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Original Article

Dietary tryptophan intervention on growth and stress of Asian seabass, *Lates calcarifer* reared in recirculating aquaculture system

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Abstract
The rapid expansion of recirculating aquaculture system (RAS) aquaculture demands precise dietary strategies. Determining optimal tryptophan levels and understanding their combined effects on growth, blood responses, and stress regulation in Asian seabass remain limited. Addressing this knowledge gap, an experiment was conducted to evaluate the effects of L-tryptophan supplementation on growth, hematobiochemical responses and stress regulation of Asian seabass in RAS. Five isonitrogenous and isocaloric treatment diets were formulated to contain graded levels of L-tryptophan at 0.47 (T1), 0.53 (T2), 0.59 (T3), 0.66 (T4) and 0.72% (T4) of diet. At the end, an increase was observed in the growth of fish fed the T1 diet, after which no variation was observed. Dietary L-tryptophan had no effect on FCR across all the diet groups. Second order polynomial regression analysis of weight gain against dietary tryptophan levels indicated that the dietary tryptophan requirement for Asian seabass reared in RAS was 0.61% of the diet. No differences were observed in whole body composition and haematological responses among the treatment groups. Cholesterol and HDL-C levels followed trend similar to the growth performance. Cortisol level was significantly lowered in T1 than the control, whereas no variation was observed among other groups. Second order polynomial regression analysis of HDL-C and cortisol against dietary tryptophan levels showed that the dietary tryptophan requirement were 0.61% and 0.62% of the diet respectively. Overall, L-tryptophan at 0.61–0.62% maximizes growth, stress responses, and tryptophan–serotonin–cholesterol axis regulation in Asian seabass in RAS culture systems, optimizing performance.

Keywords: Asian seabass; L-tryptophan; recirculating aquaculture system; serotonin–cholesterol pathway; stress physiology

1 | INTRODUCTION

Cannibalism is an intra-specific predatory behavior influenced by environmental factors (structure, temperature, and light intensity) and population factors (density and

size dimorphism) (Kestemont *et al*. 2003). Food availability influences the extent of cannibalism. For instance, in carnivorous fish, a scarcity of food leads to a notable rise in cannibalism among young fish and fry, as larger fish become more aggressive in preying on smaller ones when they are not fed regularly (Hecht and Appelbaum 1988; Hecht and Pienaar 1993; Baras and Jobling 2002; Baras 2013). High stocking density affects fish physiology and behavior in intensive culture (Ashley 2007), causing stress from social interactions (Bolasina *et al*. 2006). This stress can deplete brain serotonin, increasing aggression, cannibalism, and poor growth (Lefrançois *et al*. 2001; Mojjada *et al*. 2013; Manley *et al*. 2014; Król and Zak 2016). While size grading and frequent feeding can mitigate cannibalism, these methods increase stress and reduce water quality (Pham *et al*. 2020). Dietary intervention offers an alternative strategy for controlling cannibalistic behavior (Król and Zak 2016; Kumar *et al*. 2017) in intensive systems, such as recirculating aquaculture systems (RAS).

Asian seabass (*Lates calcarifer*), which tolerates salinity from freshwater to seawater (Plaiptech *et al*. 2014; Chaklader *et al*. 2019), is ideal for intensified culture. The fish grows to 1.0 kg in 6–8 months using pellet feed (Yazid *et al*. 2021). Hatchery-produced seed and formulated pellet feed ensure a year-round supply, enabling easy adoption by farmers. However, under such conditions, stress and aggressive behavior, including cannibalism, can negatively affect growth and survival. These responses are closely associated with serotonergic activity, highlighting the importance of dietary tryptophan as a potential nutritional strategy to modulate stress and improve performance (Ashley 2007; Baras 2013). This behavior is linked to decreased serotonin levels. Serotonin (5-hydroxytryptamine, 5-HT) stabilizes mood, relieves stress, and reduces aggression (Hseu *et al*. 2003; Höglund *et al*. 2005; Krol and Zak 2016). Dietary supplementation with tryptophan, serotonin's precursor, can increase serotonin levels (Kumar *et al*. 2017; Höglund *et al*. 2019). The dietary tryptophan requirement for teleosts ranges from 0.3% to 1.3% of dietary protein (Hoseini *et al*. 2019). Tryptophan converts to serotonin and enters the kynurenic pathway under stress (reducing serotonin availability and elevating cortisol) while favoring serotonin synthesis that suppresses HPI-axis activity and stabilizes HDL-C, as evidenced by our cortisol and lipid results (Höglund *et al*. 2019). Under stress, the kynurenic pathway is activated while suppressing serotonin production, reducing brain serotonin levels, and increasing other tryptophan-based substances. The dietary tryptophan requirement for juvenile Asian seabass has previously been reported as 0.41% for normal growth (Coloso *et al*. 2004). However, such estimates were derived under conventional rearing conditions and may not account for stress associated with intensive culture systems such as recirculating aquaculture systems (RAS), where higher stocking densities and environmental fluctuations can alter amino acid requirements. Therefore, re-evaluation of tryptophan requirements under RAS conditions is essential, particularly considering its role in stress modulation and serotonergic activity. Tryptophan supplementation in feed enhances brain serotonergic activity, improving stress resistance at higher stocking densities and reducing aggression (Johnston *et al*. 1990; Winberg *et al*. 2001; Lepage *et al*. 2002).

Despite these insights, the optimal dietary levels of tryptophan for reducing cannibalism and stress in high-density RAS for carnivorous species like Asian seabass remain largely unexplored, especially considering its specific serotonin and cortisol dynamics under intensified culture. Cortisol acts as a crucial stress marker in fish, being the primary corticosteroid present in their circulation (Mommsen *et al*. 1999). It is synthesized by the inter renal cells located in the head kidney and subsequently enters the bloodstream (Gamperl *et al*. 1994; Sadoul and Geffroy 2019), with its plasma concentration significantly increasing during periods of stress (Mommsen *et al*. 1999). Studies have shown that tryptophan supplementation suppresses plasma cortisol levels (Tejpal *et al*. 2009; Akhtar *et al*. 2013; Kumar *et al*. 2014; Morandini *et al*. 2015). Morandini *et al*. (2015) found suppressed cortisol and increased brain serotonergic activity after 4 weeks of tryptophan in *Cichlasoma dimerus*. Studies by Tejpal *et al*. (2009), Akhtar *et al*. (2013) and Kumar *et al*. (2014) showed that tryptophan administration under stressful conditions suppressed cortisol responses and improved growth in *Cirrhinus mrigala* and *Labeo rohita*, indicating higher tryptophan needs for maximum growth under stress. Given the rising demand for these species along with the substantial growth of intensive systems, limiting the stress induced by intensified culture through dietary interventions is warranted. Therefore, the present study aimed to determine the optimal dietary tryptophan level for Asian seabass reared in a RAS, with specific reference to growth performance, feed utilization, haemato-biochemical responses, and stress indicators such as cortisol and HDL-C levels.

2 | METHODOLOGY

2.1 Preparation of experimental diets

Five treatment diets, each with the same protein content (43% DP), lipid content (12%), and caloric value (19 MJ kg^{-1}), were prepared with varying amounts of L-tryptophan at 0.0, 0.07, 0.14, 0.21, and 0.28% of the diet. This resulted in tryptophan concentrations of 0.47, 0.53, 0.59, 0.66, and 0.72% in the diets, which were named T1, T2, T3, T4, and T5, respectively (Table 1). The diets were prepared in the Aquafeed extrusion mill, Directorate of Incubation and Vocational Training in Aquaculture (DIVA), Tamil Nadu Dr J. Jayalalithaa Fisheries University (TNJFU), Chennai, India. The ingredients were individually ground and sieved using a 100-micron mesh. The ingredients were

mixed using a horizontal mixer (Jinan Sunpring Machinery Co. Ltd.), as per the formulation, at 960 rpm for 5 min, along with the required amount of water. Slow-sinking 2 mm pellets were then processed using a twin-screw extruder (Jinan Sunpring Machinery Co. Ltd.), maintaining the steam at five bars and a screw temperature of 90–105°C. Pellets were air-dried, vacuum-coated with the required levels of tryptophan in a vacuum coater, and stored in airtight containers. The proximate and amino acid compositions of the experimental diets are shown in Table 2.

TABLE 1 Ingredient composition of the experimental diets (g / 100g of diet).

Ingredients	Dietary inclusion level				
	T1	T2	T3	T4	T5
Fishmeal	40.00	40.00	40.00	40.00	40.00
Squid meal	3.00	3.00	3.00	3.00	3.00
Soybean meal	21.00	21.00	21.00	21.00	21.00
Corn gluten	12.00	12.00	12.00	12.00	12.00
Wheat flour	6.80	6.80	6.80	6.80	6.80
Broken rice	5.50	5.44	5.36	5.29	5.22
Fish oil	6.00	6.00	6.00	6.00	6.00
Soy lecithin	2.00	2.00	2.00	2.00	2.00
Vitamin premix	1.00	1.00	1.00	1.00	1.00
Mineral premix	1.00	1.00	1.00	1.00	1.00
Vitamin C	0.20	0.20	0.20	0.20	0.20
DCP	0.20	0.20	0.20	0.20	0.20
DL-methionine	0.30	0.30	0.30	0.30	0.30
Binder	0.50	0.50	0.50	0.50	0.50
Chromic oxide	0.50	0.50	0.50	0.50	0.50
L-Tryptophan	0.00	0.07	0.14	0.21	0.28

2.2 Experimental fish and system

A total of 2500 Asian seabass fry, each weighing 0.75±0.1 g, were acquired from the Rajiv Gandhi Centre for Aquaculture (RGCA), Sirkazhi, Tamil Nadu, India, and reared for one month at DIVA, Chennai, India. Fish were initially fed with 0.8 mm sinking commercial feed (520 g kg^{-1} protein, 120 g kg^{-1} lipid; Uni-President Vietnam Co. Ltd., Vietnam) at the time of rearing and gradually acclimatized to 2.0 mm commercial feed pellets (470 g kg^{-1} protein, 100 g kg^{-1} lipid; Uni-President Vietnam Co. Ltd, Vietnam).

A total of 15 experimental tanks (500 L capacity) were used which comprising five dietary treatments with three replicates per treatment (n = 3). Each tank was stocked with 50 fish (initial weight: 5.14±0.10 g) and considered as an independent experimental unit for all growth and biochemical analysis. The replicate tanks corresponding to each dietary treatment were connected to a dedicated common filtration system, resulting in five independent recirculatory aquaculture system (RAS) units (one per treatment). Thus, water was recirculated only among the three replicate tanks within each treatment, with no water exchange between treatments.

Water pumped from the creek (25±3 ppt) to the reservoir tank, adjusted for salinity with freshwater, and chlorinated with 33% active chlorine, served as the source for the experiment. Before use, the water was dechlorinated, agitated, and aerated vigorously to remove residual chlorine and stored in an overhead tank until further use. During the experiment, water loss due to evaporation and filtration discharge was compensated. Water quality parameters, such as temperature (27±1.0°C), pH (7.9±0.2), dissolved oxygen (5.5±0.5 ppm), salinity (25±1 ppt), TAN (0.32±0.2 ppm), nitrite-N (0.05±0.03 ppm), and nitrate-N (10.0±5.0 ppm), were measured daily and maintained within the optimal range throughout the experiment.

TABLE 2 Analysed nutrient composition of ingredients and experimental diets (g / 100g dry matter).

Composition	Dietary inclusion level				
	T1	T2	T3	T4	T5
Dry matter	88.88	88.79	89.31	89.02	88.94
Crude protein	47.07	47.14	47.05	47.2	47.15
Crude lipid	12.33	12.35	12.25	12.30	12.29
Ash	10.17	10.10	10.21	10.17	10.15
Crude fibre	1.90	2.09	1.92	2.10	1.84
Gross energy (MJ/kg)	21.06	21.12	21.10	21.06	21.21
Ca	1.98	1.98	1.98	1.98	1.98
P	1.33	1.33	1.33	1.33	1.33
Essential amino acids					
Arginine	3.07	3.08	3.06	3.08	3.07
Histidine	1.16	1.15	1.16	1.17	1.16
Isoleucine	1.81	1.80	1.82	1.84	1.82
Leucine	4.64	4.64	4.68	4.65	4.65
Lysine	2.89	2.86	2.87	2.89	2.90
Methionine	1.70	1.70	1.72	1.70	1.73
Phenylalanine	1.86	1.85	1.85	1.89	1.87
Threonine	1.85	1.86	1.86	1.87	1.86
Tryptophan	0.47	0.53	0.59	0.66	0.72
Valine	2.09	2.10	2.08	2.11	2.11
Nonessential amino acids					
Alanine	3.00	3.01	3.02	3.01	3.00
Aspartic acid	3.99	3.98	3.97	3.98	3.99
Cysteine	0.61	0.62	0.60	0.62	0.61
Glutamic acid	6.64	6.64	6.65	6.68	6.64
Glycine	3.02	3.05	3.04	3.02	3.03
Serine	1.85	1.82	1.85	1.86	1.84
Tyrosine	3.21	3.20	3.26	3.24	3.25

2.3 Analyses of proximate, amino acid and growth parameters

The dry matter, crude protein, crude lipid, ash, energy, and phosphorus contents of the prepared diets and whole fish bodies were estimated according to the protocols (AOAC, 1990) in the Animal Feed Analytical and Quality Assurance Laboratory (AFAQAL), Veterinary College and Research Institute, Namakkal, India. A sample of 30 Asian seabass (5.14±0.09 g) was collected at the begin-

ning of the experiment, and 10 fish were randomly sampled from each replicate tank at the end of the experiment. Samples from both stages were pooled, euthanized (MS-222) and homogenized to obtain composite samples for whole-body chemical composition analysis. The amino acid profile of the prepared diets was analyzed using ultra-high-resolution liquid chromatography (UPLC; Waters ACQUITY-UPLC, Waters, Massachusetts, USA), as described by Ishida *et al*. (1981).

At the start and end of the experiment, the fish were weighed to determine their initial and final weights, respectively. Mortality was monitored daily to determine survival and feed intake during the trial. Growth parameters and bio-indices were calculated using the following equations.

Mean weight gain (g/fish) = Final weight (g) – Initial weight (g)

Survival (%) = (Final number of fish / Initial number of fish) × 100

Feed conversion ratio (FCR) = Dry feed fed (g) / Weight gain (g)

Protein efficiency ratio (PER) = Body weight gain (g) / Protein intake (g)

Thermal-unit growth coefficient (TGC) = [Final weight (1/3) – Initial weight (1/3)] / [Σ (Water temperature × duration in days)] × 100

Condition factor (CF, g / cm^3) = (Body weight (g) / Body $length^3$ (cm)) × 100

2.4 Haemato-biochemical analyses

At the end of the experiment, blood samples were collected from each replicate tank using a 2-mL tuberculin syringe with a 26-G needle at the caudal vein of anesthetized fish. To determine the haematological parameters, the samples were immediately transferred to heparinized tubes and analyzed using an automatic haematological analyzer (Zybio Z3 Inc.). To determine the serum biochemical parameters, the samples were transferred to non-heparinized tubes and allowed to clot for 2 h at 40°C. The serum was separated by centrifugation at 3500 × g for 25 min at 4°C in a refrigerated centrifuge (Eppendorf Centrifuge 5804 R), and the values were measured photometrically using a biochemical analyzer (Cytokine SK3002B, Cytokine Healthcare Pvt Ltd.) with the respective commercial kits (Sigma-Aldrich, USA) and their accredited methodologies.

2.5 Anti-oxidative stress enzymes

SOD activity was estimated using the method described by Misra and Fridovich (1972). Liver tissue was used to analyze SOD activity. The assay was based on the oxidation of epinephrine adrenochrome transition by the enzyme. The reaction mixture consisted of 50 µL of the sample, 1.5 mL of phosphate buffer, and 0.5 mL of epinephrine. The solution was mixed well, and the change in OD at 480 nm for 2 min was observed using a UV spectrophotometer. One unit of SOD activity is the amount of protein required to provide 50% inhibition of epinephrine auto-oxidation.

CAT activity was estimated according to the method described by Takahara *et al*. (1960). Liver tissue was used to analyze CAT activity. In 2.45 mL of phosphate buffer (50 mM, pH 7.0), 50 µL of the tissue homogenate was added, and the reaction was initiated by the addition of 1.0 mL H_2O_2 solution. The decrease in absorbance was measured at 240 nm at 30-s intervals for 2 min. The enzyme blank was run simultaneously with 1.0 mL of distilled water instead of H_2O_2. CAT activity was expressed as nmoles of H_2O_2 decomposed/min/protein.

2.6 Data analysis

All collected and calculated data were analyzed using the statistical software package, Statistical Package for the Social Sciences (version 26.0; SPSS, Chicago, IL, USA), and are presented as means ± standard deviation (SD) of three replicates. All data were tested for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test). Arcsine transformation was performed for all percentage values prior to statistical analysis. The effects of dietary treatments were analyzed using one-way ANOVA with Tukey's test at a significance level of 0.05. The dietary L-tryptophan requirement was estimated by second-order polynomial regression analysis (Y = a + bX + cX2) (Prabu *et al*. 2020) based on weight gain, HDL-C and cortisol values.

3 | RESULTS

3.1 Growth and feed utilisation

The growth performance of Asian seabass fed graded levels of tryptophan is presented in Table 3. The final weight was higher ($p < 0.05$) in the T1 group than in the control diet groups, and was not affected ($p > 0.05$) in the other Tryptophan-supplemented diet groups. All diet groups recorded the highest survival, and no influence ($p > 0.05$) was observed with the inclusion of tryptophan. There was no significant difference ($p > 0.05$) in FCR, PER, and TGC among the dietary treatment groups. Second-order polynomial regression analysis of weight gain against dietary tryptophan levels showed that the dietary tryptophan requirement for Asian seabass reared in RAS was 0.61% of the diet (Figure 1).

3.2 Haemato-biochemical parameters of Asian seabass

The haematological parameters, such as hemoglobin, erythrocytes, leukocytes, MCH, MCHC, MCV, and hematocrit, and the biochemical parameters, such as total protein, albumin, and globulin, showed no significant variations with the supplementation of graded levels of tryptophan in the diets (Table 4). The HDL-C values of the different diet groups were similar to those of the results

obtained in growth, where T1 was higher than the control diet group, and was not significantly affected among the other Tryptophan-supplemented diet groups. Whereas there was an inverse result in cortisol values with control diet group with significantly higher values and not significantly affected among the Tryptophan-supplemented diet groups (Figure 2). Second-order polynomial regression analysis of HDL-C and cortisol against dietary tryptophan levels showed that the dietary tryptophan requirement for Asian seabass reared in RAS was 0.61% and 0.62% of the diet, respectively.

TABLE 3 Growth and feed utilisation of Asian seabass fed experimental diets.

Parameters	Diets					*F*-value	*p*-value
	T1	T2	T3	T4	T5		
Initial body weight (g/fish)	5.18±0.11	5.32±0.18	5.32±0.07	5.28±0.16	5.29±0.18	0.455	0.767
Final weight (g/fish)	41.20±1.04^{b}	44.71±0.82^{a}	44.68±0.62^{a}	44.57±1.32^{a}	44.35±0.46^{a}	8.474	0.003
Weight gain (g/fish)	36.01±1.14^{b}	39.39±0.98^{a}	39.36±0.68^{a}	38.62±1.02^{a}	38.39±0.54^{ab}	7.095	0.006
Survival (%)	99.33±1.16	99.33±1.16	100±0.00	99.33±1.16	99.33±1.16	0.250	0.903
Feed conversion ratio	1.17±0.03	1.13±0.04	1.15±0.03	1.19±0.01	1.16±0.03	1.980	0.174
Feed intake (g/fish)	42.09±2.38^{b}	44.60±0.57^{ab}	45.41±1.21^{ab}	45.96±0.79^{a}	44.40±0.43^{ab}	4.004	0.034
PER	1.82±0.05	1.87±0.07	1.84±0.07	1.78±0.02	1.83±0.05	1.683	0.230
Thermal-unit growth coefficient (TGC)	0.110±0.003	0.115±0.003	0.115±0.001	0.115±0.003	0.114±0.002	2.401	0.119

Values expressed as mean ± SD of three replicate tanks per treatment (n = 3). Values within a row with different superscript values indicate significant difference ($p < 0.05$) as determined by one way ANOVA followed by Tukey's test.

TABLE 4 Haemato-biochemical parameters of Asian seabass fed experimental diets.

Parameters	Diets					*F*-value	*p*-value
	T1	T2	T3	T4	T5		
Haematological parameters							
Hb (g/dl)	10.90±0.53	10.87±1.10	10.07±1.01	10.23±0.31	11.00±0.01	1.066	0.422
Leuk (1000/mm^3)	15.28±2.13	15.37±2.22	13.11±1.82	14.29±2.07	14.72±0.21	0.739	0.586
Lym	13.44±1.73	13.70±2.01	11.18±1.36	12.53±1.88	13.00±0.20	1.192	0.372
Mid	0.16±0.14	0.08±0.01	0.27±0.11	0.05±0.01	0.13±0.03	3.555	0.057
Ery (million/ mm^3)	3.51±0.16	3.53±0.22	3.14±0.30	3.30±0.085	3.57±0.044	2.941	0.076
MCV (ft)	132.90± 2.42	134.633±5.47	137.10±2.78	133.77±5.78	136.17±11.02	0.223	0.919
MCH	30.93±0.12	30.80±1.13	32.03±0.25	30.93±0.15	30.67±0.41	2.918	0.077
MCHC (pictogram)	23.23±0.49	23.53±1.50	23.37±0.38	22.30±0.53	22.10±1.52	1.245	0.353
Ht (%)	46.67±1.27	46.13±1.69	43.13±4.89	45.77±0.64	48.77±3.61	1.465	0.284
PLT	125.67±14.57	142.67±13.61	166.00±30.05	138.00±1.14	141.33±17.62	1.852	0.196
Biochemical parameters							
Total protein (mg/dl)	4.69±0.23	4.37±0.17	4.60±0.30	4.60±0.45	4.82±0.33	0.830	0.536
Albumin (g/dl)	1.24±0.19	1.28±0.27	1.28±0.15	1.36±0.10	1.41±0.04	0.551	0.703
Globulin (g/dl)	3.46±0.05	3.09±0.41	3.32±0.16	3.25±0.44	3.40±0.30	0.656	0.636
A:G ratio	35.73±4.89	42.81±15.35	38.34±2.64	42.29±6.52	41.70±2.96	0.435	0.781
CHO	117.19±6.44	125.93±5.09	127.99±1.48	126.00±4.98	126.57±6.08	2.112	0.154
HDL-C (mg/dl)	65.82±1.28^{b}	78.77±4.64^{a}	77.23±1.40^{a}	81.27±1.52^{a}	80.69±4.07^{a}	13.623	<0.001
TG (mg/dl)	75.05±2.94	71.15±2.08	72.85 ±2.98	73.01±3.44	73.54±4.64	0.534	0.714
Cortisol	12.69±0.60^{a}	10.11±0.54^{b}	9.76±0.24^{b}	9.47±0.50^{b}	9.54±0.97^{b}	14.386	0.317
ALT	26.53±1.99	29.17±3.35	27.65±0.88	26.28±1.57	26.07±0.28	1.353	0.317
AST	53.54±12.95	67.91±7.81	53.89±3.19	66.37±15.19	67.05±7.96	1.524	0.268

Values expressed as mean ± SD of three replicate tanks per treatment (n = 3). Values within a row with different superscript values indicate significant difference ($p < 0.05$) as determined by one way ANOVA followed by Tukey's test.

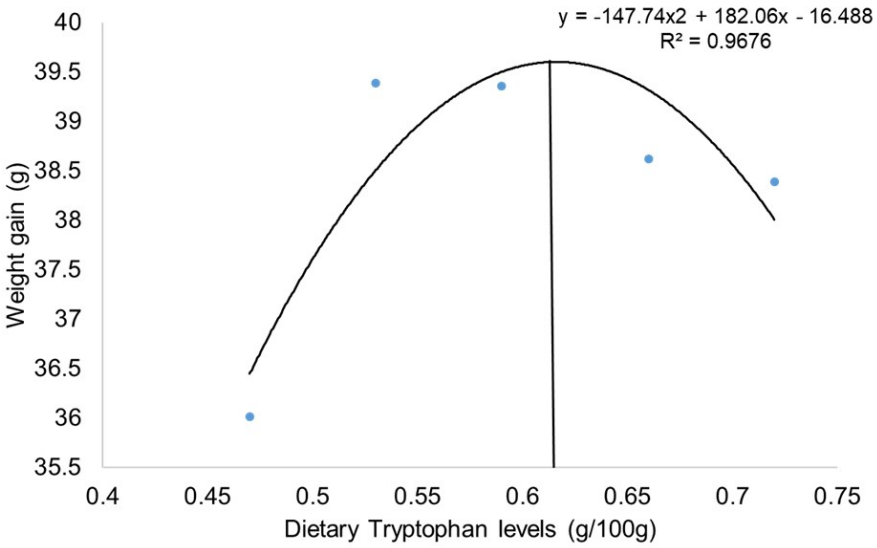

FIGURE 1 Second-order polynomial regression analysis of weight gain against graded dietary tryptophan levels.

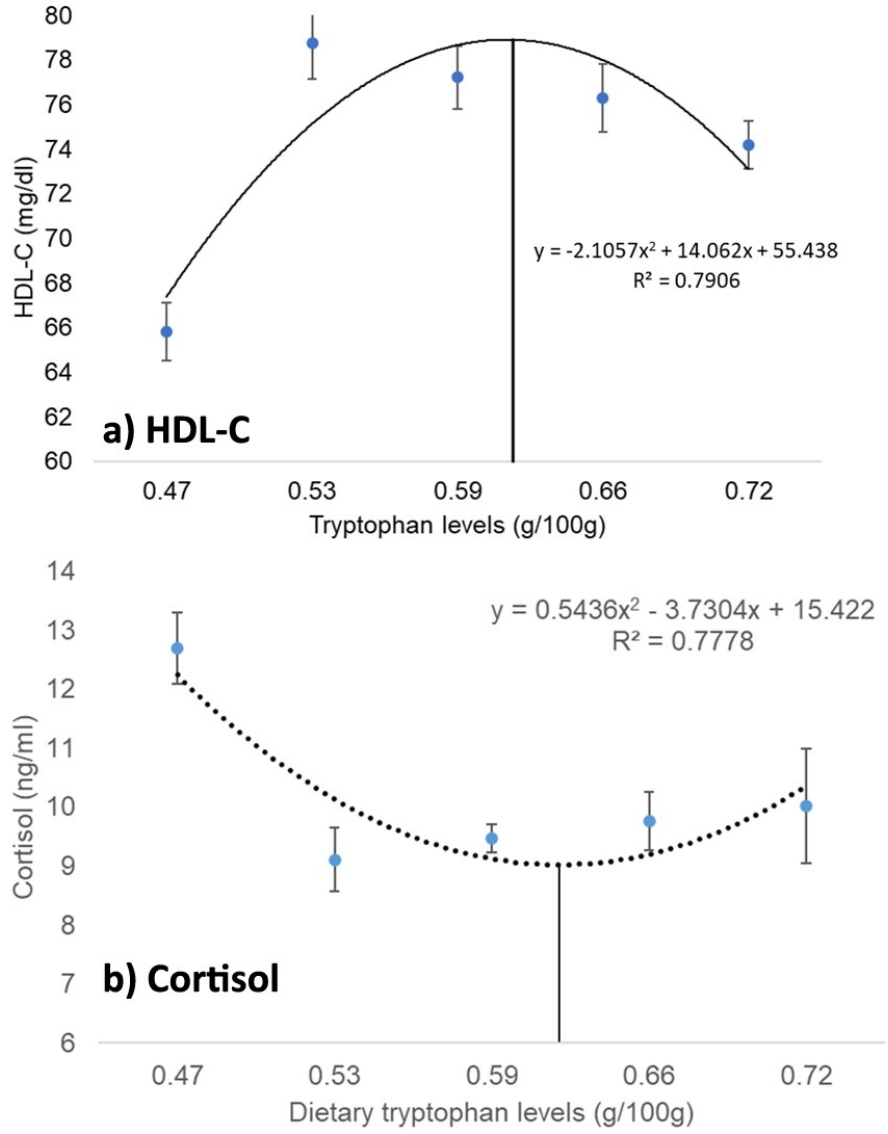

FIGURE 2 Second-order polynomial regression analysis of HDL-C (a) and cortisol (b) against graded dietary tryptophan levels.

3.3 Whole body chemical composition

The whole-body chemical composition of Asian seabass fed graded levels of tryptophan is shown in Table 5. Compositions, such as crude protein, lipid, and ash contents, were not affected by the graded levels of tryptophan in the diets of Asian seabass.

TABLE 5 Whole body chemical and amino acid composition (*g/100g wet weight basis*) of Asian seabass fed experimental diets.

Composition	Initial %	Diets					*F*-value	*p*-value
		T1	T2	T3	T4	T5		
Moisture (as is)	73.91	72.01±0.72	72.53±0.51	71.80±0.51	71.99±1.60	71.86±0.17	0.342	0.844
Crude protein	18.46	18.79±0.23	18.49±0.45	18.66±0.31	18.66±0.81	19.92±0.35	0.425	0.787
Crude lipid	4.09	4.47±0.22	4.34±0.15	4.22±0.12	4.75±0.30	4.47±0.18	2.852	0.081
Ash	2.71	2.27±0.19	2.13±0.11	2.27±0.22	2.37±0.27	2.27±0.12	0.589	0.678

Values are expressed as mean ± SD of three replicate tanks per treatment (n = 3).

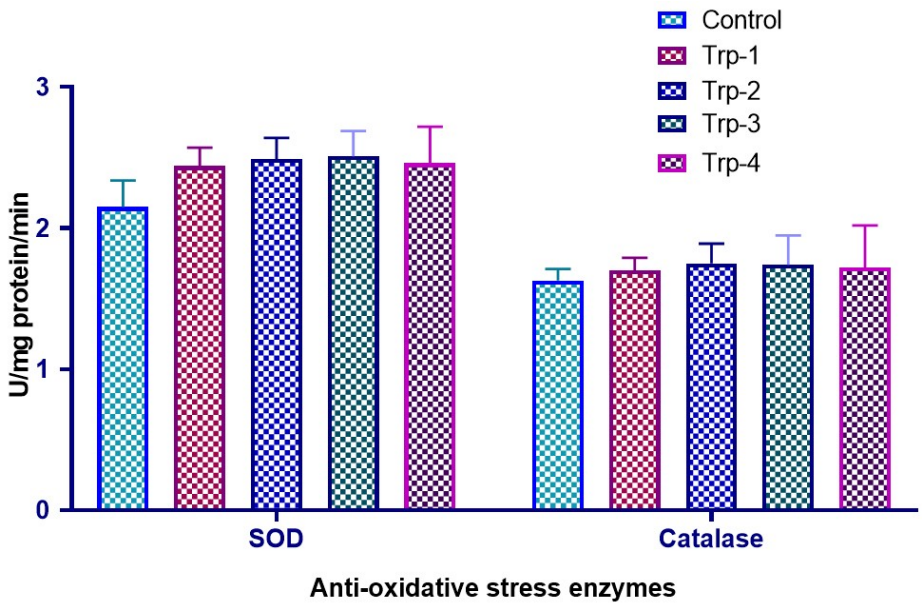

FIGURE 3 Anti-oxidative stress enzyme activities: (a) SOD and (b) catalase of Asian seabass fed graded levels of tryptophan.

3.4 Anti-oxidative stress enzyme activities

The antioxidative stress responses in Asian seabass, such as SOD and catalase, are shown in Figure 2. There were no changes in the stress enzyme activities of Asian seabass fed graded levels of tryptophan in the diets.

4 | DISCUSSION

Tryptophan, an aromatic amino acid essential for fish growth, has gained importance because of its versatility and role in protein synthesis (Hoseini *et al*. 2019). Tryptophan is considered a precursor to important indoleamines, such as serotonin (5-hydroxytryptamine, 5-HT) and melatonin (N-acetyl-5-methoxytryptamine), which positively affect fish during stress. However, it also forms a precursor for kynurenine and related compounds, such as kynurenic acid and quinolinic acid, which negatively affect fish. Because of the complexity of tryptophan metabolism, it is directly or indirectly involved in a wide array of physiological pathways.

In the present study, growth performance was higher in fish fed the T1 diet compared to the control, while no significant differences were observed among the other tryptophan-supplemented groups. This indicates that moderate supplementation of tryptophan may support growth under the present experimental conditions. A previous study on Asian seabass reported a dietary tryptophan requirement of 0.21% of the diet, corresponding to approximately 0.47% of dietary protein for optimal growth in 5 g fish (Coloso *et al*. 2004). In the present study, the optimal tryptophan level ranged between 0.53% and 0.59% of the diet, which corresponds to approximately 1.12–1.25% of dietary protein (based on 43% crude protein). Thus, when expressed on a protein basis, the tryptophan requirement observed in the present study is higher than previously reported values. This variation may be attributed to differences in dietary composition, protein levels, fish size, and culture conditions such as the recirculating aquaculture system employed in this study. The highest growth obtained in the previous study was 35.04 g in 12 weeks, whereas the control group in the present study was 41 g in 8 weeks. Similarly, another study on tryptophan requirements in Asian seabass documented that tryptophan supplementation decreased fish growth due to decreased appetite and that the level

may be around 0.19% of the diet (Chi *et al*. 2018). The differences in tryptophan requirement observed between studies could be related to variations in dietary composition, nutrient levels, fish size, and culture conditions, which have been reported to influence amino acid requirements in fish. However, as these factors were not directly investigated in the present study, this interpretation remains speculative. Therefore, the inclusion levels of essential amino acids should also be dependent on culture systems and dietary ingredients.

Studies have also mentioned that increased inclusion of tryptophan could cause a reduction in feed intake due to reduced appetite and, therefore, a reduction in growth (Hseu *et al*. 2003; Chi *et al*. 2018; Khan *et al*. 2023). Similar to the findings of previous studies, although the feed intake was not significantly different, it was reduced in T4 (0.28% supplementation), and growth was also lower. Survival was not influenced by the dietary supplementation of tryptophan and all the diet groups had higher survival percentages. Survival was not influenced by dietary tryptophan supplementation, and all treatment groups exhibited high survival rates. The high survival observed may be attributed to adequate feeding and optimal culture conditions maintained throughout the experimental period, which likely minimized aggressive interactions among fish. Similar studies with adequate feeding and reduced aggressive behavior have been observed in Atlantic salmon (Jones *et al*. 2010), gilthead seabream, and European seabass (Oikonomidou *et al*. 2019).

When fish are stressed, the brain activates the hypothalamus–pituitary–interrenal (HPI) axis. This axis produces the stress hormone cortisol in the head kidney and releases it into the bloodstream, which is a major corticosteroid in mammals and fish (Gamperl *et al*. 1994; Sadoul and Geffroy 2019). The production of cortisol is stimulated when fish are stressed due to confinement, toxicants, handling, or heat shock. In the present study, cortisol levels were higher in the control diet groups and significantly lower in the T1 diet groups; thereafter, no significant changes were observed. This indicates that the supplementation of tryptophan in the diets reduced the production of cortisol, which indirectly implies that the stress of the fish was reduced. Similar studies on tryptophan supplementation have reported reduced cortisol levels in rainbow trout (Winberg *et al*. 2001; Lepage *et al*. 2002), European sea bass (Carnevali *et al*. 2006), Mrigal (Tejpal *et al*. 2009), gilthead seabream (Castanheira *et al*. 2013), Nile tilapia (Martins *et al*. 2013), and GIFT (Prabu *et al*. 2020). The HDL-C levels in the present study were significantly enhanced in the tryptophan-supplemented diet groups than in the control diet groups. This confirms the complexity of the cholesterol–serotonin–tryptophan pathway. Low HDL-C levels may be related to structural changes and the composition of the cell membrane, which have secondary effects on neurotransmitters, such as serotonin (Muldoon *et al*. 1993). The present study corroborated the results of Aguiar and Giaquinto (2018), who observed low plasma cholesterol levels, leading to increased aggression.

5 | CONCLUSIONS

The present study demonstrates that dietary tryptophan supplementation can improve growth performance, feed utilization, physiological responses in Asian seabass under RAS conditions and also significantly modulated HDL-C and cortisol levels, indicating its role in physiological stress response. This effect may be associated with the tryptophan–serotonin pathway, where increased dietary tryptophan can enhance serotonin synthesis via tryptophan hydroxylase 2 in raphe neurons. Although serotonergic activity has been linked to reduced aggression in fish, the present study did not include direct behavioral assessments. Therefore, while the observed physiological responses suggest a potential role of tryptophan in stress modulation, its application in controlling cannibalism in intensive aquaculture systems remains speculative and requires further validation through targeted behavioral studies.

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ETHICAL APPROVAL

All applicable international, national and/or institutional guidelines for the care and use of animals were followed in this study.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

Kalidoss Manikandan: original draft, data curation, writing – review and editing; Nathan Felix: investigation, conceptualization, methodology, validation; Elangovan Prabu: supervision, methodology, visualization.

DATA AVAILABILITY STATEMENT

The data underlying the findings of this study are presented in this article.

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