibited significant weight and improved specific growth rate (SGR) compared to the control group ($P < 0.05$). Additionally, the inclusion of LP in the basal diet significantly enhanced the feed conversion ratio (FCR) ($P < 0.05$). Polynomial regressions analysis revealed that dietary LP inclusion had a significant effect ($P < 0.05$) on final weight (FW), weight gain (WG), SGR, and FCR (Fig. 1). Based on the quadratic polynomial regression, the optimal dietary inclusion levels of LP were estimated to be (47.19 g kg^{-1} for FW (Figs. 1a), 47.02 g kg^{-1} for WG (Figs. 1b), 46.42 g kg^{-1} for SGR (Figs. 1c), and 49.16 g kg^{-1} for FCR (Fig. 1d).

Table 5
Growth performances and feed utilization of Nile tilapia after 4 and 8 weeks feeding with longan peel diets.

	LP0	LP10	LP20	LP40	LP80
IW (g)	13.72 ± 0.06^{a}	13.72 ± 0.03^{a}	13.68 ± 0.08^{a}	13.67 ± 0.03^{a}	13.70 ± 0.10^{a}
FW (g)					
4 weeks	28.34 ± 0.85^{b}	29.87 ± 0.14^{ab}	30.77 ± 1.11^{ab}	32.02 ± 1.92^{a}	30.02 ± 1.70^{ab}
8 weeks	56.68 ± 0.88^{c}	58.83 ± 1.38^{bc}	61.12 ± 1.17^{ab}	63.10 ± 1.93^{a}	59.99 ± 1.72^{abc}
WG (g)					
4 weeks	14.63 ± 0.87^{b}	16.15 ± 0.17^{ab}	17.09 ± 1.11^{ab}	18.16 ± 1.90^{a}	16.22 ± 1.61^{ab}
8 weeks	42.96 ± 0.89^{c}	45.11 ± 1.39^{bc}	47.43 ± 1.19^{ab}	49.23 ± 1.91^{a}	46.19 ± 1.70^{abc}
SGR (%/day)					
4 weeks	2.42 ± 0.11^{b}	2.59 ± 0.02^{ab}	2.70 ± 0.12^{ab}	2.79 ± 0.19^{a}	2.59 ± 0.17^{b}
8 weeks	2.37 ± 0.03^{c}	2.43 ± 0.04^{bc}	2.49 ± 0.04^{ab}	2.53 ± 0.05^{a}	2.45 ± 0.05^{b}
FCR					
4 weeks	1.22 ± 0.02^{a}	1.19 ± 0.04^{ab}	1.17 ± 0.02^{b}	1.09 ± 0.01^{c}	1.18 ± 0.02^{b}
8 weeks	1.27 ± 0.01^{a}	1.24 ± 0.01^{ab}	1.22 ± 0.01^{ab}	1.15 ± 0.02^{c}	1.21 ± 0.03^{b}
SR (%)					
4 weeks	98.33 ± 2.89^{a}	95.00 ± 5.00^{a}	98.33 ± 2.89^{a}	98.33 ± 2.89^{a}	98.33 ± 2.89^{a}
8 weeks	98.33 ± 2.89^{a}	95.00 ± 5.00^{a}	95.00 ± 5.00^{a}	96.67 ± 5.77^{a}	96.67 ± 2.89^{a}

LP: longan peel, IW: initial fish weight, FW: final fish weight, WG: weight gain, SGR: specific fish growth rate, FCR: feed conversion ratio, SR: survival rate. Different letters in the same row represent significant differences ($P < 0.05$).

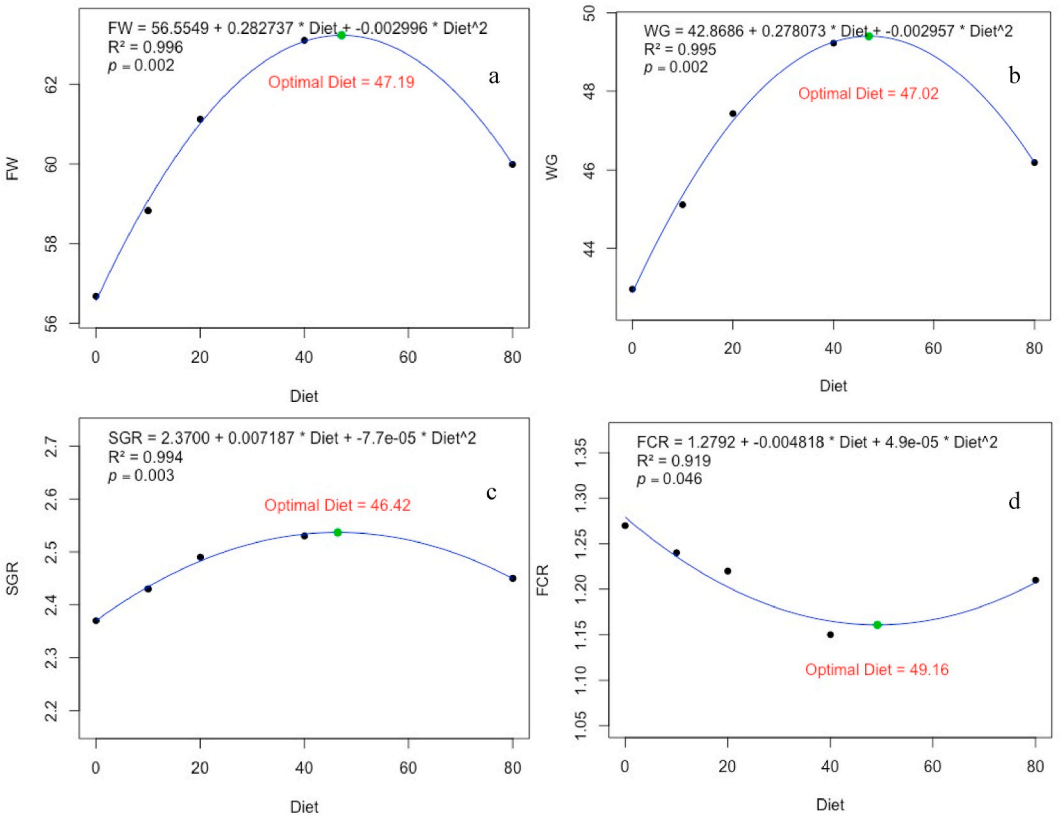

Fig. 1. Significant quadratic relationships and polynomial regressions analysis ($P < 0.05$) between final body weight (a), weight gain (b), specific growth rate (c), and feed conversion ratio (d) of Nile tilapia and dietary levels of Longan peel (LP) powder. Values expressed as means ± SE.

3.2. Lysozyme and peroxidase activities

The effects of LP on the innate immune defense mechanisms of Nile tilapia were evaluated by measuring lysozyme and peroxidase activities in fish skin mucus. Fish fed LP-supplemented diets demonstrated significantly higher ($P < 0.05$) skin mucus lysozyme activity (SMLA) and skin mucus peroxidase activity (SMPA) at both four and eight weeks compared to the control. Among the treatments, fish fed the LP40 diet exhibited the highest SMLA and SMPA levels, followed by those on the LP20, LP80, and LP10 diets (Table 6).

Serum innate immune activities were also assessed in Nile tilapia fed diets supplemented with varying concentrations of LP. Overall, LP-supplemented diets significantly increased serum lysozyme (SL) and serum peroxidase (SP) activities compared to the control ($P < 0.05$). Similar to the skin mucus results, fish fed LP40 diet showed the highest SL and SP levels, followed by those on LP20, LP80, and LP10 (Table 7).

3.3. mRNA expression of LP treated fish

As shown in Fig. 2, fish fed an LP-enriched diet exhibited significantly higher expression levels of both immune-related and

Table 6
Skin mucus lysozyme and peroxidase activities of Nile tilapia after 4 and 8 weeks feeding with experimental longan peel diets.

		LP0	LP10	LP20	LP40	LP80
4 weeks	SMLA	1.16 ± 0.07^{b}	1.24 ± 0.10^{ab}	1.31 ± 0.05^{ab}	1.37 ± 0.10^{a}	1.26 ± 0.05^{ab}
	SMPA	0.13 ± 0.01^{c}	0.15 ± 0.01^{bc}	0.16 ± 0.01^{ab}	0.18 ± 0.02^{a}	0.15 ± 0.00^{bc}
8 weeks	SMLA	2.96 ± 0.37^{b}	3.33 ± 0.18^{ab}	3.43 ± 0.21^{ab}	3.65 ± 0.27^{a}	3.36 ± 0.18^{ab}
	SMPA	0.17 ± 0.01^{b}	0.19 ± 0.02^{ab}	0.20 ± 0.02^{ab}	0.22 ± 0.01^{a}	0.19 ± 0.02^{ab}

SMLA: Skin mucus lysozyme activity (μg mL^{-1}); SMPA: Skin mucus peroxidase activity (μg mL^{-1}). Different letters in the same row indicate significant differences ($P < 0.05$).

The serum innate immune activity was also performed on the Nile tilapia treated with different concentration of LP-supplemented diets. Overall, LP-supplemented diets resulted in fish with serum lysozyme (SL) and serum peroxidase (SP) values significantly higher than the control ($P < 0.05$). LP40-treated fish exhibited the highest values, followed by LP20, LP80, and LP10 (Table 5).

Table 7
Serum lysozyme and peroxidase activities of Nile Tilapia after 4 and 8 weeks feeding with experimental longan peel diets.

		LP0	LP10	LP20	LP40	LP80
4 weeks	SLA	2.80 ± 0.15^{b}	3.24 ± 0.79^{ab}	3.53 ± 0.25^{ab}	3.89 ± 0.35^{a}	3.23 ± 0.40^{ab}
	SPA	0.12 ± 0.02^{d}	0.14 ± 0.02^{cd}	0.18 ± 0.01^{ab}	0.20 ± 0.01^{a}	0.16 ± 0.01^{bc}
8 weeks	SLA	4.19 ± 0.37^{c}	4.98 ± 0.43^{b}	5.00 ± 0.40^{b}	5.69 ± 0.20^{a}	4.94 ± 0.12^{b}
	SPA	0.21 ± 0.01^{c}	0.24 ± 0.01^{bc}	0.26 ± 0.02^{abc}	0.31 ± 0.04^{a}	0.28 ± 0.04^{ab}

SLA: Serum lysozyme activity (μg mL^{-1}); SPA: Serum peroxidase activity (μg mL^{-1}). Different letters in the same row denote significant differences ($P < 0.05$).

antioxidant genes in the liver compared to the control group ($P < 0.05$). In the LP10 treatment, while the expression of *IL-8* and *LBP* genes did not differ significantly from the ($P > 0.05$), the other genes showed increased expression, albeit lower than those observed in fish fed the other LP-supplemented diets. Among all treatments, fish in the LP40 group demonstrated the highest expression levels.

Fig. 3 illustrates the effect of dietary LP supplementation on the expression levels of immunological and antioxidant genes in the intestine of Nile tilapia fed diets containing longan peel powder. Compared to the control group, LP supplementation significantly enhanced intestinal gene expression ($P \geq 0.05$). Among the treatments, the LP40 diet resulted in the highest expression levels of *IL-1*, *IL-8*, *LBP*, *GPX*, *GSTα*, and *GSR* genes ($P < 0.05$), followed by LP80, LP20, and LP10, with no significant ($P \geq 0.05$) difference among these three groups.

4. Discussion

In recent years, the use of agricultural by-products and waste for various applications has garnered increasing attention [34]. Due to their abundant availability, low cost, and the presence of compounds, agricultural by-product holds great potential as a valuable resource for multiple industries [35,36]. Incorporating additive materials derived from agricultural by-product into aquafeed production not only reduces cost but also enhances growth performance and health in cultured aquatic species.

Our study aimed to evaluate the effects of incorporating longan peel (LP) as a feed additive in the diet of the Nile tilapia, focusing on growth, innate immune activity, and immune and antioxidant related gene expression. After 4 and 8 weeks of the feeding trial, Nile tilapia fed the LP-supplemented diet showed significantly improved growth and feed efficiency, with the highest values observed in the group receiving 40 g kg^{-1}. To the best of our knowledge, no previous studies have specifically assessed dietary LP supplementation in fish species. The observed growth improvements in Nile tilapia can be attributed to the nutrient profile and bioactive compounds present in LP. Longan peel contains carbohydrates, proteins, lipids, minerals, and fiber [21], with a composition of 7.0–7.1 % protein, 5.7 % ash, and 0.56 % carbohydrates per 100 g of dry matter (DM). Additionally, LP is rich in fibers (33.4–83.9 %), with a low-fat content (0.4–0.9 %). Notably, the fiber composition includes 18.7 % lignin and 2.1 % hemicellulose, compounds shown to enhance growth in Atlantic salmon (*Salmo salar* L) [37] and rainbow trout (*Oncorhynchus mykiss*) [38]. In terrestrial farmed species, lignin and hemicellulose are recognized as promising prebiotic sources capable of inhibiting pathogenic intestinal microbiota, improving hindgut microflora, and promoting feed utilization and growth [39,40]. These effects may extend to aquatic species as well. Our findings align with studies on other fish species. For instance, growth and feed efficiency improvements have been reported in common carp (*Cyprinus carpio*) fed apricot kernel-enriched diets [41] and pomegranate peel [42]; black rockfish (*Sebastes schlegelii*) fed yacon by-products [43]; bagrid catfish (*Mystus nemurus*) fed sweet orange peel waste [6]; Nile tilapia (*Oreochromis niloticus*) fed fruit wastes

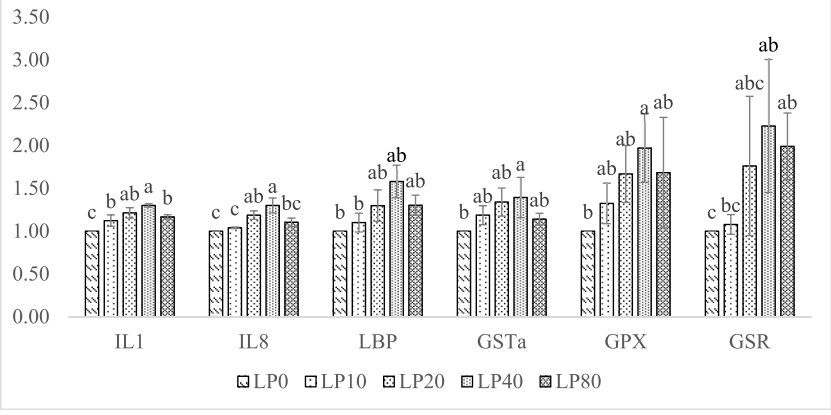

Fig. 2. Gene expression profiling of Nile tilapia liver post treatments with LP for 8 weeks. The modulated expression of immune genes post treatments LP in *O. niloticus* was measured by RT-qPCR at different concentrations of feed (10 (LP10), 20 (LP20), 40 (LP40) and 80 (LP80) g kg^{-1}). IL1 = Interleukin 1; IL8 = Interleukin 8; LBP = lipopolysaccharide-binding protein; GSTa = Glutathione S-transferase; GPX = Glutathione peroxidase; GSR = glutathione reductase). Statistical analysis performed as ANOVA. Different letters above each column represents significant differences ($P < 0.05$).

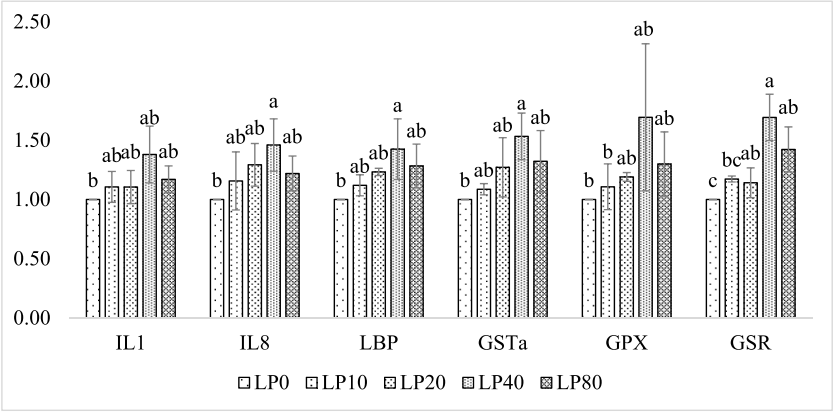

Fig. 3. Gene expression profiling of Nile tilapia intestine post treatments with LP for 8 weeks. The modulated expression of immune genes post treatments LP in *O. niloticus* was measured by RT-qPCR at various doses of feed (10 (LP10), 20 (LP20), 40 (LP40) and 80 (LP80) g kg^{-1}). IL1 = Interleukin 1; IL8 = Interleukin 8; LBP = lipopolysaccharide-binding protein; GSTa = Glutathione S-transferase; GPX = Glutathione peroxidase; GSR = glutathione reductase). Statistical analysis performed as ANOVA. Different letters above each column represents significant differences ($P < 0.05$).

[44] and banana flower powder [45], and prickly pear (*Opuntia ficus-indica*) peel [46]. These findings underscore the potential of fruit by-products like LP as effective feed additives in aquaculture.

The positive impact of agricultural by-products as aquafeed additives on fish health, particularly on the innate immune system, is well-documented. The nonspecific immune response plays a critical role as the first line of defense against invading pathogens, forming the backbone of the innate immune system [47]. Lysozyme, a proteolytic enzyme, protects fish by breaking down bacterial cell walls and inducing immunological responses, such as complement activation and phagocytosis [48]. Similar, peroxidases are antioxidant enzymes widely distributed in microbes and animal tissues [49]. These enzymes are essential in combating reactive oxygen species (ROS) during oxidative stress, serving as a critical component of the innate immune system's defense [50,51]. This study is the first to demonstrate that dietary LP significantly enhances lysozyme and peroxidase activities in both skin mucus and serum of the Nile tilapia. These findings are consistent with previous studies on various fish species, including Nile tilapia fed rambutan peel [52]; common carp (*Cyprinus carpio*) fed apricot kernel-enriched diets [41] and pomegranate peel [42]; black rockfish (*Sebastes schlegelii*) fed yacon by-products [43]; bagrid catfish (*Mystus nemurus*) fed sweet orange peel waste [6]; Nile tilapia (*Oreochromis niloticus*) fed fruit wastes [44] and banana flower powder [45], and prickly pear (*Opuntia ficus-indica*) peel [46]. Metabolomic analysis reveals that LP is rich in bioactive molecules, particularly phenolic compounds such as flavonoids, alkaloids, and tannins [20]. Phenolic compounds used as feed additives in aquaculture diets have been shown to enhance growth parameters [8,53] in species such common carp (*C. carpio*) [54], beluga sturgeon (*Huso huso*) [55], barramundi (*L. Calcarifer*) [56]. Additionally, polyphenols are known to strengthen fish immune responses by enhancing nonspecific defense activities through improved cellular and humoral immunity [57]. In Nile tilapia, polyphenols derived from chestnut shell have been reported to improve lysozyme and peroxidase activities [58], supporting the hypothesis that phenolic compounds may be responsible for the enhanced lysozyme and peroxidase activities observed in this study. Furthermore, polysaccharides found in LP, such as *l*-arabinofuranose, *d*-glucopyranose, *d*-glucopyranose, *d*-galactopyranose, and *d*-galacturonic acid [59,60], may also contribute to the improvement of immunological activity indices of the Nile tilapia. Polysaccharides are considered potential prebiotics [61,62], benefiting fish immune systems through various mechanisms, including promoting beneficial gut microbiota and enhancing immune function [63,64]. This multifaceted interaction highlights the potential of LP as a functional feed additive for improving health and immune response in aquaculture species. *IL-1* and *IL-8* are key pro-inflammatory cytokines involved in the inflammatory response, enabling organisms to respond to stress by attracting neutrophils, releasing histamine, enhancing neutrophil adhesion to endothelial cells, and facilitating migration to inflammatory sites. In this study, dietary LP supplementation significantly upregulated the expression of *IL-1* and *IL-8* genes in both the liver and intestine. To date, no studies have specifically evaluated the effect of LP on immune-related gene expression. However, similar upregulation of *IL-1* and *IL-8* has been reported in Nile tilapia treated with mango peel [65] and banana flower powder [45]. This study also revealed a significant increase in the expression of the lipopolysaccharide binding protein (LBP) gene in the liver and intestine of LP-fed Nile tilapia. LBP, a member of the lipid transfer/LBP (LT–LBP) family, plays a crucial role in detecting of bacterial components in phagocytic cells, thereby modulating cellular signaling pathways [66]. Given the limited research on LP's mechanism of action in fish immune regulation, the phytochemical substances present in LP may offer an explanation for these findings. Phenolic compounds such as phenolic acids, flavonoids, and tannins, which are abundant in LP [20], are known to process pharmacological and immunostimulatory properties in fish [67,68]. Additionally, as a natural prebiotic, LP polysaccharides may indirectly influence immune responses by promoting a favorable shift in gut microbiota composition, particularly by increasing beneficial bacteria lactic acid bacteria [62,69]. Despite these insights, further investigations are required to elucidate the precise mechanisms underlying LP's effects on immune-related genes and its broader immunomodulatory role in aquaculture species.

GPX, GSTα, and *GSR* are key antioxidant enzymes with free radical scavenging abilities that are enhanced by dietary antioxidants

[70,71]. In this study, the expression of these antioxidant genes in the liver and intestine of Nile tilapia was significantly upregulated in the fish fed LP-supplemented diets. Similar findings have been reported in other species, such as rohu (*Labeo rohita*) fed grape pomace flour and lemon peel [72]; common carp (*C. carpio*) fed cherry fruit extract [73], and Nile tilapia (*O. niloticus*) fed passion fruit peel and phytogenic mixture [65]. The observed antioxidative capacity is linked to the strong free radical scavenging capability of polyphenolic derivatives, which help mitigate oxidative stress [74]. Notably, LP is known to contain a substantial amount of polyphenols [75], which likely contribute to the enhanced antioxidant gene expression in this study. Conversely, mango peel supplementation has been reported to have no significant effects on antioxidant gene expressions in Nile tilapia [65]. These discrepancies may be attributed to differences in experimental conditions, such as fish species, diet composition, culture duration, and water quality parameters. Further studies are needed to better understand these variations and their underlying mechanisms.

5. Conclusion

In conclusion, supplementing Nile tilapia diets with LP significantly improved growth performance, enhanced innate immunological parameters in skin mucus and serum, and upregulated antioxidant gene expression in the liver and intestine, with 40 g kg^{-1} identified as the most effective concentration. Polynomial regression analysis further suggests that the optimal inclusion levels rage from 46 to 49 g kg^{-1}. The results of this study suggest that longan peel has the potential to serve as a functional feed additive, promoting sustainable Nile tilapia aquaculture.

CRediT authorship contribution statement

Supreya Wannavijit: Methodology, Investigation. **Piyatida Outama:** Methodology, Investigation. **Chinh Le Xuan:** Investigation, Formal analysis. **Camilla Maria Fontana:** Writing – review & editing. **Marina Paolucci:** Writing – review & editing. **Md Afsar Ahmed Sumon:** Writing – review & editing, Methodology, Formal analysis. **Ehab El-Haroun:** Writing – review & editing. **Hien Van Doan:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Ethical statements

Animals used in this study were cared for and used in accordance with animal ethical treatment guidelines. All animal experiments comply with AAALAC guide-lines approval by Chiang Mai University Committee (Approval No. RAGIACUC002/2565).

Data availability statement

The data presented in this study are available on request from the corresponding author.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hien Van Doan reports financial support was provided by National Research Council of Thailand. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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