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Research article

Effect of dietary supplementation with Lamduan (*Sphaerocoryne lefevrei* (Baill.) D.M. Johnson & N.A. Murray) fruit powder on the growth, digestive enzymes, non-specific immune system, and skin pigmentation of Siamese fighting fish (*Betta splendens* Regan, 1910)

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Abstract

Various parts of Lamduan (*Sphaerocoryne lefevrei* (Baill.) D.M. Johnson & N.A. Murray) have long been utilized in traditional medicine to treat a range of human ailments. Nonetheless, its potential as a bioactive feed supplement in aquaculture production remains underexplored. This research assessed the impact of incorporating Lamduan fruit powder (LFP) into the diet on the growth performance, digestive enzymatic functions, mucosal immune activity, and pigmentation of Siamese fighting fish (*Betta splendens* Regan, 1910). A total of 300 juvenile male short-tail *B. splendens* (average initial weight: 0.08 ± 0.01 g; total length: 1.97 ± 0.01 cm; two-month-old) were assigned to four dietary treatments with three replicates each and fed experimental diets containing 0, 10, 30, and 50 g/kg of LFP for eight weeks. Results revealed that the group receiving 50 g/kg exhibited significantly enhanced growth metrics ($p < 0.05$), while parameters such as feed efficiency, survival rate, and viscerosomatic index remained statistically unchanged ($p > 0.05$). Enzyme-specific activities, including amylase, protease, and lipase, along with mucus-based immune markers such as total protein, IgM, anti-protease, myeloperoxidase, superoxide dismutase, catalase, and total protease, were markedly improved in fish fed LFP-enriched diets. Moreover, antioxidant capacity and carotenoid deposition in skin and muscle tissues were significantly elevated. Based on second-order polynomial regression, an LFP inclusion of 27.18–28.58 g/kg is recommended as a practical level to maximize pigmentation while maintaining acceptable growth. The findings highlight the potential of LFP as a functional dietary supplement to enhance both health status and coloration in ornamental fish.

Keywords: Annonaceae, Feed additives, Mucosal immunity, Ornamental fish, Pigmentation.

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INTRODUCTION

The global demand for ornamental fish has been rising steadily (Chapman et al., 1997). To address this growing market, farmers have adopted semi-intensive and intensive aquaculture systems for rearing various species of ornamental fish (Domínguez and Botella, 2014), and Thailand stands out as a leading producer among Southeast Asian countries. In 2017, the country exported approximately 66 million ornamental fish, generating a revenue of 430 million baht (Son-ong, 2019). The most frequently exported species included Siamese fighting fish, guppies, and cichlids. Major export destinations included Europe, the Americas, and the Asia-Pacific region (Son-ong, 2019).

Siamese fighting fish (*Betta splendens* Regan, 1910) are regarded as a prominent and highly popular freshwater ornamental species in Thailand. In 2020, around 20 million individuals were exported, yielding an estimated value of 200 million baht (Department of Fisheries, 2025). Key international markets for this species include Singapore, China, Hong Kong, France, Japan, and the United States (Ariyasantichai et al., 2024). Owing to its prominent coloration and ease of cultivation, *B. splendens* is cultured extensively for its ornamental value in the aquaculture industry (Monvises et al., 2009). However, traditional farming practices still dominate in many parts of Thailand, posing sustainability challenges for large-scale production (Monvises et al., 2009). As consumer demand continues to rise, farming methods have shifted toward commercial-scale operations. Nevertheless, production efficiency and competitiveness in Thailand remain constrained by issues such as inadequate nutritional qualities, low fry survival, disease susceptibility, and faded skin pigmentation (Khrueahong et al., 2022).

The body size and pigmentation of ornamental fish are key factors influencing their commercial attractiveness and market value (Hoseinifar et al., 2023). Among various factors, skin pigmentation in fish is predominantly influenced by carotenoids (Nakano and Wiegertjes, 2020). These pigments are typically divided into carotenes and xanthophylls. In ornamental species, carotenoids not only enhance external coloration but also support physiological functions such as growth, oxidative stress tolerance, immune function, and antioxidant defense (Amaya et al., 2007). As fish are unable to synthesize carotenoids endogenously, they must acquire them from dietary sources and subsequently deposit them in tissues (Yuan et al., 2010). In commercial aquaculture, synthetic carotenoids (astaxanthin, β-carotene, and lycopene) are frequently incorporated into feed to promote pigmentation (Yuan et al., 2010). Nonetheless, reliance on synthetic additives raises production costs, limits pigmentation diversity, reduces pigment retention, and generates concerns over health and environmental safety (Tran et al., 2025). These limitations have elicited increasing interest in natural alternatives derived from algae, plants, fungi, and animals for use in aquafeed formulations (Pereira da Costa and Campos Miranda-Filho, 2019; Luo et al., 2021). Natural pigments are considered safer, more sustainable, and environmentally friendly compared to synthetic compounds (Luo et al., 2021).

Lamduan (*Sphaerocoryne lefevrei* (Baill.) D.M. Johnson & N.A. Murray), commonly known as devil tree or white cheesewood, is a native fruit-bearing perennial shrub widely distributed across Southeast Asia. Its yellow flowers are valued for their fragrance and are commonly used in perfumery, while the ripe bluish-black fruits are edible (Sakulnarmrat and Konczak, 2022). The plant is known to contain a wide range of phytochemicals, including terpenoids, alkaloids, steroids, flavonoids, phenolics, anthocyanins, and essential oils (Mongkol et al., 2016; Sakulnarmrat and Konczak, 2022). In pharmacological research, Lamduan has been reported to exhibit multiple bioactivities, including anti-inflammatory, antifungal, antiproliferative, and antioxidant effects (Mongkol et al., 2016; Do and Sichaem, 2022; Mohamed et al., 2024). In traditional Thai medicine, Lamduan has been employed in the treatment of various conditions such as cardiovascular ailments, skin disorders, pain, inflammation, and fever (Mongkol et al., 2016). The

fruit is also processed into beverages, jams, and wines (Sakulnarmrat and Konczak, 2022). Recent studies have investigated the use of microencapsulation techniques for anthocyanin-rich extracts derived from Lamduan fruit, aiming at their utilization in food-related applications (Sakulnarmrat and Konczak, 2022).

To evaluate the efficacy of nutraceutical feed ingredients in ornamental fish, several biological parameters are commonly calculated. Improvements in growth performance are frequently assessed using metrics such as weight gain and specific growth rate (Bulfon et al., 2013). In aquatic animals, the digestion of nutrients and growth are directly linked to digestive enzymes. Dietary supplementation with functional additives has been shown to enhance digestive enzymatic activities, improving nutrient uptake and utilization (Karataş, 2024). Fish skin mucus, a critical component of innate immunity, contains diverse bioactive molecules essential for protection against oxidative stress, pathogens, and diseases (Giri et al., 2024). Evidence suggests that natural feed additives enhance fish mucus immunity and antioxidant defenses, leading to improved disease resilience (Bulfon et al., 2013; Giri et al., 2024; Karataş, 2024). Additionally, fish pigmentation is strongly correlated with carotenoid deposition in chromatophores. Given that aquatic organisms cannot synthesize carotenoids, dietary intake is essential for pigmentation development (Yuan et al., 2010). Consequently, investigations into natural or synthetic dietary supplements that improve coloration are increasingly relevant to the advancement of ornamental fish culture (Nakano and Wiegertjes, 2020; Hoseinifar et al., 2023; Tran et al., 2025).

Several scientific reports confirm the pharmacological potential of Lamduan (Mongkol et al., 2016; Do and Sichaem, 2022; Sakulnarmrat and Konczak, 2022; Mohamed et al., 2024). Acute oral toxicity studies in rats have shown that Lamduan leaf extract, administered at doses up to 4,000 mg/kg body weight, did not induce any signs of toxicity, mortality, or abnormal behavior (Mohamed et al., 2024). These results support the traditional use of the plant in treating various health conditions (Mongkol et al., 2016; Do and Sichaem, 2022; Sakulnarmrat and Konczak, 2022; Mohamed et al., 2024). Despite its established bioactive properties, the use of Lamduan in aquaculture, particularly as a feed additive for improving growth, immunity, and pigmentation in ornamental fish, remains limited. Furthermore, Lamduan fruits are locally abundant in Thailand, but large quantities are wasted annually due to their short shelf life and limited commercial use. Importantly, they contain diverse bioactive compounds, including phenolics, flavonoids, tannins, terpenoids, carotenoids, and anthocyanins, which are associated with antioxidant, anti-inflammatory, and immunomodulatory activities. These unique properties, together with their availability as a sustainable local resource, provide the rationale for selecting Lamduan fruits as a promising functional feed additive for aquaculture. Addressing this gap, this study investigates the impact of incorporating Lamduan fruit powder (LFP) into the diet on growth performance, mucosal immunity, and pigmentation in *B. splendens*.

MATERIALS AND METHODS

Plant sample preparation

Lamduan specimens were taxonomically verified by a botanist, and a voucher specimen (Munglue 0015) was archived at the Program of Biology, Faculty of Science, Ubon Ratchathani Rajabhat University. Mature fruits were harvested from Mueang District, Sisaket Province, Thailand. After washing them thoroughly with running tap water, seeds were removed, and the pulp was subjected to drying at 60°C for 72 hours using a hot air oven (UN260, Memmert GmbH, Germany). The dried pulp was ground into fine powder, sealed in plastic bags, and preserved at −4°C for subsequent experimentation.

Phytochemical analysis

Before phytochemical and antioxidant analyses, 1 g of LFP was extracted with 10 ml of 70% ethanol, vortexed for 5 min, and sonicated for 30 min at room temperature. The mixture was centrifuged at 4,000 ×*g* for 10 min, and the supernatant was collected and filtered through Whatman No. 1 paper. The filtrate was used for all subsequent phytochemical and antioxidant activity assays. Total flavonoids were quantified via the aluminum chloride colorimetric assay (Matan et al., 2024), with absorbance measured at 510 nm and expressed as mg quercetin equivalent per gram of LFP (mg QE/g LFP). Total phenolics were determined using the Folin–Ciocalteu reagent method (Matan et al., 2024), with absorbance read at 765 nm and results reported as mg gallic acid equivalents (mg GAE/g LFP). Tannins were also analyzed using the Folin–Ciocalteu method (Ponsin et al., 2025) at 725 nm, expressed as mg tannic acid equivalents per gram (mg TAE/g LFP). Terpenoids were assessed by the spectrophotometric method (Ponsin et al., 2025), with results expressed in mg linalool equivalents (mg LNOLE/g LFP) based on absorbance at 538 nm. Anthocyanins were evaluated via the pH-differential method of Shao et al. (2014), with results given in mg/l. Carotenoids were determined by using the method of Matan et al. (2024) and reported in µg/g. β-Carotene content was estimated following Biswas et al. (2011) and presented in µg/g. All analyses were performed in triplicate using a microplate reader (SPECTRO Star Nano, BMG LabTech, Germany).

Antioxidant activity assays

The antioxidant properties of LFP were determined using DPPH, ABTS, and FRAP assays (Biswas et al., 2011; Matan et al., 2024). The DPPH and ABTS radical scavenging activities were expressed as % inhibition using the formula:

$$\% \text{ inhibition} = \frac{(\text{Abcontrol} - \text{Absample})}{\text{Abcontrol}} \times 100$$

where $Ab_{control}$ is the absorbance of the control, and Ab_{sample} is the absorbance of the sample.

For FRAP analysis, antioxidant capacity was expressed in mmol ascorbic acid equivalents per gram (mmol AAE/g) of LFP, using the equation:

$$\text{FRAP value (mmol AAE/g LFP)} = \frac{Concentration\ of\ AAE\ from\ the\ standard\ curve\ \left(\frac{mmol}{l}\right) \times Volume\ of\ extract\ (l)}{\text{Weight of LFP sample (g)}}$$

Diet preparation

Commercial floating pellets (High Grade 9006T, Charoen Pokphand Foods PCL) served as the basal diet. In this study, dietary inclusion levels ranging from 0 to 50 g LFP/kg diet were selected based on preliminary feeding trials and prior reports on similar fruit-based feed additives used in ornamental fish to evaluate both the efficacy and safety of LFP in *B. splendens* diets (Promprom et al., 2024a). Cassava starch was used as a binder at a fixed level of 1% (w/w) across all diets. All ingredients were mixed thoroughly, moistened with 30% distilled water, and pelleted using a 1.5 mm die mincer. The pellets were oven-dried at 60°C for 12 hours and stored at 4°C until use. The proximate composition of the diets (crude protein, crude lipid, ash, moisture, fiber, and nitrogen-free extract) was analyzed according to standard AOAC (2005) procedures, and the results are presented in Table 1.

Table 1 Proximate composition of the basal diet.

Proximate chemical analysis	g per 100 g diet
Crude protein	43.38
Crude lipid	6.21
Nitrogen free extract	30.85
Ash	10.45
Moisture	7.92
Fiber	1.29

Note: Crude protein, crude lipid, ash, and fiber contents were determined through experimental analysis, whereas the nitrogen-free extract (NFE) was calculated based on the difference method. All values are expressed on an as-fed basis.

Feeding trial in Siamese fighting fish

A total of 300 juvenile male short-tail *B. splendens* (average initial weight: 0.08 ± 0.01 g; total length: 1.97 ± 0.01 cm; two-month-old) were obtained from the Ubon Ratchathani Provincial Fisheries Cooperative. Only healthy fish with normal morphology and swimming behavior were selected for the feeding trial. The experiment followed a completely randomized design (CRD) consisting of four dietary treatments (0, 10, 30, and 50 g/kg LFP), with three replicates per treatment. Each replicate contained 24 fish, individually housed in plastic beakers (8 cm diameter × 11.5 cm height) containing 250 ml of dechlorinated water under a 12 h light:12 h dark photoperiod. Water quality was maintained with the following parameters: temperature < 28°C, pH 7.21–7.82, hardness 50–100 mg/l, alkalinity 25–100 mg/l, BOD 3–20 mg/l, ammonia ≤ 0.025 mg/l, nitrite ≤ 0.02 mg/l, nitrate 0.1–4.5 mg/l, and dissolved oxygen 5.7–6.5 mg/l (Thongprajukaew et al., 2011). Water was renewed every two days. Fish were fed to apparent satiation twice daily (08:00 and 16:00) for eight weeks. After the feeding trial, 12 fish per treatment group were randomly sampled for growth and enzymatic analyses, while four fish per replicate were used for mucus immune, antioxidant, and pigmentation assessments. All experimental protocols were complied with the guidelines set by the National Research Council of Thailand and were approved by the Institutional Animal Care and Use Committee (IACUC) of Ubon Ratchathani Rajabhat University (Approval No. AN643009).

Assessment of growth, feed utilization, and survival

Growth parameters, feed efficiency, and survival rates were calculated using standard equations, as detailed in the subsequent section.

Weight Gain (WG, g) = Final weight (g) – Initial weight (g)

$$\text{Specific Growth Rate (SGR, \%/day)} = \frac{ln\ \text{Final weight (g)} - ln\ \text{Initial weight (g)}}{\text{Experimental duration (days)}} \times 100$$

$$\text{Average Daily Gain (ADG, g/day)} = \frac{\text{Weight gain (g)}}{\text{Experimental duration (days)}}$$

$$\text{Feed Intake (FI, g/fish)} = \frac{\text{Feed consumed by fish (g)}}{\text{Final number of fish (fish)}}$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

$$\text{Condition Factor (CF, g/cm}^3\text{)} = \frac{\text{Final weight (g)}}{\text{Final length (cm)}^3} \times 100$$

$$\text{Survival Rate (SR, \%)} = \frac{\text{Final number of fish (fish)}}{\text{Initial number of fish (fish)}} \times 100.$$

Evaluation of digestive enzyme-specific activity

To prepare for sampling, fish were subjected to a 24-hour fasting period. Anesthesia was performed using clove oil at a concentration of 5 ml/l. Following anesthesia, the fish were dissected to obtain digestive organs, which were weighed and subsequently homogenized using a Dounce homogenizer in 0.2 M buffer solution (pH 8.0) at a ratio of 1:10 (w/v). The resulting homogenates were centrifuged at 15,000 ×*g* for 30 minutes at –4°C. The supernatant was collected

and stored at −20°C until enzyme-specific activity assays were performed. The relative weight of internal organs was calculated using the following formula:

$$\text{Viscerosomatic index (VSI, \%)} = \frac{\text{Internal organ weight (g)}}{\text{Fish body weight (g)}} \times 100$$

Amylase activity was assessed using the 3,5-dinitrosalicylic acid (DNS) method, as described by Ghafarifarsani et al. (2023). A 1% soluble starch solution served as the substrate, and absorbance was recorded at 540 nm. Enzymatic activity was calculated based on a maltose standard curve. Protease activity was measured using a non-specific protease assay (Promprom et al., 2024b), while casein was used as the substrate. Absorbance was determined at 660 nm and compared against a tyrosine standard curve. Lipase activity was evaluated via a spectrophotometric assay (Promprom et al., 2024b), employing *p*-nitrophenyl palmitate (*p*-NPP) as the substrate. The reaction product was quantified by measuring absorbance at 410 nm and referencing a *p*-nitrophenol calibration curve. The total protein concentration in each sample was quantified using the Folin–Lowry method (Lowry et al., 1951), with absorbance read at 750 nm and results standardized against bovine serum albumin (BSA). All digestive enzyme-specific activities were normalized to protein content and expressed in units per milligram of protein (U/mg protein) using the following equation:

$$\text{Enzyme-specific activities (U/mg protein)} = \frac{\Delta\text{Abs} \times \text{Vtotal}}{\varepsilon \times \text{Vsample} \times t} \times \frac{\text{ml}}{\text{mg protein}}$$

where ΔAbs refers to the change in absorbance observed at a specific wavelength, ε indicates the molar extinction coefficient of the reaction product, V_{total} corresponds to the total reaction volume, and V_{sample} denotes the volume of the enzyme extract used in the assay. The variable *t* represents the duration of the enzymatic reaction.

Assessment of skin mucus components and antioxidant activities

Following a 24-hour fasting period, fish were anesthetized using clove oil at a concentration of 5 ml/l. Individual fish were transferred into low-density polyethylene (LDPE) bags containing 10 ml of 50 mM NaCl solution and gently agitated for 1 minute. After removal, the mucus-enriched solution was centrifuged at 1,500 ×*g* for 10 minutes at 4°C. The collected supernatant was stored at −20°C for subsequent biochemical analyses.

Total protein levels were determined using the Folin–Lowry assay at 750 nm with a BSA calibration curve (Lowry et al., 1951). Immunoglobulin M (IgM) concentrations were analyzed according to Yadav et al. (2014), involving precipitation with 12% polyethylene glycol (PEG) and calculation of the difference in protein content before and after treatment. Lysozyme activity was assessed using a turbidimetric assay based on the lysis of *Micrococcus lysodeikticus* (Sigma-Aldrich, MO, USA), with one unit defined as a 0.001 absorbance unit decrease per minute per µl of mucus (Giri et al., 2024). Activities of total antiprotease and myeloperoxidase (MPO) were evaluated following the procedure outlined by Yadav et al. (2014). Alkaline phosphatase (ALP) activity was measured as described by Promprom et al. (2024a), while total protease activity was quantified using the azocasein hydrolysis method according to Guardiola et al. (2017).

Antioxidant defense markers in the mucus were also evaluated. Superoxide dismutase (SOD) and catalase (CAT) activities were determined using standard assay procedures (Wangkahart et al., 2024). Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels via the thiobarbituric acid reactive substances (TBARS) method according to Wangkahart et al. (2024). The radical scavenging activities of DPPH and ABTS in the mucus were measured following

the methods of Phrompanya et al. (2023). Additionally, total antioxidant capacity (T-AOC) was quantified using the protocol of Ponsin et al. (2025).

Determination of total carotenoid content

Fish were dissected using sterile instruments to isolate the skin and muscle tissues. Approximately 3 mg of each tissue type was weighed and immersed in 1 ml of 90% acetone within a test tube. The samples were mixed thoroughly and left to extract for three days in the dark, with gentle shaking performed daily. After the extraction period, samples were centrifuged at 5,000 ×*g* for 10 minutes. The resulting supernatant was analyzed spectrophotometrically at 474 nm using 90% acetone as the blank. The total carotenoid content was determined using the formula established by Thongprajukaew et al. (2012) and expressed in micrograms per gram of tissue sample (µg/g tissue).

Statistical analysis

A completely randomized design (CRD) was applied for the test. Before statistical comparisons, data were assessed for normal distribution using the Kolmogorov–Smirnov test and for homogeneity of variance using Levene's test. Subsequently, one-way analysis of variance (ANOVA) was conducted to evaluate significant differences among the treatments. When significance was detected, Duncan's New Multiple Range Test (DMRT) was used for post hoc comparisons at a significance level of $p < 0.05$. Quadratic second-order polynomial regression analysis was performed to estimate the optimal dietary inclusion level of LFP for growth and pigmentation parameters.

RESULTS

Phytochemical composition and antioxidant capacity of LFP

The concentrations of phytochemical constituents and antioxidant activities of LFP are summarized in Table 2. Overall, LFP exhibited high levels of phenolics and flavonoids, followed by moderate amounts of tannins and terpenoids, along with detectable quantities of carotenoids, β-carotene, and anthocyanins. The extract also demonstrated strong antioxidant potential in DPPH, ABTS, and FRAP assays, indicating substantial radical-scavenging and reducing capacities.

Table 2 Phytochemical composition and antioxidant properties of Lamduan fruit powder (LFP)

Parameters	Results
Phytochemicals	
Total flavonoid content (mg QE/g LFP)	110.91 ± 8.38
Total phenolic content (mg GAE/g LFP)	273.01 ± 6.81
Tannins (mg TAE/g LFP)	41.37 ± 0.76
Terpenoids (mg LNOLE/g LFP)	9.01 ± 0.57
Anthocyanin (mg/l)	342.92 ± 3.19
Carotenoids (µg/g LFP)	3.30 ± 0.22
β-carotene (µg/g LFP)	1.42 ± 0.12
Antioxidant activity	
DPPH (IC_{50}, µg/ml)	1,532 ± 7
ABTS (IC_{50}, µg/ml)	6,332 ± 2
FRAP (mmol AAE/g LFP)	1.78 ± 0.80

Note: Values are expressed as mean ± SEM, with a sample size of $n = 3$ for each assay. QE: quercetin equivalent; GAE: gallic acid equivalent; TAE: tannic acid equivalent; LNOLE: linalool equivalent; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2-azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid); FRAP: ferric reducing antioxidant power; AAE: ascorbic acid equivalent.

Growth performance, feed utilization, and survival

The growth parameters of *B. splendens* fed diets containing different levels of LFP are shown in Table 3. Results demonstrated that fish fed diets containing LFP at 50 g/kg exhibited significantly higher FW, WG, SGR, and ADG compared with the control group and other treatments ($p < 0.05$). In contrast, FCR was significantly lower in the 50 g/kg group, indicating improved feed utilization efficiency. No significant differences were observed in SR or VSI among treatments ($p > 0.05$). Quadratic second-order polynomial regression analysis suggested an optimal inclusion level of approximately 15.00–22.80 g/kg LFP for improving growth performance (Figure 1).

Table 3 Effects of Lamduan fruit powder (LFP) supplementation on growth performance and survival rate of *B. splendens*

Parameters	LFP (g/kg)			
	0	10	30	50
IW (g)	0.08 ± 0.01	0.08 ± 0.00	0.08 ± 0.01	0.08 ± 0.00
FW (g)	0.76 ± 0.00^{b}	0.73 ± 0.01^{b}	0.76 ± 0.00^{b}	0.80 ± 0.01^{a}
IL (cm)	1.93 ± 0.13	1.88 ± 0.06	2.10 ± 0.08	2.00 ± 0.10
FL (cm)	3.56 ± 0.10^{b}	3.96 ± 0.09^{a}	3.81 ± 0.08^{a}	3.90 ± 0.12^{a}
WG (g)	0.68 ± 0.02^{b}	0.64 ± 0.01^{b}	0.66 ± 0.03^{b}	0.73 ± 0.02^{a}
SGR (%/day)	4.15 ± 0.28^{b}	3.88 ± 0.12^{b}	3.69 ± 0.28^{b}	4.45 ± 0.13^{a}
ADG (g/day)	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.02 ± 0.00^{a}
FI (g/fish)	1.06 ± 0.01^{c}	1.04 ± 0.01^{c}	1.09 ± 0.02^{b}	1.10 ± 0.01^{a}
FCR	1.57 ± 0.06	1.60 ± 0.05	1.59 ± 0.04	1.53 ± 0.04
CF (g/cm^{3})	1.71 ± 0.24	1.19 ± 0.30	1.40 ± 0.20	1.39 ± 0.35
SR (%)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
VSI (%)	5.88 ± 0.35	5.98 ± 0.24	5.66 ± 0.22	5.91 ± 0.13

Note: Different superscript letters within the same row indicate statistically significant differences among treatment groups at $p < 0.05$. Values are expressed as mean ± SEM. IW: initial weight (g); FW: final weight (g); IL: initial length (cm); FL: final length (cm); WG: weight gain (g); SGR: specific growth rate (%/day); ADG: average daily gain (g/day); FI: feed intake (g/fish); FCR: feed conversion ratio; CF: condition factor (g/cm^{3}); SR: survival rate (%); VSI: viscerosomatic index (%).

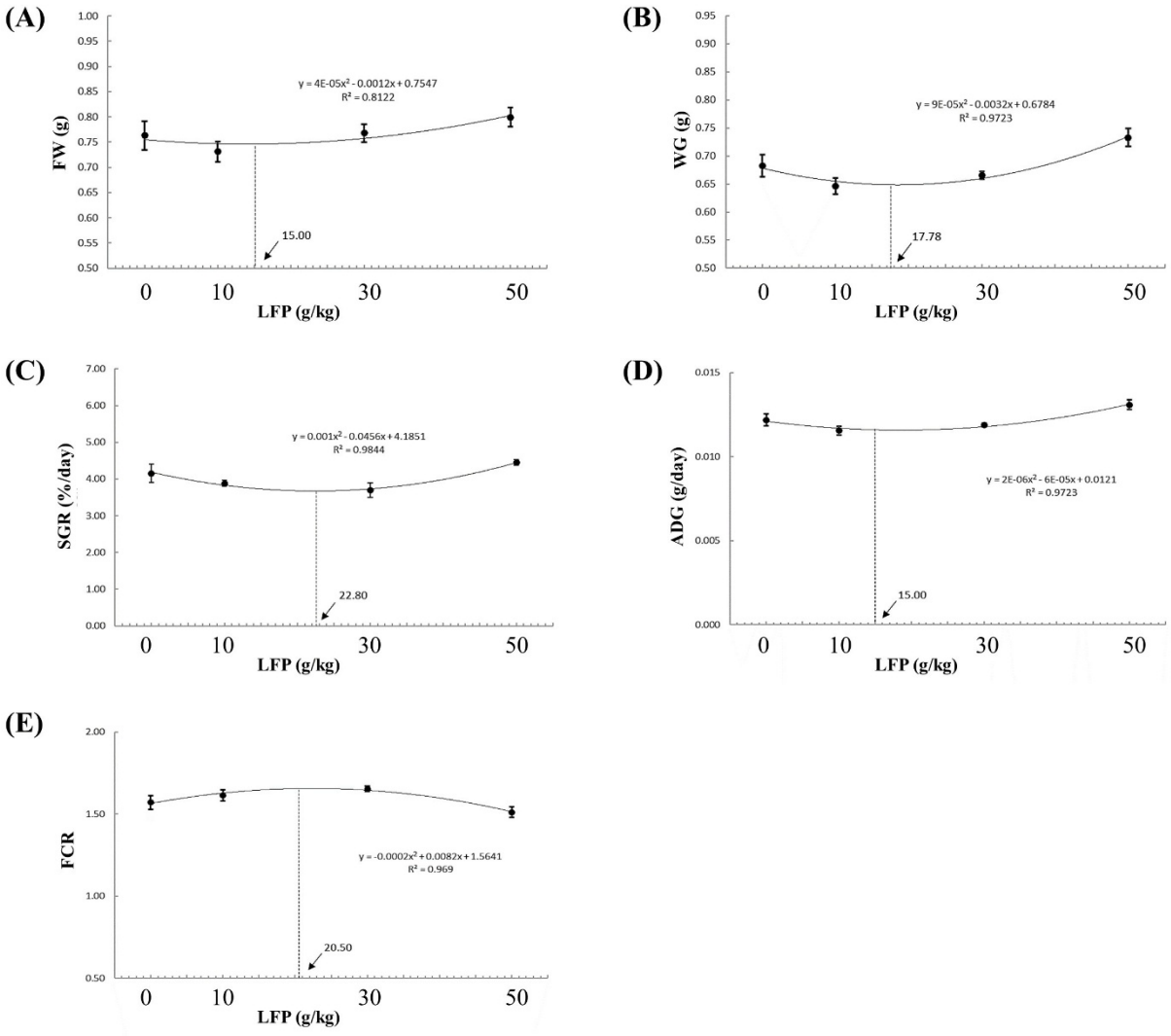

Figure 1 Quadratic second-order polynomial regression analysis of final weight (A), weight gain (B), specific growth rate (C), average daily gain (D), and feed conversion ratio (E) of *B. splendens* fed diets supplemented with Lamduan fruit powder (LFP) over an 8-week period.
Note: Data are presented as mean ± SEM.

Digestive enzyme-specific activity

Amylase, protease, and lipase specific activities were significantly increased ($p < 0.05$) in fish fed diets containing 50 g/kg LFP compared with the other groups (Figure 2).

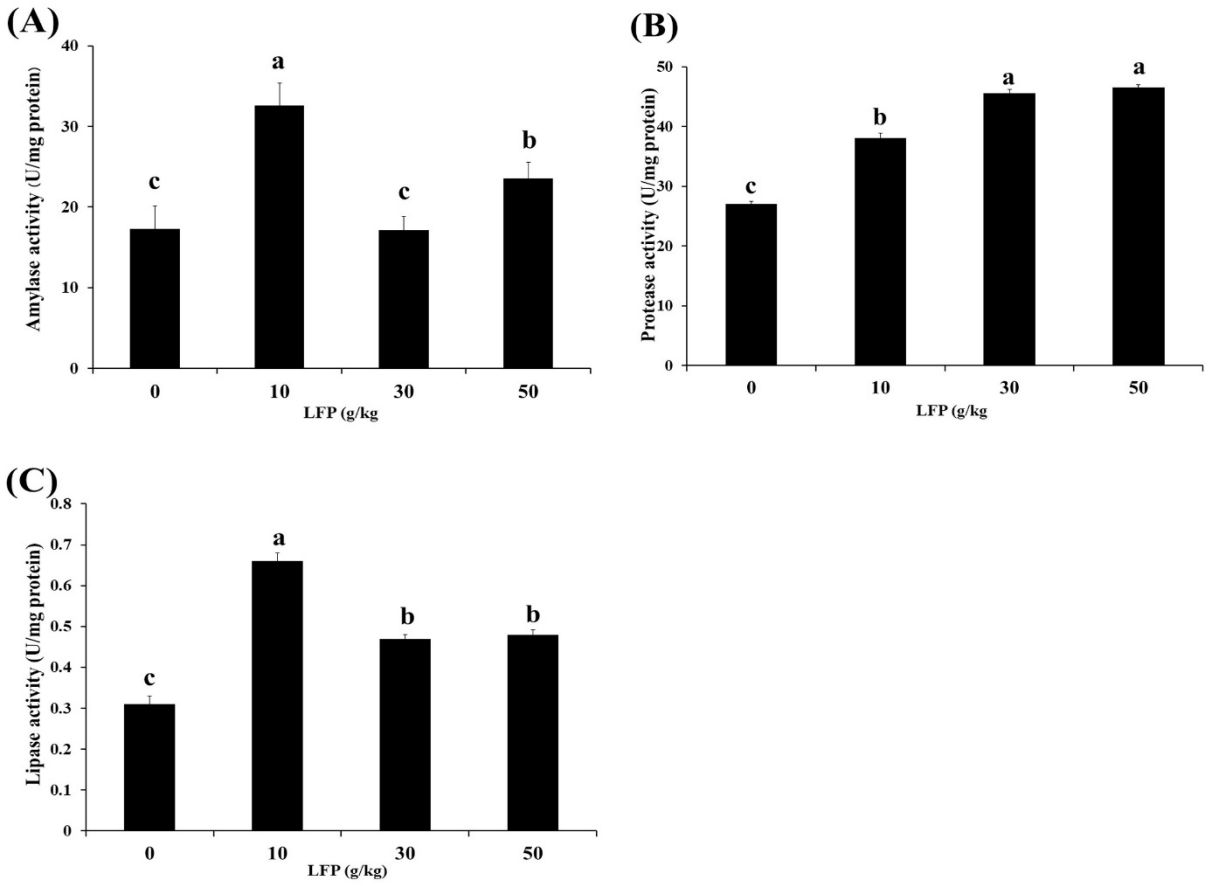

Figure 2 Amylase (A), protease (B), and lipase (C) activities in the digestive tract of *B. splendens* fed diets supplemented with Lamduan fruit powder (LFP) over an 8-week period.
Note: Different superscript letters within the same row indicate statistically significant differences among treatments at $p < 0.05$. Values are expressed as mean ± SEM. Sample size: $n = 4$ replicates per treatment, with 12 fish per treatment group.

Skin mucus immune response

As presented in Table 4, fish consuming LFP-supplemented diets exhibited notable increases in skin mucus immune parameters, including total protein, IgM, lysozyme, total antiprotease activity, MPO, ALP, and total protease levels, relative to the control group ($p < 0.05$).

Table 4 Effects of Lamduan fruit powder (LFP) supplementation on non-specific immune parameters in the skin mucus of *B. splendens*

Parameters	LFP (g/kg)			
	0	10	30	50
Total protein (mg/dl)	12.88 ± 4.10^{b}	66.28 ± 6.88^{a}	56.91 ± 7.66^{a}	49.71 ± 2.34^{a}
IgM (mg/dl)	0.10 ± 0.02^{b}	0.44 ± 0.06^{a}	0.46 ± 0.07^{a}	0.41 ± 0.09^{a}
Lysozyme (U/ml)	0.77 ± 0.01^{c}	1.98 ± 0.03^{b}	2.27 ± 0.04^{a}	2.48 ± 0.05^{a}
Total antiproteases activity (U/ml)	10.11 ± 2.34^{b}	66.50 ± 6.69^{a}	89.88 ± 2.97^{a}	71.33 ± 1.20^{a}
MPO (Abs 450 nm)	1.80 ± 0.22^{b}	2.69 ± 0.15^{a}	2.62 ± 0.12^{a}	2.55 ± 0.11^{a}
ALP (U/l)	0.44 ± 0.01^{c}	0.61 ± 0.03^{a}	0.58 ± 0.02^{a}	0.51 ± 0.06^{b}
Total protease activity (U/ml)	22.29 ± 0.55^{c}	33.98 ± 1.77^{b}	39.40 ± 0.70^{a}	39.73 ± 0.47^{a}

Note: Different superscript letters within the same row indicate statistically significant differences among treatments at $p < 0.05$. Values are expressed as mean ± SEM. Sample size: $n = 4$ replicates per treatment, with 12 fish per treatment group. IgM: immunoglobulin M (mg/dl); MPO: myeloperoxidases (Abs 450 nm); ALP: Alkaline phosphatase (U/l).

Antioxidant activity in skin mucus

The antioxidant defense profile of fish skin mucus is displayed in Figure 3. Fish receiving 10, 30 and 50 g/kg LFP diets exhibited significantly higher SOD than the control group ($p < 0.05$). Increased CAT levels were observed in fish fed diets containing 30 and 50 g/kg LFP compared with the control and 10 g/kg LFP groups. All LFP-treated groups exhibited markedly lower MDA levels compared to the control. Additionally, antioxidant capacities measured by DPPH, ABTS, and T-AOC assays were significantly greater in the mucus of LFP-fed fish than in the control.

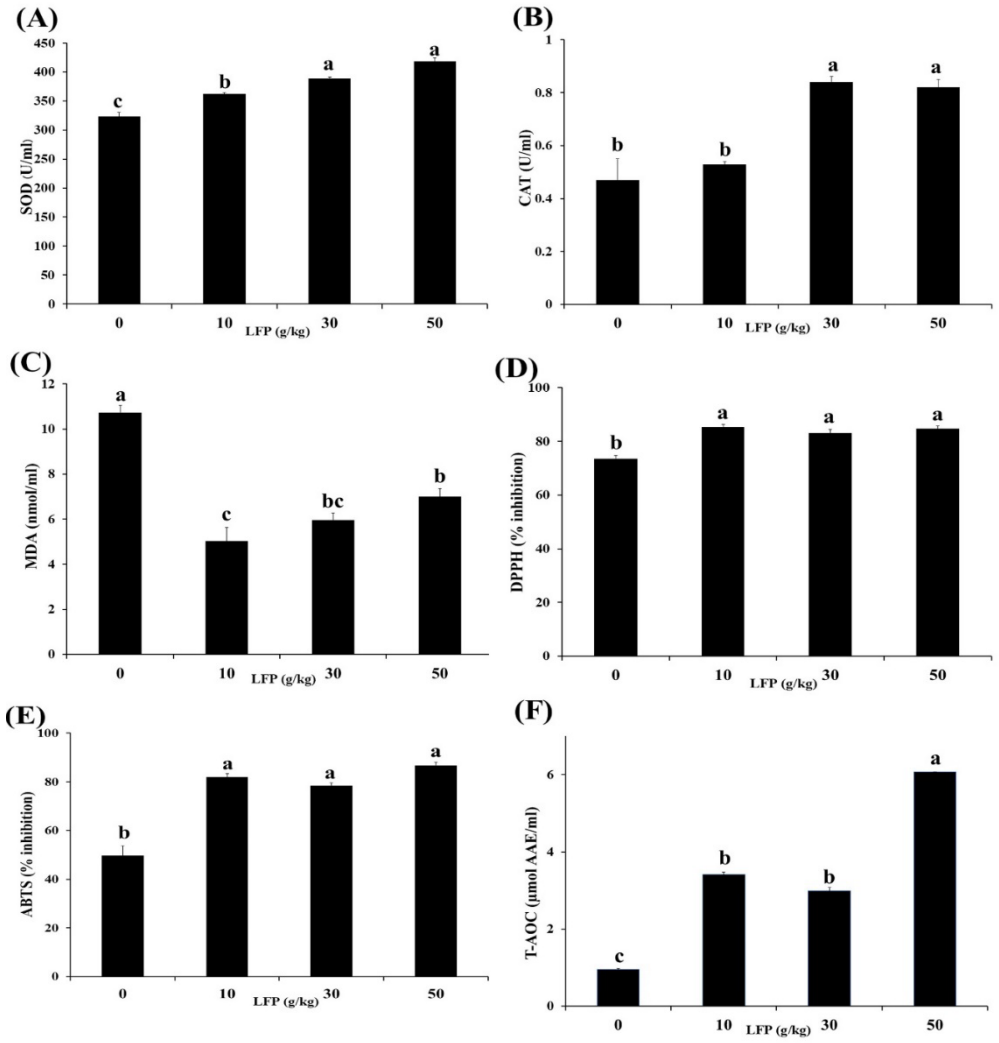

Figure 3 Superoxide dismutase (A), catalase (B), malondialdehyde (C), DPPH radical scavenging activity (D), ABTS radical scavenging activity (E), and total antioxidant capacity (F) in the skin mucus of B. splendens fed diets supplemented with Lamduan fruit powder (LFP) over an 8-week period.
Note: Different superscript letters within the same row indicate statistically significant differences among treatments at $p < 0.05$. Values are expressed as mean ± SEM. Sample size: $n = 4$ replicates per treatment, with 12 fish per treatment group.

Carotenoid deposition in skin and muscle

Table 5 details the effect of dietary LFP on carotenoid levels in *B. splendens*. The fish receiving LFP at the dose of 30 g/kg diet exhibited significantly higher carotenoid concentrations in both the skin and muscle compared to the control

group ($p < 0.05$). However, the supplementation of LFP at 50 g/kg resulted in a slight reduction in carotenoid content, which was not statistically significant ($p > 0.05$). As illustrated in Figure 4, quadratic second-order polynomial regression determined the optimal LFP inclusion for enhanced pigmentation to be within 27.18–28.58 g/kg.

Table 5 Effects of Lamduan fruit powder (LFP) supplementation on carotenoid content (μg/g tissue) in *B. splendens*

Parameters	LFP (g/kg)			
	0	10	30	50
Skin	223.53 ± 24.73^{b}	367.91 ± 48.08^{a}	419.25 ± 44.59^{a}	318.71 ± 32.15^{ab}
Muscle	195.38 ± 44.93^{b}	291.25 ± 32.97a^{ab}	345.96 ± 63.71^{a}	256.01 ± 19.37^{ab}

Note: Different superscript letters within the same row indicate statistically significant differences among treatments at $p < 0.05$. Values are expressed as mean ± SEM. Sample size: $n = 4$ replicates per treatment, with 12 fish per treatment group.

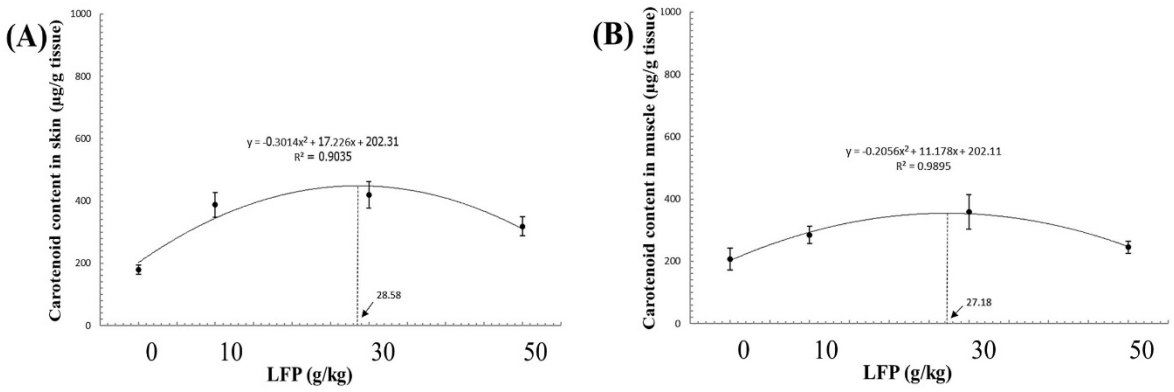

Figure 4 Quadratic second-order polynomial regression analysis of the carotenoid contents in the skin (A) and muscle (B) of *B. splendens* fed LFP-supplemented diets over an 8-week period. Note: Values are expressed as mean ± SEM. Sample size: $n = 4$ replicates per treatment, with 12 fish per treatment group.

DISCUSSION

The results of this study demonstrated that dietary supplementation with LFP effectively improved growth performance, digestive enzyme-specific activities, mucosal immune responses, antioxidant capacity, and pigmentation in *B. splendens*. These findings indicate that LFP acts as a functional feed additive capable of promoting both physiological performance and health status in ornamental fish. The observed improvements are attributed to the diverse phytochemicals present in LFP, including phenolics, flavonoids, terpenoids, tannins, anthocyanins, and β-carotene, which may synergistically enhance metabolism and immunity. These findings highlight the potential of LFP as a promising functional feed additive for ornamental fish.

Phytochemical composition and antioxidant capacity of LFP

Numerous bioactive substances and pigments found in plants have been shown to promote the growth and well-being of ornamental fish (Giri et al., 2024). The antioxidative capabilities of such botanicals can defend fish against oxidative stress, inflammation, and associated pathologies (Karataş, 2024). Prior studies

have reported that aqueous extracts from Lamduan fruits possess high anthocyanin concentrations, measuring approximately 280.6 ± 3.42 mg cyanidin 3-glucoside equivalent/g dry weight (Sakulnarmrat and Konczak, 2022). Importantly, eight bioactive compounds have been isolated from Lamduan flowers, including 1-hexacosanol, β-sitosterol, and melodorinone B (Mongkol et al., 2016). In the present investigation, LFP was found to contain phenolics, flavonoids, terpenoids, tannins, carotenoids, β-carotene, and anthocyanins, all of which may enhance growth, appetite stimulation, immunity, and physiological functions in fish (Promprom et al., 2024a; Ponsin et al., 2025). Due to their pigmentation-promoting properties, carotenoids and anthocyanins in LFP have the potential to serve as natural replacements for artificial colorants in aquafeeds (Tran et al., 2025; Pereira da Costa and Campos Miranda-Filho, 2019). Additionally, antioxidant assays such as DPPH suggest that LFP supplementation may help strengthen health and mitigate stress-related disorders in cultured fish (Wangkahart et al., 2022; Karataş, 2024). It is well documented that the phytochemical profile and bioactivity of botanicals can vary depending on the plant part, developmental stage, environmental conditions, and extraction methods (Mongkol et al., 2016; Sakulnarmrat and Konczak, 2022). These results suggest that LFP may be used in ornamental aquaculture as a multipurpose phytogenic feed addition.

Growth performance, feed utilization, and survival

As shown by the results, the highest growth indices and lowest FCR observed in the group fed 50 g/kg LFP confirm that LFP enhances feed efficiency and nutrient utilization in *B. splendens*. This aligns with previous studies reporting that fruit-derived phytogenic additives improve growth by stimulating digestive enzyme activity and promoting intestinal health (Ponsin et al., 2025). Although the regression model predicted an optimal inclusion level around 15 – 23 g/kg, the best practical response was achieved at 50 g/kg, suggesting that the biological benefits of LFP may extend beyond the modeled range without adverse effects. Interest in using medicinal plants in aquaculture has increased, primarily due to their roles in promoting growth and supporting immune function (Ramezani et al., 2021). Such beneficial effects are largely attributed to the presence of phenolics, flavonoids, tannins, and terpenoids, which act through mechanisms including improved feed palatability, nutrient digestibility, antioxidant enhancement, microbial modulation in the gut, immune stimulation, gene regulation, and disease prevention (Awad and Awaad, 2017; Ramezani et al., 2021; Wangkahart et al., 2022). A lower FCR indicates better feed efficiency, whereas feed consumption represents the total quantity of feed ingested by fish. In this study, FCR values decreased significantly with increasing LFP inclusion levels, indicating improved feed utilization, while total feed intake remained relatively stable among treatments. Given the observed phytochemical richness and antioxidant activities of LFP, its use may support both growth performance and feed efficiency. Further molecular investigations are warranted to elucidate the specific gene expression pathways associated with LFP-mediated growth enhancement in *B. splendens*.

Digestive enzyme-specific activity

The specific activity of digestive enzymes plays a vital role in determining both the growth efficiency and feed utilization in aquatic species. In the current research, the enhancement of digestive enzyme-specific activities in fish fed LFP-supplemented diets suggests that LFP promotes nutrient utilization efficiency in *B. splendens*. The significantly higher amylase, protease, and lipase activities observed in the 50 g/kg group, compared with the control, indicate improved carbohydrate, protein, and lipid digestion, respectively. These results agree with previous findings in other fish species, where phytogenic compounds were found to stimulate digestive function by increasing enzyme secretion and intestinal absorption efficiency (Thongprajukaew et al., 2011). The presence of flavonoids

and terpenoids in LFP may play a key role in modulating digestive physiology and gut health (Ramezani et al., 2021; Wangkahart et al., 2022; Ghafarifarsani et al., 2023; Karataş, 2024). In contrast, the 10 and 30 g/kg inclusion levels did not produce a significant change, suggesting that a threshold concentration is required for observable enzymatic enhancement. Although enzyme-specific activities increased with higher LFP supplementation (50 g/kg), these biochemical enhancements did not correspond linearly with FCR values. This discrepancy may be attributed to metabolic compensation, where enzyme activation exceeds the actual requirement for digestion once nutrient assimilation reaches an optimal level (Quintino-Rivera et al., 2023). Furthermore, enzyme activity can be modulated by the presence of phytochemicals, even when feed utilization efficiency remains stable (Promprom et al., 2024a). Similar findings have been reported in studies where increases in digestive enzyme activity did not directly translate to proportional improvements in feed efficiency (Promprom et al., 2024a). Thus, the improved enzyme activities observed here suggest enhanced digestive potential, while the FCR reflects the overall balance between nutrient intake, assimilation, and growth.

Immune response of skin mucus

Fish skin mucus is a vital component of innate immunity, containing diverse bioactive molecules such as proteins, immunoglobulins, enzymes, and antimicrobial factors that protect against pathogens and environmental stressors (Awad and Awaad, 2017; Reverter et al., 2018; Harikrishnan et al., 2021). In this study, dietary supplementation with LFP significantly enhanced key mucus immune parameters, including total protein, IgM, lysozyme, antiprotease, MPO, ALP, and protease activity. These improvements are consistent with previous studies reporting that plant-derived supplements can modulate innate immune responses by influencing cytokine expression and enhancing antimicrobial defenses (Wangkahart et al., 2022; Giri et al., 2024; Karataş, 2024). The observed effects are likely attributable to phytochemicals in LFP, such as flavonoids, phenolics, and terpenoids, which are known to exert immunomodulatory activities (Awad and Awaad, 2017). Overall, the findings indicate that LFP can strengthen mucosal immunity in *B. splendens*, thereby improving disease resilience and health status. Further studies focusing on the molecular mechanisms, particularly the gene expression pathways influenced by LFP, are recommended to confirm these immunological benefits.

Antioxidant activity in skin mucus

The antioxidant defense mechanism in fish comprises two integral components: enzymatic and non-enzymatic systems. The enzymatic defense includes key enzymes such as SOD, CAT, and glutathione peroxidase (GPx), while the non-enzymatic system involves various antioxidant compounds including carotenoids, flavonoids, amino acids, and vitamins C and E. Notably, SOD and CAT play crucial roles in controlling oxidative stress by neutralizing reactive oxygen species and supporting innate immune function (Li et al., 2018). SOD facilitates the dismutation of superoxide anions (O_2^-) into hydrogen peroxide (H_2O_2), which is then broken down into water and oxygen by CAT. The presence of MDA is widely utilized as a biomarker for lipid peroxidation and oxidative cellular damage. It is documented that T-AOC serves as a comprehensive indicator of antioxidant status, reflecting the combined activity of enzymatic and non-enzymatic components (Li et al., 2018). In addition, DPPH and ABTS methods are commonly performed to assess antioxidant capacity because of their reliability, rapidity, and ease of use (Phrompanya et al., 2023). In this study, dietary inclusion of LFP at 30 and 50 g/kg markedly enhanced the activities of SOD and CAT. The T-AOC level was significantly higher in fish fed the 50 g/kg LFP diet compared to the other groups. Moreover, fish receiving LFP-supplemented diets exhibited improved skin mucus radical scavenging capacity, as evidenced by the DPPH and ABTS assays.

Conversely, MDA concentrations were noticeably reduced in fish receiving LFP-enriched diets, suggesting decreased lipid peroxidation. Thus, these findings support the potent antioxidant capacity of LFP. Similar results have been reported in previous research, where dietary supplementation with karonda fruit powder significantly was found to significantly elevate SOD, CAT, and T-AOC levels in the skin mucus of *B. splendens* (Ponsin et al., 2025). Also, the inclusion of *Aegle marmelos* extract in the diet was found to enhance antioxidant enzyme activities in Nile tilapia (*Oreochromis niloticus*) (Wangkahart et al., 2024). Moreover, *Salvadora persica* (miswak) elevated SOD, CAT, and GPx, while significantly lowering MDA concentrations in Nile tilapia (Abd El-latif et al., 2021). The increased SOD and CAT activities observed may result from phytochemicals in LFP, such as flavonoids and carotenoids, which enhance antioxidant systems through the regulation of gene expression and molecular signaling (Assar et al., 2023; Mathew et al., 2025). Furthermore, the elevated T-AOC levels imply effective modulation of the non-enzymatic antioxidant system by these plant-derived compounds (Li et al., 2018). In order to neutralize free radicals, several of these phytochemicals can transfer electrons or hydrogen atoms due to structural characteristics, including aromatic rings and conjugated double bonds (Muscolo et al., 2024). This electron donation stabilizes reactive species, thereby reducing oxidative stress and cellular damage (Li et al., 2018). Nonetheless, additional investigations are necessary to elucidate the molecular mechanisms through which LFP regulates the antioxidant defense pathways in fish.

Carotenoid deposition in skin and muscle

In ornamental fish, external traits such as skin coloration, fin configuration, tail morphology, and body dimensions are widely recognized as key indicators of aesthetic quality and economic value (Tran et al., 2025). Among these, the intensity and distribution of skin pigmentation are primarily regulated by genetic factors and the intake of dietary pigments (Gomes et al., 2002; Tang et al., 2022). As a result, ornamental fish maintained under intensive aquaculture or in controlled environments often require dietary supplementation with color-enhancing pigments to achieve desirable coloration (Gomes et al., 2002). The use of natural pigment sources in aquafeeds is considered an eco-friendly and cost-effective strategy that can support the sustainable production of ornamental fish while minimizing adverse health impacts (Pereira da Costa and Campos Miranda-Filho, 2019; Tran et al., 2025). In this study, the inclusion of LFP in the diet resulted in a marked elevation of carotenoid levels in both the skin and muscle tissues of *B. splendens* relative to the control group. A second-order polynomial regression model identified the optimal LFP inclusion level for enhancing carotenoid pigmentation at approximately 27 – 29 g/kg of feed. Notably, supplementation exceeding 30 g/kg did not yield additional improvements in pigmentation. It is hypothesized that excessive LFP levels (e.g., 50 g/kg) may impair carotenoid uptake across the intestinal epithelium or redirect pigment deposition to non-target tissues, thereby reducing visible carotenoid levels in the skin and muscle (Mukherjee et al., 2009; Fawzy et al., 2022; Sathyaruban et al., 2024). Mukherjee et al. (2009) observed that the optimal inclusion rate of turmeric in fantail guppy (*Poecilia reticulata*) diets was 0.8 g/kg of feed, while higher levels (1–2 g/kg) resulted in a decline in astaxanthin content. Similarly, Sathyaruban et al. (2024) found that dietary inclusion of palmyrah fruit pulp above 10 g/kg failed to enhance skin pigmentation in guppies and was associated with reduced color intensity. Additionally, evidence suggests that the supplementation of natural plant-based supplements can upregulate the genes involved in pigment synthesis and accumulation in red tilapia (*Oreochromis* spp.), supporting carotenoid deposition in various tissues (Judan Cruz et al., 2021). Multiple factors affect carotenoid accumulation in fish, including pigment source, metabolism, age, sex, reproductive and health status, as well as environmental aspects like light intensity and water quality (Miranda-Filho, 2019; Luo et al., 2021; Pereira da Costa and Campos Tran

et al., 2025). It was found that LFP contains high levels of natural pigments, including anthocyanins, β-carotene, and carotenoids, which are likely responsible for the improved skin pigmentation seen in *B. splendens*. Despite these promising results, the precise mechanisms underlying pigment absorption, transport, and deposition in the tissues of *B. splendens* fed LFP-enriched diets remain poorly understood. Further examination is suggested to elucidate the molecular pathways involved in pigment metabolism and distribution in this species.

Practical recommendation

The incorporation of natural feed additives into ornamental fish diets has emerged as a fundamental approach to support sustainable aquaculture by promoting growth, enhancing health status, and improving pigmentation quality (Pereira da Costa and Campos Miranda-Filho, 2019; Tran et al., 2025). According to the present results, regression analysis indicated that the most effective LFP inclusion rate for enhancing growth and nutrient utilization in *B. splendens* is between 15.00 and 20.50 g/kg of diet. For effective enhancement of skin and muscle carotenoid deposition, the recommended supplementation level is slightly higher, ranging from 27.18–28.58 g/kg. However, dietary inclusion of LFP at 50 g/kg was associated with a reduction in carotenoid concentrations, suggesting that excessive supplementation may negatively influence pigment absorption and distribution. To maximize coloration, a key market trait in ornamental fish, while maintaining acceptable growth, an inclusion level around 27.18–28.58 g/kg is advisable. Although this dosage slightly exceeds the growth-optimized range, it remains within a safe margin, as no adverse effects were recorded at this level (Yanar et al., 2008). Based on these observations, it is recommended that LFP be strategically incorporated into the diet of *B. splendens* during critical phases such as the juvenile-to-adult transition, pre-stress conditions (e.g., handling, transport, or disease exposure), and before marketing or exhibition. It may be possible to improve color intensity and carotenoid deposition by administering LFP at the appropriate concentration during these periods, which would increase the aesthetic appeal and commercial value of the fish (Sathyaruban et al., 2024; Ponsin et al., 2025).

Compared to the widely used marigold (*Tagetes erecta*) flower extract, which serves as a natural carotenoid source rich in lutein and zeaxanthin (Yanar et al., 2007), Lamduan fruit powder offers a broader spectrum of bioactive compounds, including phenolics, flavonoids, terpenoids, anthocyanins, and β-carotene. These constituents not only enhance pigmentation but also support antioxidant defense and immune function in fish (Awad and Awaad, 2017; Reverter et al., 2018; Harikrishnan et al., 2021). Furthermore, Lamduan fruits are locally available in Thailand and can be processed sustainably from underutilized plant resources, making LFP a cost-effective alternative to imported marigold extracts. Therefore, the incorporation of LFP into ornamental fish diets represents a multifunctional strategy that promotes coloration, health, and sustainability in aquaculture feed development.

CONCLUSIONS

This study demonstrates that dietary supplementation with Lamduan fruit powder (LFP) significantly enhances multiple biological parameters in *B. splendens*, including growth performance, digestive enzyme activities, skin mucus immunity, and pigmentation. The optimal inclusion level for achieving maximum efficacy was identified within the range of 27.18–28.58 g/kg of feed According to these findings, LFP may be used as a natural feed supplement to enhance the health and appearance of ornamental fish. This research provides valuable evidence supporting the sustainable application of plant-derived additives in aquaculture nutrition. Notably, this is the first report to demonstrate the aquaculture benefits of

LFP supplementation, thereby introducing a novel functional ingredient for ornamental fish production. Further investigations are warranted to evaluate its physiological influences across other ornamental and food fish species, with particular emphasis on pigmentation pathways and the expression of genes related to growth and immunological control.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Wilawan Promprom: Funding acquisition (lead); review and editing (equal); resources (supporting).
Wannachai Chatan: Review and editing (equal); data analysis (supporting); review and editing (equal).
Kajita Somnate: Data analysis (lead); formal analysis (lead); data curation (supporting); review and editing (equal).
Phukphon Munglue: Conceptualization (lead); data curation (lead); formal analysis (supporting); resources (lead); review and editing (lead); writing-review and editing (lead).

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