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Effects of dietary tryptophan on cannibalism, survival and growth of *Wallago attu* (Bloch & Schneider, 1801) juveniles

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Abstract. This study aims to evaluate the effects of supplemented commercial diets with tryptophan (TRP) on plasma serotonin, *cannibalism, survival rate, and growth of Wallago attu (*helicopter catfish). After one week of acclimation, 3,200 juveniles of helicopter catfish (BW = 2.5 ± 0.27 g, total length = 5.6 ± 0.43 cm) were randomly assigned for the experiment in fifteen plastic tanks (200 L). The experiment was conducted with five treatments (CT – 0 g TRP/kg – control group, T1 – 5 g TRP/kg, T2 – 10 g TRP/kg, T3 – 20 g TRP/kg, and T4 – 40 g TRP/kg) in a completely randomized design with three replications for four weeks. The results from the enzyme-linked immunosorbent assay reveal that TRP-supplemented diets effectively increased the serotonin level (5-HT) in the plasma of the catfish. The serotonin level increased with the dose of TRP added to the feed. Furthermore, a higher TRP level significantly decreased cannibalism and improved the final survival of the fish. However, the fish's growth rate among treatments T1, T2, T3, and T4 was not significantly different, but there was a statistical difference between the experimental treatments and the CT treatment. The findings of this study suggest that TRP could be supplemented at a dose of 20 $g \cdot kg^{-1}$ to the feed to reduce cannibalism and improve the final survival of helicopter catfish.

Keywords: cannibalism, growth, tryptophan, serotonin (5-HT), survival rate, *Wallago attu*

1 Introduction

*Wallago attu (*helicopter catfish) is an ideal species for aquaculture in several countries because of its fast growth and high nutritional value [1, 2]. This species is categorized as vulnerable species, listed as "nearly threatened", with a rapidly decreasing trend in the natural population, according to IUCN [3]. However, the high rate of cannibalism is one of the major challenges for the commercial culture of this species [4] because of its highly predatory nature [1]. Based on the anatomical features of incurved vomerine teeth and swimming tactic, helicopter catfish is believed to inherit aggressive behaviours that cause high mortality during the larval stage [4].

Numerous factors affect the rate of cannibalism in teleost fish. These factors include fish size and size variation in a cohort [5, 6], feeding [7], shelter [8], stocking density [9], and light intensity [10, 11]. Several solutions were implemented to reduce the rate of cannibalism of helicopter catfish at larval stages. Particularly, low stocking density, size-grading, and segregation of larvae during the rearing period can improve the survival rate and reduce the cannibalism rate [4, 12]. However, these methods require time, space, high labour costs, and additional stressors for fish.

Serotonin (5-HT) is a key hormone contributing to regulating aggressive behaviours in aquatic animals [13, 14]. In particular, an increase in brain serotonergic activity inhibits

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aggressive behaviour in numerous vertebrate species, including fish [15]. The fish occupying low positions in the dominance hierarchy are characterized by a stress-induced increase in brain serotonin turnover [16]. In addition, aggressive behaviours in cichlid fish (*Aequidens pulcher*) and knife fish (*Apteronotus leptorhynchus*) were inhibited when they were injected with 5-HT [17, 18]. Intraperitoneal injection with p-chlorophenyl alanine (PCPA), a 5-HT synthesis inhibitor, stimulated the aggression of male fire mouth cichlid (*Thorichthys*) [19].

Tryptophan (TRP) is a large neutral amino acid, the precursor of the monoaminergic neurotransmitter 5-HT [20]. Adding TRP to the diet improved the production of 5-HT in the fish brain and stress tolerance [21, 22] or reduced aggression [13]. Recently, the supplementation of TRP in feed has been proven to decrease the cannibalism of some farmed fish species. For example, oral administration of TRP resulted in lower cannibalism in pikeperch post-larvae (*Sander lucioperca*) [23] and Asian seabass (*Lates calcarifer*) fry [24]. Similar results were found for other fish, such as rainbow trout (*Oncorhynchus mykiss*) [22], orange-spotted grouper (*Epinephelus coioides*) juvenile [13], and Atlantic cod (*Gadus morhua*) [25].

Previous efforts to regulate cannibalism in helicopter catfish mainly focused on the routine of size-grading and stocking density [4, 12]. Therefore, the present study aims to investigate the effects of dietary tryptophan supplementation on cannibalism, survival, and growth of helicopter catfish juveniles.

2 Material and methods

2.1 Experimental fish

A total of 3,200 juveniles of helicopter catfish were obtained from the Hatchery Centre, Quang Tri province, Vietnam (16°49′51″N, 107°04′02″E). Before the experiment, these fish were held in a recirculating tank (50 m^3) with continuous aeration for one week under simulated ambient autumn photoperiod and temperature (26–27 °C) for acclimation for one week prior to the experiment. During this period, fish were fed once daily in the morning (at 12:00 noon) with the commercial dry pellet (Nanolis C, Ocialis) at a ratio equivalent to 1–2% body weight (BW) per day. Commercial diets contained 55% protein, 6% lipid, 14% ash, and 3% fibre.

2.2 Preparation of the experimental diets

The commercial dry pellet was supplemented with TRP (tryptophan, Sigma) at different levels (5, 10, 20, and 40 g TRP/kg) according to the sprinkled method by Jaroslaw and Zdzislaw [23]. In brief, the amount of TRP for each treatment was weighed and dissolved in hot water and ethanol (80%). Subsequently, the TRP was sprinkled on the commercial diets, which were then dried in the oven at 37 °C for one hour, then stored at 4 °C until being used. To avoid the palatability effects, we sprinkled the control treatment (without TRP supplementary) with an ethanol solution.

2.3 Experimental design

After one week of acclimation, 3,000 juveniles of helicopter catfish (BW = 2.5 ± 0.27 g, total length (TL) = 5.6 ± 0.43 cm) were randomly selected and assigned to fifteen plastic tanks (200 juveniles/1,000 L) under the same conditions as the stock tank. They were kept in the tanks without feeding for one day before the experiment commenced. Subsequently, these tanks were randomly assigned to five experimental groups (CT – 0 g TRP/kg – control group, T1 – 5 g TRP/kg, T2 – 10 g TRP/kg, T3 – 20 g TRP/kg, and T4 – 40 g TRP/kg) with the completely randomized design method with three replicates. The fish were reared for four weeks and fed three times a day (at 8 a.m., 1 p.m., and 6 p.m.) until apparent satiation. The satiation level was determined based on apparent visual satiety. The uneaten feed and faecal matter were removed by siphoning for 30 minutes after feeding each meal every day. The temperature, pH, and DO in experimental tanks were measured with a thermometer, a pH meter (Model: HI 98127; Manufacturer Hanna Instruments, USA), and a DO meter (Model: YSI 550A; Manufacturer: Yellow Spring Instrument Company, Ohio, USA). Water samples were collected daily at 8 a.m. and 6 p.m. from the tanks to determine $N–NH_4^+$ and $N–NO_2^-$. Water quality parameters (mean ± SD) were recorded during the experimental period as follows: water temperature 27.5 ± 0.5 °C, pH 7.5 ± 0.3, DO 5.8 ± 0.4 $mg·L^{-1}$, ammonia and nitrite nitrogen < 0.1 and 0.05 $mg·L^{-1}$.

2.4 Sampling protocols

At the beginning and the end of the experiment, thirty fish in each tank were randomly collected. Fish were anaesthetized in 10 $mL·m^{-3}$ AQUI-S®. Subsequently, the total length (TL) and weight of the fish (BW) were measured to determine the growth rate before collecting blood from the caudal vein by using a syringe. Blood collection was only conducted at the end of the experiment. The blood was stored in 1.5 mL Eppendorf tubes containing 5 µL of 200 $mg·mL^{-1}$ ethylenediaminetetracetic acid (EDTA) in an ice box to prevent blood from clotting until the sampling session was finished. Blood was then centrifuged at 4 °C at 1,000 × g for 15 minutes to collect plasma. Plasma was aspirated and stored at –80 °C until the serotonin assay.

2.5 Serotonin assay

The level of serotonin (5-HT) was quantified in plasma samples with an enzyme-linked immunosorbent assay (ELISA) according to the kit instruction (Fish 5-hydroxytryptamine/serotonin (5HT/ST) Elisa kit) from My BioSource Company. A volume of 50 µL of each plasma sample and the standard solution was added to the wells of a microplate. The intra- and inter-assay coefficients of variation were 8 and 10%.

2.6 Calculation and statistical analysis

The growth parameters of fish were estimated by determining body weight (final body weight) and total length (final total length) at the beginning and the end of the experiment. The dead fish was recorded daily. The natural mortality rate (NMR) is the percentage of the fish dying during the experimental period for unknown reasons. The cannibalism rate (CR) is calculated as the percentage of individuals that died without carcasss (absence of fish).

All the parameters were calculated as follows:

CR,% = $[(F_s - F_e - F_d)/F_s] \times 100$;

NMR,% = % total mortality rate – % cannibalism rate (CR);

Specific growth rate (SGR %/day) = $(L_n \times W_e - L_n \times W_s) \times 100/t$.

Coefficient of variation, CV, % = (Standard deviation of individual /Mean of n observation) × 100.

Survival rate (SR) % = $F_e \times 100/F_s$ [13, 26].

where F_s is the initial number of fish; F_e is the number of remaining fish; F_d is the number of naturally died fish; W_s and W_e are the weight of fish at the beginning and end of the experiment; and t is the experimental time in days.

The data were processed as mean values of three replicates (n = 3) by using biological statistics on Microsoft Office Excel 2016 and SPSS software (version 20.0 for Windows). The influence of the TRP supplement on cannibalism, growth, and survival of fish was analyzed in terms of ANOVA (multi-comparisons Tukey-Kramer HSD posthoc test). Statistical comparison tests were conducted at a level of significance of 0.05.

3 Results and discussion

3.1 Plasma serotonin (5-HT) levels of fish

ELISA shows that TRP-supplemented diets caused an increase in the level of 5-HT in the plasma of helicopter catfish (Fig. 1). The 5-HT concentration increased proportionally with the TRP dose. The highest and lowest 5-HT concentrations were in the T4 and CT treatments. The 5-HT concentration significantly differed among the treatments ($p < 0.05$). However, there was no significant difference in the concentration of 5-HT between T3 and T4 ($p > 0.05$). Furthermore, the plasma 5-HT level and the amount of TRP-supplemented diets were significantly positively correlated ($r = 0.917$, $F = 463.86$, $p = 0.001$, $R^2 = 0.841$).

The results of the present study confirmed the report of Johnston et al. [21] that a higher amount of tryptophan added to food leads to an increase in blood tryptophan, thereby increasing the availability of tryptophan for absorption into the brain. Tryptophan is converted to 5-HT by the action of tryptophan hydroxylase and aromatic amino acid decarboxylase in the presence of vitamin B6. Tryptophan hydroxylase is not saturated by its substrate; therefore, increasing tryptophan intake causes an increase in 5-HT in the fish brain [21]. Some previous studies reported that tryptophan supplementation increased 5-HT [27-29]; however, these studies dealt with short-term TRP-supplemented diets (7–15 days). According to Rubio et al. [30], the concentration of 5-HT in the plasma of fish varied with TRP intake, stress, and fish culture conditions.

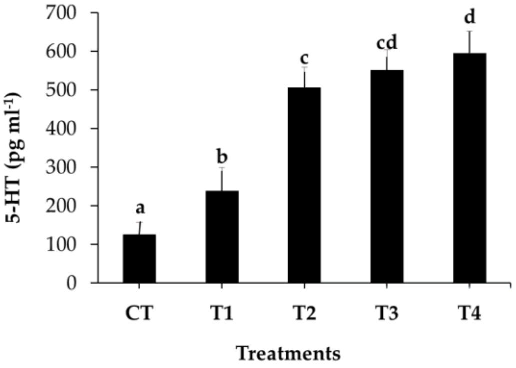

Fig. 1. Effect of supplementary dietary tryptophan on plasma serotonin (5-HT) level in juveniles

Different letters indicate significant differences ($p < 0.05$).

3.2 Cannibalism, natural mortality, and survival rate

The addition of TRP to fish feed was effective in reducing CR. Fig. 2 shows that the CT treatment had the highest CR (48.7%), significantly different from the other treatments ($p < 0.05$). The CR of treatment T3 (9.5%) and T4 (8.3%) was substantially lower than that of T1 and T2 ($p < 0.05$). For the natural mortality rate (Fig. 3) among the levels of TRP supplementation, there was no significant difference ($p > 0.05$).

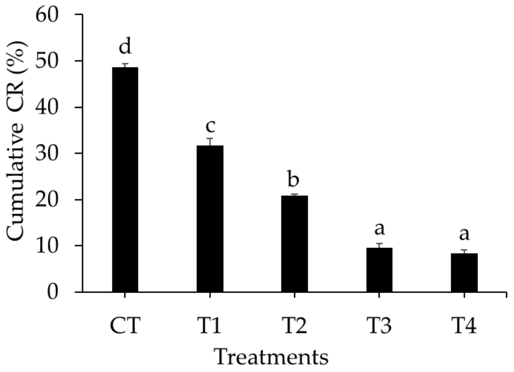

Fig. 2. Effect of supplementary dietary tryptophan on cannibalism rate of helicopter catfish juveniles

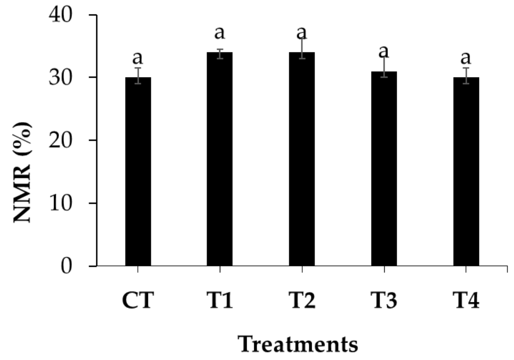

Fig. 3. Effect of supplementary dietary tryptophan on cumulative natural mortality (%) of helicopter catfish juveniles
Data with the same letter are not significantly different among the treatments ($p < 0.05$).

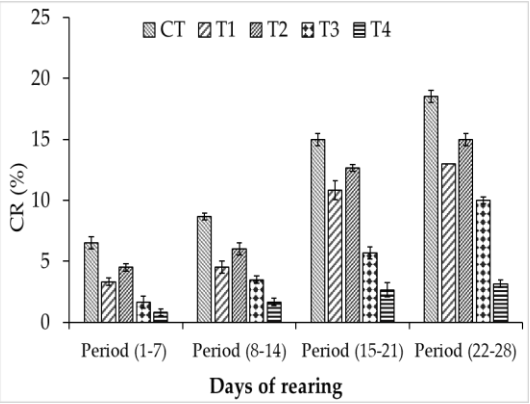

Fig. 4. Weekly cumulative cannibalism rate (%) of helicopter catfish juveniles

Giri et al. [31] and Sahoo et al. [32] reported that cannibalism was the leading cause of mortality in helicopter catfish. Fig.s 1 and 2 show that serotonin levels increase proportionally with the amount of TRP added to the feed, and the CR decreases when the level of TRP increases. Our study reveals that dietary supplementation of TRP increases the 5-HT level in plasma and, thus, reduces catfish's aggressive behaviour. This result is consistent with that of De et al. [33], who reported the influence of serotonin concentration on the behaviour of goldfish (*Carassius auratus*). Likewise, Hseu et al. [13] concluded that adding TRP to the feed increased 5-HT concentration and reduced cannibalism in grouper (*Epinephelus coioides*). Similar conclusions were found for rainbow trout (*Oncorhynchus mykiss*) and European seabass (*Dicentrarchus labrax*) [34, 35].

The weekly cumulative CR in all treatments gradually increased over time in the experiment (Fig. 4). In the first two weeks of culturing, the CR ranged from 14.2 to 22.8%, reaching around 38–48% after four weeks. The CT treatment had the lowest survival rate of fish (21.3%) (Fig. 5). There was a significant difference in the survival rate of fish among treatments ($p < 0.05$), except T3 and T4 ($p > 0.05$). The survival rate of fish in treatments increased with the increasing amount of TRP supplements. This result agreed with previous studies reporting that the leading cause of the low survival rate in the predatory species was aggressive cannibalistic behaviour [36, 37].

Cannibalism depends on the size heterogeneity and density of predators in the aquarium [38]. Therefore, to reduce CR, frequent grading or separating of oversized fish of the cohort should be conducted [36, 37]. In this study, the fish of considerable size were not separated, possibly causing a quick increase in the CR. According to Baras and Jobling [38], reducing aggressive behaviour at the earlier stage was necessary, and it could lead to a decreasing CR. In addition, optimizing feeding schedules in all

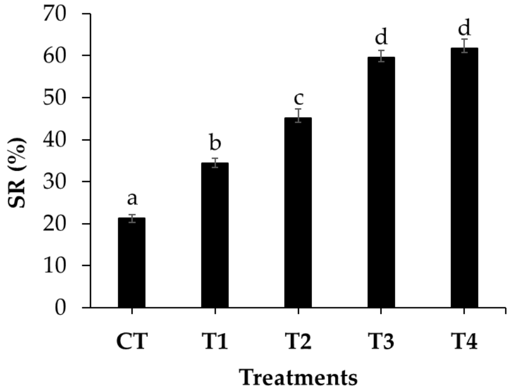

Fig. 4. Cumulative survival rate of fish in different supplementary dietary tryptophan treatments

Different letters in the bar indicate the significant difference among treatments ($p < 0.05$).

nutritional aspects and management capacity can minimize losses due to cannibalism in intensive fish farming [39].

The dietary supplementation of TRP is believed to reduce aggressive behaviour in several fish species, such as Atlantic cod (*Gadus morhua*) [25], the rainbow trout (*Oncorhynchus mykiss*) [22], and orange-spotted grouper (*Epinephelus coioides*) [13]. The studies above suggested that the reduction of aggressive behaviour in fish originated from higher brain plasma 5-HT activities resulting from the presence of TRP in the feed supply.

3.3 Fish growth parameters

In this study, the TRP addition to the feed affected the growth of the experimental fish (Table 1). The total body length and mean body weight among treatments (T1, T2, T3, and T4) were not significantly different ($p > 0.05$), but there was a statistical difference between the experimental treatments and the CT treatment ($p < 0.05$).

The fish growth rate in length (SGR_L) in the TRP treatments was significantly higher than that of the CT treatment ($p < 0.05$), whilst the growth rate in weight (SGR_W) did not differ significantly among the groups ($p > 0.05$). This suggests that the additional level of TRP did not significantly affect the weight growth but could improve the survival rate and reduce the CR compared with the control treatment ($p < 0.05$). Similarly, the size heterogeneity of fish (CV_L and CV_W) in the experimental groups (T1, T2, T3, and T4) was much lower than that of the CT group ($p < 0.05$). This difference is probably caused by the size heterogeneity in the control group, where the larger fish predate the smaller ones. Sahoo et al. [12] demonstrated that sizing and separating oversized fish improved the growth and survival rates compared with the control group. According to Folkvord and Ottera [40], strict size grading enhanced the survival rate in Atlantic cod (*Gadus morhua*). Therefore, it is recommended to develop suitable habitats and reasonable density to improve the survival rate of fish [41].

Table 1. Growth performance parameters and coefficient of variation of helicopter catfish juveniles during four weeks of experiment

Parameters	TRP supplemented experimental diets				
	CT	T1	T2	T3	T4
TL (cm)	8.40 ± 1.61^a	13.5 ± 0.80^b	14.6 ± 0.87^b	14.8 ± 0.55^b	15.2 ± 0.42^b
BW (g)	16.0 ± 2.99^a	20.5 ± 0.96^b	21.4 ± 0.80^b	22.1 ± 0.61^b	23.0 ± 0.46^b
SGR_L (%/day)	1.41 ± 0.82^a	3.13 ± 0.15^b	3.42 ± 0.18^b	3.46 ± 0.30^b	3.56 ± 0.09^b
SGR_w (%/day)	6.59 ± 0.95^a	7.53 ± 0.38^a	7.68 ± 0.42^a	7.79 ± 0.37^a	7.93 ± 0.27^a
CV_L (%)	19.6 ± 3.69^a	5.97 ± 0.35^b	5.98 ± 0.35^b	3.73 ± 0.14^b	2.75 ± 0.08^b
CV_W (%)	19.2 ± 3.46^a	4.69 ± 0.22^b	3.75 ± 0.14^b	2.74 ± 0.08^b	1.99 ± 0.04^b

Note: Mean values are data of three replicates ($n = 3$) where 30 individuals of each replicate were pooled for analysis. Data with the same letters a, b in each row are not significantly different among treatments ($p > 0.05$).

Thus, the supplement of TRP to the feed effectively increased the plasma serotonin (5-HT) level, reducing the CR but not affecting the growth of helicopter catfish. According to Papoutsoglou et al. [34], a high level of serotonin tended to limit food intake and growth in fish [33, 35]. Similarly, Hseu et al. [13] observed that the groups of fish treated with TRP even expressed a lower growth rate and suggested that this could be an effect of increasing brain serotonergic activity and decreasing aggression and appetite. Also, the treatment with TRP-supplemented feed resulted in depressed growth, increased food consumption, and food conversion ratio [34, 35]. In the present study, however, there were no adverse effects of TRP supplementation on growth, although the feed intake and the food conversion ratio were high. In contrast, the growth index of TRP-supplementation treatments was higher than that of the control treatment.

This work is the first study of its kind, reporting the effect of TRP supplementation on the growth and cannibalism–survival relation in helicopter catfish. Therefore, this effect could be even more conspicuous when combined with other methods for decreasing cannibalism, such as optimal stocking densities, feeding, segregation, or refuges. The fact that nutritional requirements of these fish are still unknown leads to the unavailability of a commercial diet for these species in the market. It is advisable to determine the most suitable time for TRP supplementation when cannibalism is the highest to save costs and improve production efficiency.

4 Conclusion

This study shows that the concentration of 5-HT in the plasma of helicopter catfish increased proportionally with the dietary tryptophan supplementation, and the cannibalism rate decreased. The increasing level of tryptophan improved the survival rate of helicopter catfish but did not affect the fish's growth. Our findings suggest that tryptophan could be supplemented at a dose of 20 $g \cdot kg^{-1}$ to the feed to reduce cannibalism and improve the final survival of helicopter catfish.

The certificate of animal ethics approval

This study was carried out under the recommendations in Animal Welfare and Viet Nam's code of practice for the care and use of animals for scientific research. The protocol, species and number of animals used in this study were approved by the Hue University Animal Ethics Committee (No. HU VN0005).

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Conflicts of interest

The authors declare no conflicts of interest.

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