Interactive Influence of Stocking Density and Dietary L-Tryptophan on Growth Performance, Physiological Responses and Cannibalism in Striped Snakehead (*Channa striata*) (Bloch, 1793)

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ABSTRACT

A 60-day experiment was conducted to investigate the interactive effects of stocking density and dietary L-tryptophan on the growth performance and physiological responses of striped snakehead (*Channa striata*) during the nursery rearing period. Advanced fry (0.32±0.293 g) were randomly stocked in 36 glass aquaria in a 3×4 factorial design, with three stocking densities (375, 500, and 625 fish·m^{-3}) and four dietary L-tryptophan levels (0%, 1%, 2%, 3%). Each treatment was triplicated. Results showed that fry reared at 375 fish·m^{-3} and fed 3% L-tryptophan had significantly higher weight gain (4.67±0.04 g), specific growth rate (4.68±0.09%·day^{-1}), and feed efficiency ratio (1.33±0.02), as well as lower feed conversion ratio (0.75±0.02) and cannibalism (26.7±0.30%) than other groups. This treatment also produced higher RBC (4.07±0.05), Hb (9.76±0.25), Ht (35.1±0.32) and greater digestive enzyme activities, including protease, lipase, and amylase. Higher respiratory burst activity (0.41±0.01) was recorded at 375 fish·m^{-3} with 0% L-tryptophan, while highest antioxidant enzyme activities, catalase and superoxide dismutase, were observed at 625 fish·m^{-3} with 3% L-tryptophan. Survival rate (69.93±1.11%) was observed at 375 fish·m^{-3} with 3% L-tryptophan. No abnormalities were observed in the intestine or liver of L-tryptophan fed groups, whereas severe hepatocyte vacuolations were observed in control. Therefore, 375 fish·m^{-3} with 3% L-tryptophan is the ideal combination for improving growth, health performance and survival while reducing stress and cannibalism.

Keywords: Cannibalism, *Channa striata*, L-tryptophan, Stocking density, Stress

INTRODUCTION

Global aquaculture is acknowledged as the fastest-growing food production industry, accounting for more than 50% of global fish consumption (FAO, 2024). Improving food and nutritional security is crucial, especially in Asia, where the rising demand for high-protein fish as food has driven the rapid expansion of both intensive and semi-intensive farming systems.

Among the freshwater species, snakehead (*Channa* spp.) have emerged as hardy and economically valuable candidate for aquaculture, owing to their strong market demand, excellent flesh quality, and remarkable tolerance to low dissolved oxygen and suboptimal water conditions (Laxmappa and Babu, 2014). The striped snakehead is widely cultured across tropical Asia and has gained more attention as a promising species for commercial culture among snakehead species.

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Despite its potential, large-scale farming of striped snakehead (*Channa striata*) is severely constrained by intense cannibalism during the early stages of development. This aggressive behaviour often results in substantial mortality, uneven growth, and reduced economic returns, ultimately limiting the efficiency of nursery and grow-out operations (Smith and Reay, 1991). Cannibalism in carnivorous fishes is caused by a variety of factors, including size heterogeneity, dominance hierarchies, insufficient feeding, and environmental stress. In African catfish (*Clarias gariepinus*) and European perch (*Perca fluviatilis*), intraspecific aggression has resulted in significant losses during the larval and juvenile stages (Krol and Zieliński, 2015).

Several methods have been explored to control behavioural abnormalities in aquaculture species, yet nutritional supplementation is considered one of the most effective strategies. Among functional amino acids, L-tryptophan has gained significant attention as it is the precursor of serotonin, a neurotransmitter that regulates stress, aggression, and feeding behaviour in farmed fish. A diet rich in L-tryptophan has the potential to significantly improve the welfare and performance of farmed fish since serotonin is a crucial neurotransmitter that regulates stress responses, aggressiveness, and social interactions. Lepage *et al*. (2002). Dietary supplementation of L-tryptophan has been shown to increase brain serotonin concentrations, thereby reducing aggression and improving growth and welfare in several species, including brown trout (*Salmo trutta*) (Höglund *et al*., 2007) and rainbow trout (*Oncorhynchus mykiss*) (Lepage *et al*., 2002).

In addition to nutrition, practices such as stocking density play a critical role in behavioural expression. High stocking density often intensifies stress and competition for resources, while excessively low densities may create opportunities for dominant individuals to prey on smaller conspecifics (Montero *et al*., 2001). Although both L-tryptophan supplementation and stocking density have been independently studied in rainbow trout (Walton *et al*., 1984), Asian seabass (*Lates calcarifer*) (Kumar *et al*., 2017), rohu (*Labeo rohita*) (Kumar *et al*., 2018), Nile tilapia (*Oreochromis niloticus*) (Vieira *et al*., 2021), and *Wallago attu* (Vo *et al*., 2022), their combined influence has not been adequately evaluated in striped snakehead.

This demonstrates a glaring research vacuum and emphasizes the necessity for coordinated management to address cannibalism in striped snakehead aquaculture. The current study seeks to evaluate the combined effects of dietary L-tryptophan incorporation and varying stocking densities on aggressiveness, cannibalism, survival, and growth in striped snakehead rearing throughout the transition from advanced fry to fingerling stages. The results are anticipated to lead to the development of more successful, sustainable, and economically viable approaches in striped snakehead culture, with broader relevance to other carnivorous species experiencing comparable behavioural difficulties.

MATERIALS AND METHODS

Experimental design and diet preparation

The research was carried out using advanced fry of striped snakehead with an average initial length of 3.01±0.078 cm and an average initial weight 0.32±0.293 g sourced from the Advanced Research Farm Facility (ARFF) in Madhavaram (13.15°N 80.24°E), Chennai, Tamil Nadu, India. Four iso-nitrogenous diets were prepared by incorporating L-tryptophan with final protein content of 45%. The experimental setup comprised a total of 36 glass aquarium tank, each filled with 40 L of water, in a 3×4 factorial design, combining three stocking densities (375 fish·m^{-3}, 500 fish·m^{-3}, and 625 fish·m^{-3}) and four levels of dietary L-tryptophan incorporation (0%, 1%, 2%, and 3%) in the experimental diets, formulated with combinations of treatments such as T1-375/0, T2-375/1,T3-375/2,T4-375/3,T5-500/0,T6-500/1, T7-500/2,T8-500/3, T9-625/0,T10-625/1,T11-625/2, and T12-625/3. All the dry feed ingredients were powdered and passed through a 0.3 mm sieve. These pulverized ingredients, as per the formulation, were blended together along with the required additives and thoroughly homogenized. The diet mix was made into a soft dough by adding about 400 mL of boiled and cooled water into the 1,000 g

mix, and it was then steam-cooked (at atmospheric pressure) for 20 min. Then the blend was cooled and extruded into pellets through a laboratory-scale pelletizer using a 2 mm die. Boiled and cooled water was gradually added to the dry mixture at a ratio of 35–40% (v/w), i.e., 350–400 mL of water per kg of dry feed mixture, to obtain a uniform and consistent dough. The water was previously boiled and cooled to room temperature (25–28 °C) to prevent microbial contamination. The pellets were later dried in a hot air oven at 45 °C for 24 h to reduce the moisture content to below 10% and fed as crumbles to the fry. Water temperature, salinity, pH, and dissolved oxygen were recorded daily, while total ammonia and nitrite were measured weekly (APHA, 1989). The fish were hand-fed ad libitum twice a day at 10:00 a.m. and 5:00 p.m., with the formulated experimental diets. The same feeding protocol was consistently followed for all treatments during the entire study period, irrespective of growth progression. After 30 min of feeding, the unconsumed feed was siphoned out and dried to determine the exact feed consumed by the fish. The ingredient composition of the experimental diets formulated with graded levels of L-tryptophan are presented in Table 1. All diets were iso-nitrogenous (45% crude protein).

Growth parameters

Growth monitoring of striped snakehead fry (n = 5 fish per tank) was performed at 14-day intervals by recording length and weight using digital weighing balance (0.001 g). At the end of rearing, mean length, mean weight, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), feed efficiency ratio (FER), cannibalism percentage, and survival rate were derived following the procedure of Kumar *et al*. (2017). The growth parameters were calculated with the formula as mentioned below.

Weight gain (WG) = Final body weight (g) - Initial body weight (g)

Average daily growth (ADG) = Weight gain (g)/rearing period (days)

Specific growth rate (SGR) (%·day^{-1}) = (Ln final weight - Ln initial weight)/ Rearing period (days)

Survival rate (%) = (Total number of fish harvested /Total number of fish stocked) ×100

FCR = Feed intake (g)/Weight gain (g)

FER = Weight gain (g)/Feed intake (g)

Cannibalism rate (%) = (Number of missing fish / Initial number of fish) × 100

Table 1. Experimental diet formulation and ingredient composition (g·kg^{-1}).

Feed ingredients	Control 0%	l-TRP-1%	TRP-2%	TRP-3%
Fish meal	220	220	220	220
Soybean meal	430	420	410	400
Wheat gluten	260	260	260	260
wheat flour	50	50	50	50
Ricebran	20	20	20	20
L-tryptophan	0	10	20	30
Fish oil	10	10	10	10
Dicalcium phosphate	5	5	5	5
Vitamin/mineral	5	5	5	5
Total	1,000	1,000	1,000	1,000

Digestive enzyme activity

Total protein estimation

Total protein content of the intestinal homogenate was determined to express enzyme activity as specific activity. Tissue samples were homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4) and centrifuged at 6,000 × g for 10 min at 4 °C. The supernatant was collected and used for enzyme and protein analysis. Protein concentration was estimated following the Lowry *et al*. (1951) method using bovine serum albumin (BSA) as the standard. An aliquot of the enzyme extract was mixed with alkaline copper reagent and incubated at room temperature for 10 min. Subsequently, Folin–Ciocalteu phenol reagent was added and the mixture was incubated for 30 min in the dark. The absorbance was measured at 660 nm using a UV–Visible spectrophotometer. Protein concentration was calculated from the standard curve prepared using BSA and expressed as $mg \cdot mL^{-1}$.

Sample homogenate procedure

At the end of the rearing period, five striped snakehead fingerlings were randomly selected from each tank and exposed to clove oil anaesthesia (10 ppm, Sigma-Aldrich). Dissections were carried out to collect muscle and intestinal tissues, which were processed for protein and enzyme assays. Tissue samples were homogenized in pre-chilled 0.25 M sucrose with a portable homogenizer, and the homogenate was centrifuged at 3,070×g for 5 min at 4 °C. The clarified supernatant was used in subsequent analyses. Intestinal protease, amylase, and lipase activities were assayed using the protocols of Sarath *et al*. (1989) and (Crandall and Cherry, 1932) respectively.

Haematological analysis

Blood sampling was carried out at the end of the experiment by selecting five snakehead fingerlings from each tank. Fish were anesthetized with clove oil ($0.5 mL \cdot L^{-1}$), and blood was collected from the caudal vein using sterile 1 mL syringes with 22-gauge needles. The syringes were rinsed with 2.7% EDTA to function as anticoagulant. Samples from individual fish were placed in separate EDTA vials for further haematological assessment. The parameters measured included red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb), haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). RBC and WBC counts were obtained with a Neubauer hemocytometer, Hb concentration was determined by the cyanmethemoglobin method (Drabkin, 1951), and Ht was calculated according to the micro-hematocrit method of Nelson and Morris (1989). MCV, MCH, and MCHC values were derived based on the formulae described by Wintrobe (1934):

$$MCV\ (fL) = (Ht/RBC) \times 10$$

$$MCH\ (pg) = (Hb/RBC) \times 10$$

$$MCHC\ (g \cdot dL^{-1}) = (Hb/Ht) \times 100$$

Tissue homogenate preparation and antioxidant enzyme assays

Liver samples were collected aseptically from five snakehead fingerlings per tank at the end of the experiment and homogenized in pre-chilled 0.25 M sucrose to prepare a 5% homogenate (1:19 w/v). The homogenates were centrifuged at 5,000 rpm for 10 min at 4 °C, and the clear supernatant was collected for enzymatic assays. Superoxide dismutase (SOD) activity was quantified using the Misra and Fridovich (1971) method, where absorbance changes at 480 nm were monitored for 2 min on a UV–VIS spectrophotometer (KLUV-2100). One unit of SOD was considered as the protein concentration required to suppress 50% of epinephrine auto-oxidation. Catalase (CAT) activity was analyzed by measuring the decrease in absorbance of H_2O_2 at 240 nm every 15 s for 3 min, and was expressed as nanomoles of H_2O_2 degraded per min per mg protein.

Immunological parameter

Respiratory burst activity of phagocytes was evaluated using a modified nitro blue tetrazolium (NBT) test. The sample's absorbance was measured at 540 nm with a spectrophotometer.

Histological analysis

At the end of the 60-day experimental period, three fish per tank were randomly sampled and anesthetized using clove oil (10 ppm). Liver and anterior intestine tissues were excised, rinsed with physiological saline, and fixed in 10% neutral buffered formalin for 24–48 h, and were later processed by passing through ascending grades of ethanol (70–100%) before paraffin embedding. Thin sections of 5 µm were prepared using rotary microtome and stained with hematoxylin and eosin following the method of Alyahya *et al*. (2018). For comparative evaluation, both qualitative and quantitative assessments were performed. The stained slides were examined under a light microscope (Olympus BX51) at 40× magnification, and digital images were captured using a Magcam-DC5 system for further analysis with Mag vision software. Liver sections were examined for hepatocyte arrangement, nuclear morphology, sinusoidal space, cytoplasmic vacuolation, necrosis, and congestion. A semi-quantitative scoring system (0–3 scale: 0 = normal, 1 = mild, 2 = moderate, 3 = severe alteration) was used to assess lesion severity. Mean lesion scores were calculated for each treatment. Intestinal sections were evaluated for villus height, villus width, muscularis thickness, epithelial integrity, and goblet cell density. The average values were calculated for each treatment.

Statistical analysis

Normality of data distribution was verified through the Shapiro–Wilk test, while equality of variances was checked using Levene's test. Results were reported as means of three triplicates with their corresponding standard deviations. The influence of stocking density, L-tryptophan incorporation, and their interaction was analysed using a two-way ANOVA, followed by Tukey's multiple comparison test at $p<0.05$. Statistical computations were carried out with SPSS (v16.0) (SPSS Inc., Chicago, IL, USA).

Ethical approval

This study complied with the guidelines of the national animal welfare standards (CPCSEA, Government of India). Experimental approval was formally granted by the Institutional Animal Ethics Committee, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, and the trial was initiated only after obtaining clearance.

RESULTS AND DISCUSSION

Growth performance of experimental fish

The highest weight gain (4.67±0.04 g) and specific growth rate (4.68±0.09%·day^{-1}) were observed in fish reared at a stocking density of 375 fish·m^{-3} and fed a diet incorporated with 3% L-tryptophan (Table 2). In contrast, the lowest growth was recorded in the control group at 625 fish·m^{-3}, with a weight gain of 2.29±0.02 g and a specific growth rate of 3.55±0.08%·day^{-1} fed a diet incorporated with 0% L-tryptophan. These findings suggest that growth is maximized at lower stocking densities when combined with higher L-tryptophan incorporation, likely due to reduced stress and intraspecific aggression. A significant difference was observed in the cannibalism rate ($p<0.05$). The lowest cannibalism rate was observed at a stocking density of 375 fish·m^{-3} fed with 3% L-tryptophan incorporation (26.7±0.30%), followed by 500 fish·m^{-3} (32.8±0.40%) and 625 fish·m^{-3} (37.3±0.35%) at the same incorporation level. The highest cannibalism rate (60.00±0.60%) was recorded at 625 fish·m^{-3} in the control group, (without L-tryptophan incorporation) highlighting the adverse effect of both high density and absence of dietary L-tryptophan. Final body weight (FBW) and average daily gain (ADG) were significantly influenced by stocking density and dietary L-tryptophan supplementation. Fish reared at lower stocking densities exhibited higher growth, whereas growth declined at higher densities. This reduction may be attributed to density-induced stress, increased competition for feed, and the establishment of social hierarchy, which negatively affect feed intake and metabolic efficiency (Ellis *et al*., 2002). Similar density-dependent growth suppression has been reported in carnivorous fish species (Qin and Fast, 1996). Feed utilization indices followed a similar trend. Lower FCR and higher FER and PER were observed in fish reared at lower densities and supplemented with L-tryptophan, indicating improved

nutrient conversion efficiency. Improved feed efficiency in low-stress environments has been widely documented in teleosts (Ellis *et al*., 2002). The enhancement observed with tryptophan incorporation may be linked to its role as a precursor

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Table 2. Effects of stocking density (SD) and dietary L-tryptophan (TRP) level on growth performance, feed utilization, cannibalism, and survival of striped snakehead (Channa striata) reared for 60 days under different stocking density and fed experimental diets containing different concentrations of L-tryptophan. Main effects of SD and TRP, as well as their interaction (SD × TRP), are shown with their corresponding p-values.

	IBW (g)	FBW (g)	WG (g)	FCR	FER	ADG	SGR	PER	Cannibalism (%)	Survival (%)
Main effect (SD)										
375	0.31±0.01	4.28±0.43	3.76±0.64	1.03±0.24	1.01±0.23	0.066±0.008	4.42±0.18	0.078±0.02	35.45±7.14	55.94±13.24
500	0.32±0.01	3.78±0.41	3.41±0.41	1.08±0.24	0.96±0.23	0.057±0.006	4.01±0.22	0.078±0.01	42.80±8.40	46.54±11.07
625	0.32±0.02	3.59±0.49	3.18±0.65	1.09±0.31	0.86±0.23	0.054±0.008	3.81±0.32	0.070±0.01	48.65±8.40	31.88±3.96
p-value	0.738	0.006	<0.001	0.009	0.032	<0.001	0.002	0.021	0.03	0.001
Main effect (TRP)										
0%	0.32±0.02	3.46±0.33	2.91±0.54	1.32±0.08	0.76±0.04	0.052±0.005	3.89±0.35	0.067±0.01	52.87±7.01	32.35±5.93
1%	0.31±0.01	3.60±0.39	3.34±0.14	1.28±0.07	0.78±0.04	0.054±0.006	3.94±0.36	0.067±0.01	42.70±4.60	43.52±11.88
2%	0.32±0.01	3.93±0.34	3.44±0.15	0.89±0.07	1.04±0.05	0.060±0.006	4.06±0.34	0.077±0.01	41.37±6.63	49.34±14.53
3%	0.30±0.01	4.53±0.31	4.19±0.43	0.77±0.02	1.27±0.06	0.069±0.006	4.44±0.17	0.090±0.01	32.27±4.89	53.95±16.73
p-value	0.113	0.002	<0.001	0.03	0.027	<0.001	0.032	0.041	0.042	<0.001
Interaction SD × TRP										
375/0	0.30±0.01	3.81±0.01^{d}	3.25±0.07^{c}	1.25±0.04^{d}	0.80±0.05^{f}	0.058±0.04^{cd}	4.24±0.02^{bc}	0.06±0.01^{cd}	43.4±0.60^{cd}	38.90±1.15^{f}
375/1	0.30±0.01	4.03±0.02^{c}	3.50±0.02^{b}	1.21±0.01^{d}	0.82±0.04^{f}	0.061±0.03^{abc}	4.33±0.10^{bc}	0.06±0.08^{cd}	38.2±0.70^{e}	53.26±2.23^{d}
375/2	0.32±0.01	4.31±0.03^{bc}	3.61±0.02^{b}	0.91±0.01^{f}	1.09±0.03^{c}	0.066±0.01^{c}	4.44±0.03^{b}	0.09±0.03^{ab}	33.5±0.35^{ef}	61.68±2.48^{b}
375/3	0.30±0.01	4.96±0.01^{a}	4.67±0.04^{a}	0.75±0.02^{h}	1.33±0.02^{a}	0.077±0.06^{a}	4.68±0.09^{a}	0.10±0.09^{a}	26.7±0.30^{g}	69.93±1.11^{a}
500/0	0.31±0.01	3.40±0.03^{c}	3.18±0.02^{c}	1.31±0.02^{c}	0.76±0.06^{g}	0.051±0.02^{fg}	3.87±0.06^{d}	0.08±0.01^{abc}	55.2±0.75^{b}	30.78±2.23^{i}
500/1	0.32±0.04	3.51±0.05^{c}	3.28±0.04^{c}	1.27±0.01^{d}	0.78±0.05^{g}	0.053±0.03^{de}	3.88±0.10^{d}	0.07±0.09^{bc}	42.4±0.65^{cd}	47.02±3.01^{e}
500/2	0.33±0.04	3.82±0.02^{d}	3.37±0.03^{b}	0.95±0.01^{e}	1.05±0.04^{cd}	0.058±0.01^{cd}	3.96±0.09^{c}	0.07±0.01^{bc}	40.8±0.25^{de}	53.00±2.0^{d}
500/3	0.31±0.02	4.37±0.01^{b}	4.07±0.02^{a}	0.79±0.01^{g}	1.26±0.06^{ab}	0.065±0.03^{b}	4.34±0.04^{bc}	0.09±0.01^{ab}	32.8±0.40^{ef}	55.35±2.10^{c}
625/0	0.34±0.08	3.17±0.06^{cg}	2.29±0.02^{d}	1.40±0.01^{a}	0.71±0.06^{h}	0.047±0.01^{g}	3.55±0.08^{f}	0.06±0.06^{cd}	60.0±0.60^{a}	27.36±1.48^{j}
625/1	0.32±0.02	3.27±0.04^{f}	3.25±0.06^{c}	1.35±0.02^{b}	0.74±0.02^{h}	0.049±0.01f^{g}	3.61±0.09^{ef}	0.07±0.03^{ab}	47.5±0.65^{cd}	30.28±2.23^{i}
625/2	0.32±0.02	3.65±0.01^{b}	3.33±0.09^{b}	1.01±0.02^{e}	0.99±0.06^{e}	0.055±0.02^{ef}	3.77±0.02^{e}	0.07±0.03^{cd}	49.8±0.30^{c}	33.33±1.30^{h}
625/3	0.30±0.06	4.26±0.03^{c}	3.84±0.09^{a}	0.82±0.07^{g}	1.21±0.08^{ab}	0.066±.002^{b}	4.29±0.04^{bc}	0.08±0.01^{abc}	37.3±0.35^{e}	36.56±0.90^{g}
p-value	0.101	0.003	0.02	0.002	<0.001	0.002	0.004	0.021	0.03	0.001

Note: Mean±SD (n = 5) within the same column and within the same factor followed by different lowercase superscript letters are significantly different ($p<0.05$).

of serotonin, which regulates appetite, stress response, and aggressive behaviour (Winberg and Nilsson, 1993). Reduced aggression and improved physiological stability likely contributed to better feed utilization and protein efficiency (Lepage *et al*., 2002). Survival was significantly higher at lower stocking densities and in tryptophan-supplemented groups, while high density without supplementation showed reduced survival. Increased mortality at high density is commonly associated with stress and cannibalism in carnivorous species (Qin and Fast, 1996). The improved survival in tryptophan-fed groups suggests behavioural modulation and reduced social stress.

According to the factorial analysis (Table 3), stocking density significantly affected FBW, WG, FCR, FER, ADG, SGR, PER, cannibalism, and survival, but not IBW, whereas dietary L-tryptophan significantly affected FBW, WG, FCR, FER, ADG, SGR, PER, cannibalism, and survival, but not IBW. In addition, the interaction between stocking density and dietary L-tryptophan was significant for FBW, WG, FCR, FER, ADG, SGR, PER, cannibalism, and survival, indicating that the magnitude of the response to L-tryptophan depended on stocking density. Therefore, the effects of dietary L-tryptophan should be interpreted within each stocking density rather than as an isolated main effect alone.

The present findings of improved growth performance and reduced cannibalism align with observations in other carnivorous fish species. Winberg *et al*. (2001) reported that supplementation with L-tryptophan decreased aggressive behaviour in rainbow trout by enhancing serotonergic regulation. Similarly, Höglund *et al*. (2007) demonstrated reduced aggression and cannibalism in *Gadus morhua* fed a 2.8% L-tryptophan diet. Likewise, Vieira *et al*. (2021) reported that supplementation of L-tryptophan at 1.3–2.6% levels decreased aggressive behaviour in Nile tilapia even at high stocking densities. Hseu *et al*. (2003) also reported improved growth and survival with dietary L-tryptophan in juvenile grouper (*Epinephelus coioides*), especially under lower stocking densities.

Interestingly, higher inclusion levels of L-tryptophan (≥2%) reduced growth in sea bass due to appetite suppression and altered serotonergic activity (Papoutsoglou *et al*., 2005). The present study observed no growth depression at 3% incorporation. This discrepancy may be explained by species-specific serotonin sensitivity, feeding protocols, or differences in diet formulation (Winberg *et al*., 2001). Since our fish were fed ad libitum, potential serotonin-induced anorexia might have been offset, which aligns with earlier observations in rainbow trout by Höglund *et al*. (2007). According to Vo *et al*. (2022), tryptophan administration dramatically improved survival and growth performance in *W. attu* juveniles while lowering cannibalism rates. Similarly, these effects may be associated with serotonin-mediated behavioural responses. Sepúlveda-Quiroz *et al*. (2024) showed that dietary tryptophan reduced intracohort cannibalism in tropical gar (*Atractosteus tropicus*) larvae.

Overall, the results demonstrate that dietary L-tryptophan incorporation effectively reduces cannibalism and enhances growth in striped snakehead, particularly at lower stocking densities. The significant interaction between stocking density and L-tryptophan indicates that the optimal incorporation level is context-dependent, reinforcing the importance of integrating nutritional strategies with husbandry practices. This supports the conclusions of Kestemont *et al*. (2007), who emphasized that behavioural modulation in aquaculture is most effective when multiple management approaches are combined.

Digestive enzyme analysis

The highest activities of digestive enzymes were recorded in fish reared at 375 fish·m^{-3} with 3% L-tryptophan incorporation, showing protease activity of 4.10±0.07 U·mg^{-1} protein·min^{-1}, lipase activity of 3.40±0.08 U·mg^{-1} protein·min^{-1}, and amylase activity of 2.55±0.06 U·mg^{-1} protein·min^{-1}. The lowest enzyme activities were found in the control group at 625 fish·m^{-3}, with values of protease 3.15±0.05 U·mg^{-1} protein·min^{-1}, lipase activity of 2.55±0.06 U·mg^{-1} protein·min^{-1}, and amylase activity of 1.70±0.05 U·mg^{-1} protein·min^{-1}. According to the two-way ANOVA, stocking density, dietary L-tryptophan level, and their interaction significantly

affected protease, lipase, and amylase activities ($p<0.05$). The main-effect means further indicated that digestive enzyme activities generally decreased with increasing stocking density, whereas they increased with increasing dietary L-tryptophan levels. Because the interaction between stocking density and dietary L-tryptophan was significant for all three enzymes, the response to L-tryptophan should be interpreted within each stocking density.

The enhancement in enzyme activity at lower stocking densities with higher L-tryptophan levels could be attributed to improved gut health and reduced physiological stress, which is known

Table 3. Effects of stocking density (SD) and dietary L-tryptophan (TRP) level on digestive enzyme activity of striped snakehead (*Channa striata*) reared using experimental diets with different concentrations of L-tryptophan under various stocking density for a period of 60 days. Main effects of SD and TRP, as well as their interaction (SD × TRP), are shown with their corresponding p-values.

	Protease (U·mg^{-1} protein·min^{-1})	**Lipase (U·mg^{-1} protein·min^{-1})**	**Amylase (U·mg^{-1} protein·min^{-1})**
Main effect			
(SD)			
375	3.79±0.28	3.14±0.23	2.25±0.26
500	3.67±0.30	3.03±0.26	2.15±0.26
625	3.57±0.35	2.91±0.28	2.00±0.27
p-value	<0.001	<0.001	0.002
Main effect			
(TRP)			
0%	3.30±0.15	2.70±0.15	1.83±0.13
1%	3.55±0.13	2.94±0.12	2.03±0.13
2%	3.83±0.12	3.17±0.09	2.23±0.13
3%	4.03±0.05	3.30±0.10	2.43±0.13
p-value	0.004	0.002	0.001
Interaction			
SD × TRP			
375/0	3.45±0.05^{g}	2.85±0.08^{f}	1.95±0.06^{ef}
375/1	3.67±0.06^{e}	3.05±0.07^{d}	2.15±0.05^{d}
375/2	3.95±0.05^{b}	3.25±0.09^{b}	2.35±0.07^{b}
375/3	4.10±0.07^{a}	3.40±0.08^{a}	2.55±0.06^{a}
500/0	3.30±0.06^{fgh}	2.70±0.07^{g}	1.85±0.05^{g}
500/1	3.55±0.05^{f}	2.95±0.08^{e}	2.05±0.06^{e}
500/2	3.82±0.07^{c}	3.18±0.08^{ab}	2.25±0.06^{c}
500/3	4.00±0.06^{a}	3.30±0.07^{a}	2.45±0.05^{ab}
625/0	3.15±0.05^{h}	2.55±0.06^{h}	1.70±0.05^{h}
625/1	3.42±0.06^{fg}	2.82±0.07^{fg}	1.90±0.04^{f}
625/2	3.72±0.06^{d}	3.08±0.06^{d}	2.10±0.05^{d}
625/3	3.90±0.05^{bc}	3.18±0.06^{c}	2.30±0.06^{b}
p-value	0.002	0.005	0.036

Note: Mean±SD (n = 5) within the same column and within the same factor followed by different lowercase superscript letters are significantly different ($p<0.05$).

to support digestive function (Yarahmadi *et al*., 2016). These findings are in line with Hseu *et al*. (2003) who reported increased protease and amylase activities juvenile grouper (*E. coioides*) fingerlings incorporated with 2% dietary L-tryptophan under moderate densities. In contrast, Papoutsoglou *et al*. (2005) found that 2% L-tryptophan incorporation in *Dicentrarchus labrax* did not significantly improve digestive enzyme activity, possibly due to species-specific serotonergic responses or lower feed intake under stress. However, fish were fed until they were satiated in the current investigation. Therefore, the anabolic effect of L-tryptophan on protein metabolism, together with its function in decreasing aggressiveness and improving feed absorption, may be responsible for the increased enzyme activity. This finding is in line with Winberg *et al*. (2001), who showed that dietary L-tryptophan supplementation reduces aggressive behaviour in rainbow trout and modifies central serotonergic activity.

Antioxidant enzyme activity

The highest catalase activity was observed in fish reared at a stocking density of 625 fish·m^{-3} and fed with 3% L-tryptophan (3.11±0.07 U·mg^{-1} protein), followed by a stocking density of 500 fish·m^{-3} fed with 3% L-tryptophan (2.73±0.05 U·mg^{-1} protein), and a stocking density of 625 fish·m^{-3} fed with 2% L-tryptophan (2.52±0.06 U·mg^{-1} protein). The lowest catalase activity (1.41±0.05 U·mg^{-1} protein) was recorded in the control group at 375 fish·m^{-3} (Table 4). Similarly, SOD activity followed the same trend, with the highest value (69.41±0.14 U·mg^{-1} protein) observed in fish reared at a stocking density of 625 fish·m^{-3} stocking density fed with 3% L-tryptophan, whereas the lowest SOD activity (38.03±0.13 U·mg^{-1} protein) was recorded in the control group at 375 fish·m^{-3}. The highest respiratory burst activity was observed in fish reared at a stocking density of 375 fish·m^{-3} fed without L-tryptophan (0.41±0.01), followed by a stocking density of 500 fish·m^{-3} fed without L-tryptophan (0.39±0.01), and a stocking density of 375 fish·m^{-3} fed with 1% L-tryptophan (0.35± 0.03). The lowest respiratory burst activity was recorded in fish reared at a stocking density of 625 fish·m^{-3} fed with 3% L-tryptophan (0.22±0.02). According to the two-way ANOVA, stocking density, dietary L-tryptophan, and their interaction significantly affected catalase, superoxide dismutase, and respiratory burst activity ($p<0.05$). The main-effect means showed that catalase and superoxide dismutase activities generally increased with increasing dietary L-tryptophan levels, while respiratory burst activity decreased. Across stocking densities, catalase and superoxide dismutase tended to be higher at higher stocking densities, whereas respiratory burst activity tended to be lower. Fish exposed to higher stocking densities and provided with 3% L-tryptophan in their diet exhibited the greatest antioxidant enzyme activities, which may be attributed to the interaction between increased oxidative stress and the mitigating role of L-tryptophan.

The present findings align with Zhao *et al*. (2023), who reported that *Channa argus* fingerlings fed dietary arginine exhibited significantly elevated SOD and CAT activities along with reduced malondialdehyde (MDA) levels. Similarly, Luo *et al*. (2019) demonstrated that graded tryptophan levels (up to 0.40%) enhanced plasma T-SOD, CAT, and GSH activities in *Megalobrama amblycephala*, mediated by the upregulation of *Nrf2* and downstream antioxidant-related genes. Chen *et al*. (2023) also reported a quadratic dose-response increase in antioxidant enzyme activities with dietary tryptophan in hybrid catfish (*Pelteobagrus vachelli* × *Leiocassis longirostris*), suggesting an optimal threshold for antioxidative protection. In terms of stocking density, the present study showed a clear trend of increasing CAT and SOD levels with higher stocking rates when supplemented with L-tryptophan. This aligns with Mahalakshmi *et al*. (2024), who observed significantly higher antioxidant enzyme activities in *Aulonocara* sp. reared under biofloc systems with higher stocking densities, indicating an adaptive mechanism to counterbalance increased oxidative demand. Respiratory burst activity (RBA) is an important indicator of innate immune function, reflecting the ability of phagocytes to generate reactive oxygen species (ROS) to kill invading pathogens (Biller-Takahashi *et al*., 2013). In this study, RBA varied significantly across treatments, being highest in fish at lower stocking density

without tryptophan (375/0) and declining with increased dietary L-tryptophan and higher stocking densities. A higher RBA can indicate an elevated innate immune response, which may occur under stress or increased pathogen exposure (Biller-Takahashi *et al*., 2013). The significant reduction in RBA with increasing dietary L-tryptophan suggests that tryptophan may modulate immune activation, potentially through its role in serotonergic pathways that influence stress and immune responses. Dietary L-tryptophan has been shown to affect immune status in fish, altering leukocyte function and inflammatory responses. Furthermore, high stocking densities often elevate stress and may impair immune competence, reducing several innate immune responses and, in some cases, phagocytic and oxidative burst activity (Biller-Takahashi and Urbinati, 2018; Dara *et al*., 2023). The observed interaction between stocking density and L-tryptophan indicates that nutritional modulation can partially offset density-induced changes in innate immune activation. These findings highlight that respiratory burst activity is sensitive to both environmental stressors and dietary L-tryptophan and that both can influence innate immune functions in striped snakehead.

Overall, the present study confirms that dietary L-tryptophan incorporation at 3% markedly enhances antioxidant defences in striped snakehead under higher stocking densities, thereby supporting stress mitigation and oxidative homeostasis.

Haematological analysis

The effects of stocking density and dietary L-tryptophan incorporation on the haematological indices of striped snakehead fingerlings are summarised in Table 5. A significant difference was observed in red blood cell (RBC) counts with respect to both stocking density and dietary L-tryptophan levels. The mean RBC values decreased with increasing stocking density, with the highest mean recorded at 375 fish·m^{-3} (3.57±0.41×10^6 cells·mm^{-3}), followed by 500 fish·m^{-3} (3.46±0.36×10^6 cells·mm^{-3}), and the lowest at 625 fish·m^{-3} (3.33±0.29×10^6 cells·mm^{-3}). In contrast, RBC values increased progressively with increasing dietary L-tryptophan levels, with the highest mean observed at 3% incorporation (3.90±0.19×10^6 cells·mm^{-3}) and the lowest at 0% (3.07±0.07×10^6 cells·mm^{-3}). Mean corpuscular volume (MCV) ranged from 92.40±1.38 fL to 103.23±2.09 fL across dietary treatments. Although stocking density did not exert a significant effect on MCV, a decreasing trend was observed with increasing dietary tryptophan levels, with the highest mean value recorded at 0% (103.23±2.09 fL), followed by 1% (98.29±0.84 fL), 2% (94.97±1.74 fL), and the lowest at 3% (92.40±1.38 fL). Mean corpuscular haemoglobin (MCH) values varied between 25.84±0.25 pg and 26.38±0.25 pg, with no significant differences observed among treatments. Both stocking density and dietary tryptophan did not significantly influence MCH, although slight variations were evident across groups. Mean corpuscular haemoglobin concentration (MCHC) ranged from 25.82±0.05 g·dL^{-1} to 27.97±0.20 g·dL^{-1}. While stocking density had no significant effect on MCHC, dietary L-tryptophan significantly influenced this parameter, with values increasing progressively from 0% (25.82±0.05 g·dL^{-1}) to 3% (27.97±0.20 g·dL^{-1}). Overall, these results indicate that RBC, WBC, Hb, and Ht were affected by both stocking density and dietary L-tryptophan, whereas MCV and MCHC were influenced mainly by dietary L-tryptophan, and MCH was not significantly affected by either factor. In addition, the interaction between stocking density and dietary L-tryptophan was significant only for RBC.

The present findings agree with Ahmed *et al*. (2024), who reported increased RBC production in rainbow trout fingerlings supplemented with essential amino acids, attributing it to enhanced hematopoietic activity. Similarly, Ciji *et al*. (2015) observed elevated RBC and hemoglobin (Hb) levels in rohu, following dietary L-tryptophan incorporation, confirming the conserved role of this amino acid in improving hematological health across freshwater fish. Hemoglobin levels followed a comparable trend, with the highest value recorded in fish reared at 375 fish·m^{-3} stocking density fed with 3% L-tryptophan, showing significant differences among treatments. Hematocrit (Ht) values also peaked in the same group, indicating enhanced erythrocyte volume and oxygen-carrying potential. The elevation of Hb and Ht under low

stocking densities with L-tryptophan incorporation likely reflects reduced stress levels, improved erythropoiesis, and enhanced metabolic efficiency. In *Pagrus major*, Yarahmadi *et al*. (2016) reported elevated Hb and Ht in fish fed L-tryptophan-enriched diets, highlighting the role of tryptophan in restoring normal red blood cell production through serotonin-mediated suppression of the hypothalamic-pituitary-interrenal (HPI) axis. Similarly, Mahalakshmi *et al*. (2024) observed that *Aulonocara* sp. reared under biofloc conditions and low stocking density exhibited significantly higher Hb and Ht levels compared to high-density. In contrast, higher white blood cell (WBC) counts were noted in fish reared at higher stocking densities, particularly in control groups. Stress induces leukocytosis, via activation of the HPI axis and elevated cortisol secretion, which stimulate leukocyte proliferation. Similarly,

Table 4. Antioxidant enzyme and immune response of striped snakehead (*Channa striata*) reared using experimental diets with different concentrations of L-tryptophan under various stocking density for a period of 60 days.

	Catalase U·mg protein^{-1}	**Superoxide dismutase U·mg protein^{-1}**	**Respiratory burst activity**
Main effect			
(SD)			
375	1.95±0.47	49.06±11.70	0.33±0.07
500	2.16±0.46	51.85±12.07	0.31±0.06
625	2.16±0.31	56.63±10.60	0.31±0.06
p-value	<0.001	<0.001	<0.001
Main effect			
(TRP)			
0%	1.63±0.22	41.11±3.02	0.37±0.05
1%	1.95±0.22	45.88±6.69	0.32±0.03
2%	2.33±0.19	56.05±3.24	0.27±0.03
3%	3.11±0.30	69.41±2.47	0.22±0.02
p-value	<0.001	<0.001	0.023
Interaction			
SD × TRP			
375/0	1.41±0.05^{i}	38.03±0.13^{g}	0.41±0.01^{a}
375/1	1.73±0.06^{g}	41.09±0.07^{ef}	0.35±0.03^{ab}
375/2	2.14±0.05^{efg}	53.04±0.18^{d}	0.30±0.01^{bc}
375/3	2.50±0.06^{cd}	64.07±0.17^{ab}	0.26±0.01^{de}
500/0	1.63±0.05^{h}	41.23±0.14^{ef}	0.39±0.01^{ab}
500/1	1.95±0.05^{g}	43.02±0.10^{e}	0.32±0.01^{c}
500/2	2.32±0.06^{de}	55.63±0.10^{bc}	0.27±0.01^{de}
500/3	2.73±0.05^{b}	67.52±0.12^{a}	0.25±0.01^{de}
625/0	1.85±0.06^{g}	44.07±0.10^{e}	0.31±0.01^{bc}
625/1	2.17±0.05^{ef}	53.53±0.17^{d}	0.29±0.01^{bcd}
625/2	2.52±0.06^{bc}	59.49±0.17^{b}	0.24±0.02^{ef}
625/3	3.11±0.07^{a}	69.41±0.14^{a}	0.22±0.02^{g}
p-value	0.024	0.005	0.004

Note: Mean±SD (n = 5) within the same column and within the same factor followed by different lowercase superscript letters are significantly different (p<0.05).

Vargas-Chacoff *et al*. (2018) reported increased WBC counts in Atlantic salmon under crowding stress, indicating increased immune response and physiological disturbance. Higher WBC counts in high-density groups lacking dietary L-tryptophan supplementation likely reflect a compensatory immune response to sustained stress, whereas supplementation helped maintain more stable hematological homeostasis. Overall, the findings suggest that dietary L-tryptophan incorporation at 3% under lower stocking densities improves RBC, Hb, and Ht values by supporting erythropoiesis and oxygen transport, while mitigating WBC elevation associated with crowding stress. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin

Table 5. Haematological parameters of striped snakehead (*Channa striata*) reared using experimental diets with different concentrations of L-tryptophan under various stocking density for a period of 60 days.

	RBC× (10^6 cells·mm^{-3})	**WBC (×10^5 L^{-1})**	**Hb (g·dL^{-1})**	**Ht (%)**	**MCV (fl)**	**MCH (pg)**	**MCHC (g·dL^{-1})**
Main effect							
(SD)							
375	3.57±0.41	4.66±0.37	9.09±0.58	33.81±1.08	96.68±3.83	25.97±0.24	26.84±0.82
500	3.46±0.36	4.86±0.32	8.96±0.64	33.12±1.17	96.19±4.92	25.96±0.36	27.02±1.01
625	3.33±0.29	5.19±0.32	8.73±0.60	32.25±1.19	98.80±4.86	26.46±0.20	27.02±0.88
p-value	0.012	0.004	0.001	<0.001	0.211	0.294	0.472
Main effect							
(TRP)							
0%	3.07±0.07	5.33±0.26	8.21±0.20	31.75±0.84	103.23±2.09	26.38±0.25	25.82±0.05
1%	3.29±0.16	5.04±0.25	8.71±0.20	32.56±0.83	98.29±0.84	26.37±0.27	26.73±0.07
2%	3.54±0.13	4.72±0.18	9.15±0.18	33.46±0.79	94.97±1.74	25.93±0.41	27.33±0.20
3%	3.90±0.19	4.52±0.34	9.64±0.19	34.45±0.68	92.40±1.38	25.84±0.25	27.97±0.20
p-value	0.001	0.005	0.007	0.002	0.001	0.429	0.011
Interaction							
SD × TRP							
375/0	$3.07±0.05^{g}$	5.10±0.10	8.40±0.10	32.56±0.35	101.50±3.10	26.26±1.02	25.79±0.05
375/1	$3.47±0.05^{d}$	4.71±0.09	8.90±0.10	33.33±0.35	97.64±1.32	26.07±0.49	26.69±0.02
375/2	$3.67±0.06^{bc}$	4.62±0.10	9.30±0.09	34.23±0.35	95.10±1.65	25.84±0.45	27.10±0.01
375/3	$4.07±0.05^{a}$	4.20±0.09	9.76±0.25	35.10±0.32	92.48±2.17	25.71±1.18	27.79±0.06
500/0	$3.14±0.06^{f}$	5.27±0.13	8.21±0.10	31.80±0.30	102.60±5.82	26.21±1.59	25.78±0.07
500/1	$3.21±0.18^{e}$	5.08±0.10	8.71±0.09	32.63±0.35	97.98±2.57	26.41±0.52	26.69±0.01
500/2	$3.55±0.06^{c}$	4.61±0.10	9.20±0.11	33.53±0.36	93.18±3.11	25.57±0.42	27.44±0.05
500/3	$3.93±0.06^{ab}$	4.46±0.15	9.73±0.15	34.53±0.33	90.99±3.69	25.63±0.39	28.18±0.07
625/0	$3.00±0.10^{h}$	5.61±0.11	8.01±0.10	30.90±0.40	105.60±6.04	26.68±1.08	25.88±0.01
625/1	$3.19±0.11^{e}$	5.32±0.08	8.51±0.08	31.73±0.34	99.25±4.19	26.62±1.09	26.82±0.04
625/2	$3.41±0.06^{de}$	4.92±0.09	8.96±0.11	32.63±0.33	96.63±3.36	26.37±0.84	27.46±0.01
625/3	$3.70±0.01^{bc}$	4.89±0.11	9.42±0.09	33.73±0.36	93.73±1.63	26.18±0.46	27.93±0.07
p-value	0.024	0.215	0.922	0.997	0.990	0.997	0.937

Note: Mean±SD (n = 5) within the same column and within the same factor followed by different lowercase superscript letters are significantly different (p<0.05).

concentration (MCHC) are important erythrocytic indices used to evaluate red blood cell morphology and oxygen-carrying capacity in fish. In the present study, MCV was not significantly affected by stocking density but was significantly influenced by dietary L-tryptophan. A gradual reduction in MCV with increasing tryptophan inclusion suggests improved erythrocyte stability and reduced cellular swelling, which is commonly associated with better physiological adaptation under reduced stress conditions. MCH did not differ significantly among treatments, indicating that hemoglobin content per erythrocyte remained stable despite changes in density and diet. Stable MCH values generally reflect normal erythropoiesis and absence of anemia or hemoglobin synthesis impairment.

MCHC showed significant variation with dietary L-tryptophan ($p<0.05$), with slightly elevated values in higher supplementation groups. Increased MCHC indicates improved hemoglobin concentration within erythrocytes and enhanced oxygen transport efficiency. Variations in MCV and MCHC are often linked to stress and metabolic adjustments in teleost fish (Wedemeyer and Yasutake, 1977). The observed pattern suggests that dietary L-tryptophan helped to maintain hematological homeostasis under varying stocking densities.

Histological analysis

Histological examination of liver and intestinal tissues revealed distinct differences between the control and L-tryptophan-supplemented treatments (Figure 1). Fish fed diets containing 1%, 2%, and 3% L-tryptophan exhibited normal hepatic architecture characterized by well-organized hepatocytes, intact sinusoids, and the absence of cellular degeneration. Similarly, intestinal sections from these groups showed intact mucosal layers with well-defined villi and normal goblet cell distribution, without evidence of structural damage or inflammatory changes. In contrast, liver sections from the control group exhibited diffuse and severe hepatocellular vacuolation. Intestinal sections from control fish showed a mild increase in goblet cell density along the villi compared to the supplemented groups. The normal hepatic and intestinal morphology observed in L-tryptophan-supplemented groups indicates that dietary inclusion of tryptophan did not induce tissue pathology and maintained the structural integrity of vital organs. The absence of histological abnormalities suggests adequate metabolic regulation and physiological stability under supplementation. Diffuse hepatocellular vacuolation observed in the control group is commonly associated with metabolic disturbances, lipid accumulation, or stress-related alterations in hepatic function (Hinton and Lauren, 1990). In teleost fish, hepatic vacuolation is often considered an indicator of nutritional imbalance or chronic stress exposure. The mild increase in goblet cells observed in the intestine of control fish may represent a compensatory protective response. Goblet cells secrete mucus that forms a protective barrier over the intestinal epithelium, and their proliferation is frequently linked to stress or irritation of the mucosal surface (Buddington *et al*., 1997). Such changes may reflect altered mucosal homeostasis in the absence of dietary supplementation. L-tryptophan is a precursor of serotonin, a neurotransmitter involved in stress modulation and physiological regulation in fish (Winberg and Nilsson, 1993). By potentially reducing stress-related metabolic disturbances, dietary L-tryptophan may help maintain normal hepatic and intestinal histoarchitecture.

Overall, the present findings demonstrate that dietary L-tryptophan supplementation supports the tissue integrity of the liver and intestine, whereas the absence of supplementation may predispose fish to stress-associated histopathological alterations.

Water quality

The water quality parameters recorded during the experimental period were within the optimal range for aquaculture. The mean values were as follows: pH, 7.99±0.04; temperature, 28.99±0.14 °C; dissolved oxygen, 4.85±0.11 $mg{\cdot}L^{-1}$; hardness, 333.40±2.31 $mg{\cdot}L^{-1}$; and alkalinity, 229.87±1.50 $mg{\cdot}L^{-1}$. Nitrogenous metabolites, including ammonia (0.01±0.09 $mg{\cdot}L^{-1}$), nitrate (0.01±0.02 $mg{\cdot}L^{-1}$), and nitrite (0.04±0.001 $mg{\cdot}L^{-1}$), also remained within safe limits throughout the study.

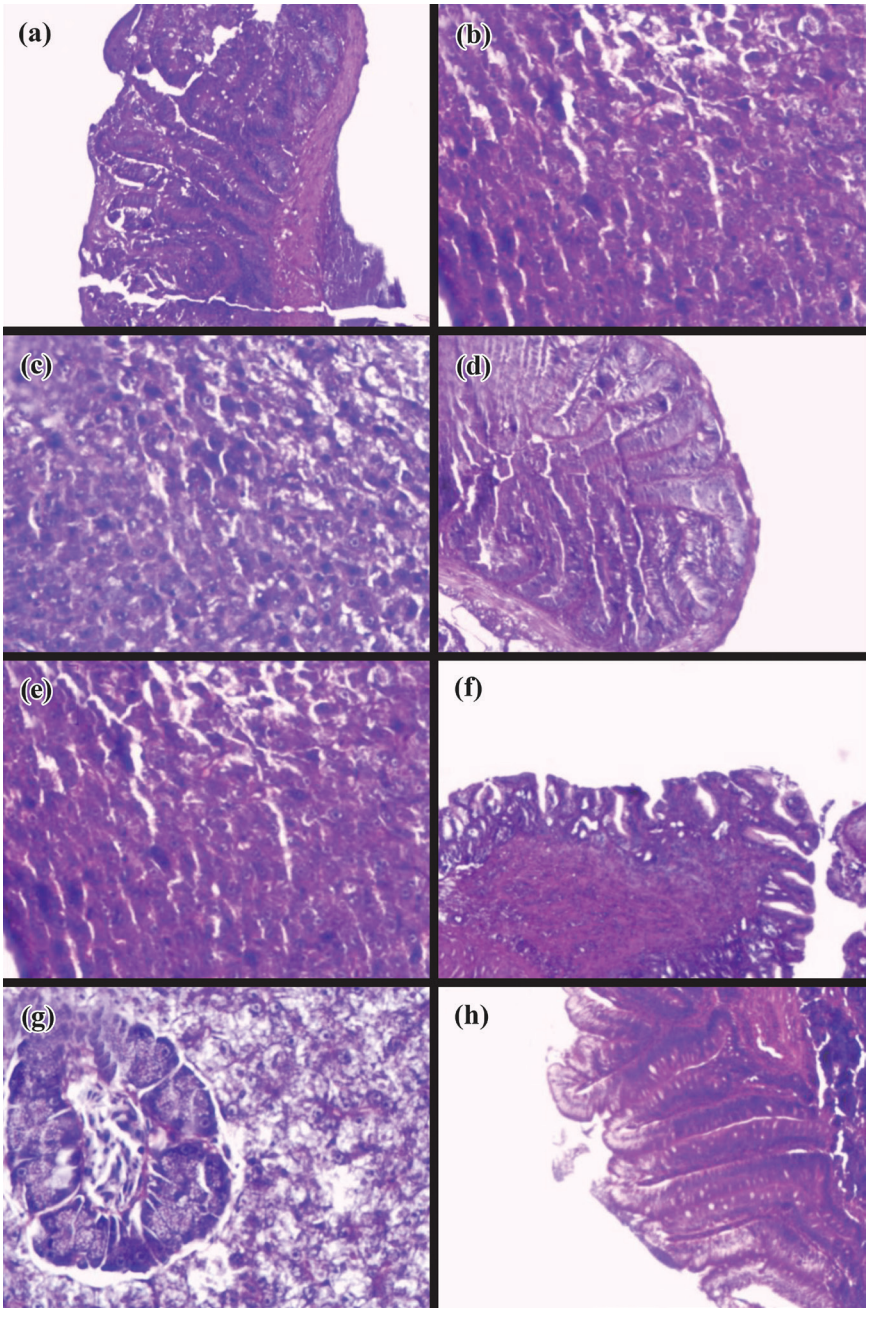


Figure 1. Histological features of the liver and intestine of striped snakehead (*Channa striata*) after 60 days of feeding diets containing different levels of L-tryptophan: (a) 3% L-tryptophan, intestine, showing no abnormalities; (b) 3% L-tryptophan, liver, showing normal hepatic architecture; (c) 2% L-tryptophan, liver, showing normal abnormalities; (d) 2% L-tryptophan, intestine, showing no histopathological abnormalities; (e) 1% L-tryptophan, liver, showing normal hepatic architecture; (f) 1% L-tryptophan, intestine, showing no histopathological abnormalities; (g) control, liver, showing diffuse severe hepatocellular vacuolations; and (h) control, intestine, showing a mild increase in goblet cell density along the villi.

CONCLUSIONS

The present study indicated that the interactive influence of dietary L-tryptophan and stocking density positively influenced the rearing performance of striped snakehead from the advanced fry to fingerling stage. The combination of 3% dietary L-tryptophan with a stocking density of 375 fish·m^{-3} significantly reduced stress and cannibalism, leading to better health and uniform growth. The study recommends the combination of 3% dietary L-tryptophan with a stocking density of 375 fish·m^{-3} as the optimal condition for nursery rearing. However, the stocking density can be increased up to 500 fish·m^{-3} with 3% L-tryptophan without compromising physiological stability.

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LITERATURE CITED

Ahmed, I., I. Ahmad and B.A. Malla. 2024. Effects of dietary tryptophan levels on growth performance, plasma profile, intestinal antioxidant capacity and growth related genes in rainbow trout (*Oncorhynchus mykiss*) fingerlings. **Aquaculture** 585: 740710. DOI: 10.1016/j.aquaculture.2024.740710.

AlYahya, S.A., F. Ameen, K.S. Al-Niaeem, B.A. Al-Saadi, S. Hadi and A.A. Mostafa. 2018. Histopathological studies of experimental *Aeromonas hydrophila* infection in blue tilapia, *Oreochromis aureus*. **Saudi Journal of Biological Sciences** 25(1): 182 –185. DOI: 10.1016/j.sjbs.2017.10.019.

American Public Health Association (APHA). 1989. **Standard Methods for the Examination of Water and Wastewater,** 17th ed. American Public Health Association, Washington, D.C., USA. 1134 pp.

Biller-Takahashi, J.D. and E.C. Urbinati. 2018. Oxidative stress and fish immune system: phagocytosis and leukocyte respiratory burst activity. **Anais da Academia Brasileira de Ciências** 90(4): 3403–3414. DOI: 10.1590/0001-3765201820170730.

Biller-Takahashi, J.D., L.S. Takahashi, M.V. Saita, R.Y. Gimbo and E.C. Urbinati. 2013. Leukocytes respiratory burst activity as indicator of innate immunity of pacu *Piaractus mesopotamicus*. **Brazilian Journal of Biology** 73(2): 425–429.

Buddington, R.K., A. Krogdahl and A.M. Bakke-Mckellep. 1997. The intestines of carnivorous fish: structure and functions and the relations with diet. **Acta Physiologica Scandinavica** 161: 67–80.

Chen, J., M. Zhuo, J. Jiang, A. Yu, D. Yu and F. Huang. 2023. Effects of dietary lipid levels on fiber quality, lipidomic profiles, antioxidant and inflammation responses in muscle of yellow catfish *Pelteobagrus fulvidraco*. **Aquaculture Reports** 33: 101855. DOI: 10.1016/j.aqrep.2023.101855.

Ciji, A., N.P. Sahu, A.K. Pal and M.S. Akhtar. 2015. Dietary L-tryptophan modulates growth and immuno-metabolic status of *Labeo rohita* juveniles exposed to nitrite. **Aquaculture research** 46(8): 2013–2024. DOI: 10.1111/are.12355.

Crandall, L.A. and I.S. Cherry. 1932. Blood Lipase, diastase and esterase in multiple sclerosis: A possible index of liver dysfunction. **Archives of Neurology and Psychiatry** 27: 367–374. DOI: 10.1001/archneurpsyc.1932.02230140121009.

Dara, M., P. Carbonara, C.L. Corte, D. Parrinello, M. Cammarata and M.G. Parisi. 2023. Fish welfare in aquaculture: Physiological and immunological activities for diets, social and spatial stress on mediterranean aquacultured species. **Fishes** 8(8): 414. DOI: 10.3390/fishes8080414.

Drabkin, D.L. 1951. Metabolism of the hemin chromoproteins. **Physiological Reviews** 31(4): 345–431. DOI: 10.1152/physrev.1951.31.4.345.

Ellis, T., B. North, A.A. Scott, N.R. Bromage, M. Porter and D. Gadd. 2002. The relationships between stocking density and welfare in farmed rainbow trout. **Journal of fish Biology** 61(3): 493–531. DOI: 10.1111/j.1095-8649.2002.tb00893.x.

Food and Agriculture Organization of the United Nations (FAO). 2024. **The State of World Fisheries and Aquaculture 2024: Blue Transformation in Action.** Food and Agriculture Organization of the United Nations, Rome, Italy. 244 pp.

Hinton, D.E. and D.J. Lauren. 1990. **Liver structural alterations accompanying chronic toxicity in fishes: potential biomarkers of exposure.** In: Biomarkers of Environmental Contamination (eds. J.F. McCarthy and L.R. Shugart), pp. 17–57. CRC Press, Boca Raton, Florida, USA.

Höglund, E., S. Øverli, O. Lepage and S. Winberg. 2007. Attenuation of stress-induced anorexia in brown trout (*Salmo trutta fario*) by pre-treatment with dietary L-tryptophan. **British Journal of Nutrition** 97: 786–789. DOI: 10.1017/S0007114507657946.

Hseu, J.R., F.I. Lu, H.M. Su, L.S. Wang, C.L. Tsai and P.P. Hwang. 2003. Effect of exogenous tryptophan on cannibalism, survival and growth in juvenile grouper (*Epinephelus coioides*). **Aquaculture** 218: 251–263. DOI: 10.1016/S0044-8486(02)00503-3.

Kestemont, P., X. Xueliang, N. Hamza, J. Maboudou and I.I Toko. 2007. Effect of weaning age and diet on pikeperch larviculture. **Aquaculture** 264(1–4): 197–204.

Kumar, P., M. Kailasam, S.N. Sethi, K. Sukumaran, G. Biswas, R. Subburaj, G. Thiagarajan, T.K. Ghoshal and K.K. Vijayan. 2017. Effect of dietary L-tryptophan on cannibalism, growth and survival of Asian seabass (*Lates calcarifer*) fry. **Indian Journal of Fisheries** 64: 28–32. DOI: 10.21077/ijf.2017.64.2.61333-05.

Kumar, P., A.K. Pal, N.P. Sahu, A.K. Jha, N. Kumar, L. Christina and P. Priya. 2018. Dietary L-tryptophan potentiates non-specific immunity in *Labeo rohita* fingerlings reared under elevated temperature. **Journal of Thermal Biology** 74: 55–62. DOI: 10.1016/j.jtherbio.2018.03.017.

Król, J. and E. Zieliński. 2015. Effects of stocking density and weaning age on cannibalism, survival and growth in European perch *Perca fluviatilis* larvae. **Polish Journal of Natural Sciences** 30(4): 403–415.

Laxmappa, B. and G.V. Babu. 2014. Present status and prospects of murrels farming in Andhra pradesh, India. **International Journal of Fisheries and Aquatic Studies** 1: 22–31.

Lepage, O., O. Tottmar and S. Winberg. 2002. Elevated dietary intake of L-tryptophan counteracts the stress-induced elevation of plasma cortisol in rainbow trout (*Oncorhynchus mykiss*). **Journal of Experimental Biology** 205: 3679–3687. DOI: 10.1242/jeb.205.23.3679.

Lowry, O., N. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. **Journal of Biological Chemistry** 193(1): 265–275.

Mahalakshmi, S., C. Antony and K. Ravaneswaran. 2024. Influence of biofloc and stocking density on physiological responses and disease resistance in peacock cichlid (*Aulonocara* sp.). **Aquaculture** 592: 741168. DOI: 10.1016/j.aquaculture.2024.741168.

Misra, H.P. and I. Fridovich. 1971. The generation of superoxide radical during the autoxidation of ferredoxins. **Journal of Biological Chemistry** 246: 6886–6890. DOI: 10.1016/S0021-9258(19)45929-2.

Montero, D., L.E. Robaina, J. Socorro, J.M. Vergara, L. Tort and M.S. Izquierdo. 2001. Alteration of liver and muscle fatty acid composition in gilthead seabream *(Sparus aurata*) juveniles held at high stocking density and fed an essential fatty acid deficient diet. **Fish Physiology and Biochemistry** 24(1): 63–72.

Nelson, D.A. and M.W. Morris. 1989. **Basic methodology.** In: Clinical Diagnosis and Management by Laboratory Methods, 17th ed. (ed. J.B. Henry), pp. 578–625. W.B. Saunders Company, Philadelphia, USA.

Papoutsoglou, S.E., N. Karakatsouli and P. Koustas. 2005. Effects of dietary L-tryptophan and lighting conditions on growth performance of European sea bass (*Dicentrarchus labrax*) juveniles reared in a recirculating water system. **Journal of Applied Ichthyology** 21: 520–524. DOI: 10.1111/j.1439-0426.2005.00661.x.

Qin, J. and A.W. Fast. 1996. Size and feed dependent cannibalism with juvenile snakehead *Channa striatus*. **Aquaculture** 144(4): 313–320.

Sarath, G., R.S. de la Motte and F.W. Wagner. 1989. **Protease assay methods.** In: Proteolytic Enzymes: A Practical Approach (eds. R.J. Beynon and J.S. Bond), pp. 25–56. IRL Press, Oxford, UK.

Sepúlveda-Quiroz, C.A., J. Contreras and E. López. 2024. Tryptophan reduces intracohort cannibalism behaviour in tropical gar (*Atractosteus tropicus*) Larvae. **Fishes** 9: 40. DOI: 10.3390/fishes9010040.

Smith, C. and P. Reay. 1991. Cannibalism in teleost fish. **Reviews in Fish Biology and Fisheries** 1(1): 41–64. DOI: 10.1007/BF00042661.

Vargas-Chacoff, L., A.M. Regish, A. Weinstock and S.D. McCormick. 2018. Effects of elevated temperature on osmoregulation and stress responses in Atlantic salmon *Salmo salar* smolts in fresh water and seawater. **Journal of Fish Biology** 93(3): 550–559. DOI: 10.1111/jfb.13683.

Vieira, B.R.M., I.I. Guermandi, M.S. Bellot, B. Camargo-dos-Santos, J. Favero-Neto and P.C. Giaquinto. 2021. The effects of tryptophan supplementation on stress and aggression in Nile tilapia. **Journal of Applied Ichthyology** 37(4): 578–584. DOI: 10.1111/jai.14186.

Vo, D.N., T. Nguyen and L. Tran. 2022. Effects of dietary tryptophan on cannibalism, survival and growth of *Wallago attu* (Bloch and Schneider, 1801) juveniles. **Hue University Journal of Science: Natural Science** 131: 67–75. DOI: 10.26459/hueunijns.v131i1D.6516.

Walton, M.J., R.M. Coloso and C.B. Cowey. 1984. The effects of dietary tryptophan levels on growth and metabolism of rainbow trout (*Salmo gairdneri*). **British Journal of Nutrition** 51: 279–287. DOI: 10.1079/BJN19840034.

Wedemeyer, G.A. and W.T. Yasutake. 1977. **Clinical Methods for the Assessment of the Effects of Environmental Stress on Fish Health,** Volume 89. U.S. Fish and Wildlife Service, U.S. Department of the Interior, Washington, D.C., USA. 18 pp.

Winberg, S. and G.E. Nilsson. 1993. Roles of brain monoamine neurotransmitters in agonistic behaviour and stress reactions, with particular reference to fish. **Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology** 106(3): 597–614.

Winberg, S., Ø. Øverli and O. Lepage. 2001. Suppression of aggression in rainbow trout (*Oncorhynchus mykiss*) by dietary L-tryptophan. **Journal of Experimental Biology** 204: 3867–3876. DOI: 10.1242/jeb.204.22.3867.

Wintrobe, M.M. 1934. Anemia: Classification and treatment on the basis of differences in the average volume and hemoglobin content of the red corpuscles. **Archives of Internal Medicine** 54: 256–280. DOI: 10.1001/archinte.1934.00160140099006.

Yarahmadi, P., H.K. Miandare, S. Fayaz and C.M.A. Caipang. 2016. increased stocking density causes changes in expression of selected stress- and immune-related genes, humoral innate immune parameters and stress responses of rainbow trout (*Oncorhynchus mykiss*). **Fish and Shellfish Immunology** 48: 43–53. DOI: 10.1016/j.fsi.2015.10.034.

Zhang, X., A. Wang, E. Chang, B. Han, J. Xu, Y. Fu, X. Dong and S. Miao. 2023. Effects of dietary tryptophan on the antioxidant capacity and immune response associated with TOR and TLRs/MyD88/NF-κB signaling pathways in northern snakehead, *Channa argus* (Cantor, 1842). **Frontiers in Immunology** 14: 1149151. DOI: 10.3389/fimmu.2023.1149151.