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Tryptophan Reduces Intracohort Cannibalism Behavior in Tropical Gar (*Atractosteus tropicus*) Larvae

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Abstract: The intracohort cannibalism present in tropical gar larvae (*A. tropicus*) generates great problems in its culture, as in other fish species around the world. The addition of tryptophan (Trp) (10, 20, and 30 g/kg) and a control diet (CD) without Trp were evaluated in *A. tropicus* larvae regarding growth, survival, cannibalism, behavior, digestive enzymatic activity, and genes related to aggressiveness and/or cannibalism in two stages: 0–13 days after hatching (DAH); and only cannibals (14–24 DAH). In the first stage, no differences were observed in growth parameters; cannibalism was lower with the use of Trp, with the lowest percentage being the 10 g/kg Trp treatment (56.75 ± 2.47%) compared to CD (64.75 ± 1.76%). In the second stage, survival was greater in 10 g/kg Trp (75.00 ± 7.07%) than in CD (23.33 ± 5.77%). Thus, cannibalism was lower with 10 g/kg Trp (20.0 ± 10.0%) compared to CD (76.66 ± 5.77%). Cannibal larvae fed with 10 g/kg Trp had a greater enzymatic activity in acid and alkaline proteases and leucine aminopeptidase, as well as the overexpression of *avpi1*, *crh*, and *htr1a* and the subexpression of *tph1*, *th*, *sstr1*, and *hdc* ($p < 0.05$). No aggressive behaviors were recorded in the larvae fed with the 10 g/kg Trp treatment, unlike those fed with CD. The use of 10 g/kg Trp improves survival and reduces cannibalism in *A. tropicus* larvae.

Keywords: intracohort cannibalism; mitigation; survival; fish larvae; behavior

Key Contribution: The use of 10 g/Kg of Trp in the diet improves survival and reduces cannibalism in *A. tropicus* larvae. Cannibal larvae of *A. tropicus* show a greater growth due to the "jumper" effect, unlike non-cannibal larvae.

1. Introduction

In fish, around 390 species have been documented that show some type of cannibalism, a behavior that occurs most of the time throughout their life or exclusively in the early stages of development (larvae and/or juvenile) [1]. Tropical gar (*Atractosteus tropicus*) shows cannibalism in the larvae stage (10 DAH (days after-hatching)) in captivity and in the

wild [2,3]. This species is considered of importance; it has economic value due to its culture and fishing, as well as ecological and cultural value in Mexico and Central America [4,5]. However, the presence of cannibalism in the early stages of development limits the growth, survival, and profitability values of the production. For example, Frías-Quintana et al. [6] reported, for *A. tropicus* in laboratory conditions, survival and cannibalism values of 24% and 33%, respectively. In another study, Palma-Cancino et al. [7] used co-feeding (commercial diet and *Artemia nauplii*) on *A. tropicus* larvae; cannibalism was recorded with complete (11 DAH) and incomplete (21 DAH) ingestion, obtaining survival values ranging from 1 to 33%. The problem is magnified because the fish that are not consumed but are injured by the attacks die. When modifying the diet of *A. tropicus* larvae with a low concentration of polyunsaturated fatty acid, similar cannibalism values (40%) and survival values ranging from 15 to 30% continued to be found [8].

Because of this, different strategies have been evaluated in several species to mitigate cannibalism in fish, one of them being the incorporation of Tryptophan (Trp) in the diet. Trp is an essential amino acid, which is the precursor of 5-hydroxytryptamine (5-HT) (serotonin) [9], a neurotransmitter responsible for controlling appetite [10], reproduction [11], and physiological processes related to immunity and intestinal homeostasis [12]. It has been observed that the administration of Trp in the fish *Aequidens pulcher* and *Apteronotus leptorhynchus* reduced their aggressiveness [13,14]. Meanwhile, the administration of the inhibitor 5-HT (p-chlorophenylalanine) increased aggressiveness in *Cichlasoma meeki* [15]. Likewise, the incorporation of Trp at concentrations of 2, 4, and 6 ppm and 2 and 3% in the diet induced a significant decrease of 50% in the cannibalism of Pabda (*Ompok bimaculatus*) larvae [16,17].

Therefore, the objective of this work is to evaluate the effect of Trp administration in the diet of *A. tropicus* larvae, as a possible mitigant of cannibalistic behavior. The rate of cannibalism, survival, growth, enzymatic activity, and the expression of genes related to cannibalism and behavior are analyzed.

2. Materials and Methods

2.1. Biological Material

The *A. tropicus* larvae were obtained for this experiment by inducing a female (3.4 kg, 91 cm) to spawn using the hormone LHRHa (Sigma-Aldrich, Taufkirchen, Germany) (30 µg/kg fish^{-1}) applied intramuscularly. Subsequently, the female and three males (1.6 kg, 35 cm, no hormone induction) were placed in a circular tank (2000 L) with raffia thread to simulate the natural spawning site. The above was conducted with the broodstock batch from the Laboratorio de Fisiología en Recursos Acuáticos (LAFIRA) from Division Academica de Ciencias Biologicas of the Universidad Juarez Autonoma de Tabasco.

2.2. Experimental Design

The present study consisted of two stages. In the first stage, three concentrations of Trp (10, 20, and 30 g/kg) and a control diet (CD; no Trp) were evaluated; this stage lasted from day 3 to 13 DAH of larvae. Larvae (n = 200, 0.018 ± 0.001 g; 1.28 ± 0.09 cm) were placed in circular-shaped 70 L tanks (2.8 larvae/L). Each treatment was carried out in triplicate. The feeding regime was as follows: after yolk sac absorption (3 DAH), co-feeding was supplied; treatment diet and Artemia nauplii were administered for additional five days. At the end of the co-feeding, fish were fed only with the formulated feed, four times per day (8:00, 12:00, 16:00, and 20:00 h) ad libitum. In the second stage, only cannibal larvae were selected and fed with Trp (10 g/kg) and CD. Treatments were carried out in triplicate with 10 fish per replicate (0.14 larvae/L). This stage lasted from 14 to 24 DAH. Fish were weighted and sized at the beginning and at the end of each stage and cannibalism was quantified. For both stages, the tanks were connected to a recirculation system powered by a 0.5 HP water pump (Jacuzzi, JWPA5D-230A, Delavan, WI, USA) and a 1500 L reservoir for solid deposition and a biological filter. A partial daily exchange of 10% water was carried out by siphoning feces and uneaten feed. The water quality parameters were daily monitored

temperature (27.36 ± 0.6 °C), dissolved oxygen (4.8 ± 0.4 mg/L, oximeter YSI 85; Yellow Springs, OH, USA), and pH (7.1 ± 0.3, HANNA HI 991001, Nusfalau, Romania).

2.3. Formulation and Preparation of the Experimental Diets

The formulation of the diets was carried out using the software MIXITWIN v.5.0. (Microsoft Windows, Washington, DC, USA), following Álvarez-González et al. [18]. The macronutrients were weighed and mixed, followed by the incorporation of micronutrients. In this step, we added tryptophan (Sigma-Aldrich, Taufkirchen, Germany reagent grade, ≥98%) in the different concentrations mentioned. This was followed by the addition of liquid ingredients. To achieve an adequate mixture, water was added 400 mL/kg per diet and mixed 15 min with each addition (a total of 60 min of mixing time per diet). The mixture was passed through a meat grinder (Torrey, M-22RI, Nuevo Leon, Mexico) and pellets were oven dried at 55 °C for 12 h (Coriat, HC-35-D, Ciudad de Mexico, Mexico). Finally, the pellets were manually ground and sieved to particles smaller than 0.5 mm (used during co-feeding) and larger than 0.7 mm (used after co-feeding). Diets were stored in hermetic plastic bags at −20 °C for later use. For all diets, the proximal components (moisture, ash, lipids, and protein) were analyzed according to AOAC [19] (Table 1), lipids (Table 2), and amino acids (Table 3).

Table 1. Composition of the experimental diets with different concentrations of Trp and CD.

Ingredients	CD	Trp (g/kg) 10	20	30
Fish meal [a]	305.4	305.4	305.4	305.4
Renderer meal [a]	300.0	300.0	300.0	300.0
Soy meal [a]	150.0	150.0	150.0	150.0
Corn starch [b]	67.1	67.1	67.1	67.1
Oil soy [c]	116.5	116.5	116.5	116.5
Cellulose [d]	30.0	20.0	10.0	0.0
Tryptophan [d]	0.0	10.0	20.0	30.0
Premix vit-min [e]	15.0	15.0	15.0	15.0
Grenetine [f]	10.0	10.0	10.0	10.0
Vit C [g]	5.0	5.0	5.0	5.0
Vit E [h]	1.0	1.0	1.0	1.0
Proximate composition (g/100 g dry matter), except energy				
Energy (kJ/g)	17.91	17.53	17.39	17.48
Protein	44.00	45.00	46.00	47.00
Ether extract	16.38	16.59	16.41	16.48
Fibre	1.05	1.12	1.07	1.01
Ash	13.43	13.23	13.02	12.93
NFE [1]	25.14	24.06	23.5	22.58

[a] Marine and agricultural proteins S.A. de C.V., Guadalajara, Jalisco; [b] IMSA Corn Industrializer S.A de C.V. Guadalajara, Jalisco, México; [c] Ragasa industries S.A. de C.V.; [d] Sigma-Aldrich Quimica S. de R.L. de C.V.; [e] Vitamin premix composition g, mg, or International Units per kg of diet: Vitamin A, 10,000,000 IU; Vitamin D3, 2,000,000 IU; Vitamin E, 100,000 IU; Vitamin K3, 4.0 g; Thiamine B1, 8.0 g; Riboflavin B2, 8.7 g; Pyridoxine B6, 7.3 g; Vitamin B12, 20.0 mg; Niacin, 50.0 g; Pantothenic acid, 22.2 g; Inositol, 0.15 mg; Nicotinic Acid, 0.16 mg; Folic Acid, 4.0 g; Biotin, 500 mg; Vitamin C, 10.0 g; Choline, 0.3 mg, Excipient q.s., 2 g; Manganese, 10 g; Magnesium, 4.5 g; Zinc, 1.6 g; Iron, 0.2 g; Copper, 0.2 g; Iodine, 0.5 g; Selenium, 40 mg; Cobalt, 60 mg; Excipient q.s., 1.5 g; [f] D'gari, food and diet products relámpago S.A. de C.V.; [g] ROVIMIX® STAY-C® 35-DSM, Guadalajara, México; [h] GELPHARMA, S.A. de C.V. NFE [1] = nitrogen-free extract: 100-(%protein-%ethereal extract-%ash-%fibre).

Table 2. Analysis of the total fatty acids in the experimental diets used for *A. tropicus* larvae.

Fatty Acids (%)	CD	Trp (g/kg) 10	20	30
C13:0	7.0	10.2	9.7	7.9
C14:0	1.2	1.3	1.2	1.2
C16:0	16.7	17.0	17.0	17.4
C17:0	ND	ND	ND	ND
C18:0	5.8	6.0	6.0	6.1
C23:0	ND	ND	ND	ND
ΣSFA	**30.7**	**34.4**	**33.9**	**32.7**
C16:1n7	2.0	2.1	2.1	2.2
C18:1n9	21.0	20.4	20.5	21.1
C18:1n7	1.6	1.8	1.8	1.8
ΣMUFAS	**24.6**	**24.3**	**24.5**	**25.1**
C18:2n6	34.9	32.0	32.3	32.6
C18:3n3	0.3	4.3	4.3	4.3
C18:4n3	4.7	ND	ND	ND
C20:3n3	0.5	0.6	0.7	0.7
C20:4n6	0.3	ND	ND	ND
C20:5n3	1.6	1.7	1.8	1.7
C22:5n3	0.4	0.4	0.4	0.4
C22:6n3	1.9	2.2	2.2	2.4
ΣPUFAS	**44.6**	**41.3**	**41.6**	**42.2**
NID	**0.0**	0.0	0.0	0.0
	100.0	100.0	100.0	100.0

Table 3. Analysis of the total amino acids in the experimental diets used for *A. tropicus* larvae.

Amino Acid	CD	Trp (g/kg) 10	20	30
Essential amino acids				
HIS	1.1	1.0	1.2	1.0
ARG	5.2	5.6	5.4	5.3
THR	1.7	1.6	1.7	1.7
VAL	1.7	1.6	1.6	1.6
MET	0.5	0.5	0.2	0.2
LYS	5.7	5.0	5.3	5.5
ILE	1.3	1.2	1.3	1.3
LEU	3.5	3.2	3.5	3.5
PHE	1.4	1.2	1.4	1.4
subtotal	22.1	20.9	21.5	21.7
Non-essential amino acids				
ASP	2.4	2.2	2.4	2.4
SER	2.3	2.2	2.4	2.3
GLU	5.8	5.7	6.0	6.0
GLY	7.0	8.2	7.1	6.9
ALA	3.4	3.7	3.6	3.5
TYR	1.5	1.3	1.4	1.5
subtotal	22.3	23.4	22.9	22.7
Others				
TAU	0.6	0.7	0.6	0.6
Total	45.0	45.0	45.0	45.0
Tryptophan (mg/g)	10.60	19.74	32.42	44.21

2.4. Growth Indexes and Feed Quality

Weight and length were measured as follows: At the beginning of the experiment (3 DAH) and at the end of the experiment (13 DAH) as well as in the second phase (14–24 DAH). The individual weight of each organism was determined by using an analytical balance (A&D Company, Limited mod.HR-250, Seoul, Republic of Korea). The total length was calculated by analyzing the photographs taken of the organisms, through a transparent container with a scale using the software ImageJ 1.51j8 (U.S. National Institutes of Health, Bethesda, MD, USA). The following biometrics were calculated as follows: Survival (S): (final fish number/initial fish number) × 100; feed intake (FI): total feed intake per experimental unit/number of rearing days; absolute weight gain (AWG): final weight (g)—initial weight (g); specific growth rate (SGR): [(ln final weight − ln initial weight)/days] × 100; feed conversion ratio (FCR): (feed intake, g dry matter)/(fish weight gain, g); condition factor (K): [(wet weight (g) × total length − 3 (cm)] × 100; and protein efficiency ratio (PER): fish live weight gain (g)/dry protein fed (g). Visually deformed organisms (scoliosis, crossbite, lower jaw reduction, and without eyes) were identified and counted by monitoring organisms with erratic swimming, high pigmentation, and visible malnutrition. These organisms were collected after death, and the deformity was confirmed visually using a stereomicroscope (Carl Zeiss mod. Stemi DV4, Göttingen, Germany). The percentage of deformed organisms was calculated using the following formula: Deformity (D) (fish with deformities/initial fish number) × 100. Also, the coefficient of variation (%) (CV) ((standard deviation of individual weight/mean individual weight) × 100) and the size heterogeneity (weight) (SH) ((final coefficient of variation/initial coefficient of variation)) were calculated.

2.5. Collection of Biological Samples

All procedures were performed according to the Official Mexican Norm (NOM-062-ZOO-1999) [20] of Animal Welfare and with the Declaration of Helsinki.

At the end of the experiment of the first stage, 15 larvae per treatment were euthanized with a cold temperature shock; larvae were in a tray and then placed in an ultra-freezer at −80 °C (Lexicon II ultra-low freezer, Singapore) for 3 min, and then dissected to determine the effect of Trp on growth, digestive enzymes, gene expression, and cannibalism. In the second stage, nine larvae per treatment were used, and the same euthanized protocol was used. For the analysis of the enzymatic activity, the larvae were preserved at −80 °C. Finally, to analyze the gene expression, the larvae were preserved in RNAlater buffer according to the manufacturer's instructions (Invitrogen, Waltham, MA, USA) at −80 °C. Whole larvae were used in all analyses.

2.6. Cannibalism

In both stages, cannibalism was measured by monitoring larvae 30 min before and 60 min after each feeding. The criteria to determine cannibalism was the following: attack by bites (a fish attacks another fish by bites without the attacker ingesting the prey), partial cannibalism (one fish partially eats another fish), and complete cannibalism (one fish entirely eats another fish). These three behaviors are classified as cannibalism. The formula (fish with cannibalistic behavior/initial fish number) × 100) was used to quantify cannibalism in each treatment. The attacking larvae (cannibal) and the attacked larvae (non-cannibal) were counted with the weight and size being registered. Five larvae were sampled for molecular analysis and five for enzymatic activity, as described earlier.

2.7. Digestive Enzyme Activity

For the quantification of digestive enzymes, we followed the Shuangyao et al. [21] protocol: The larvae were manually macerated inside 1.5 mL tubes on ice. For this, 100 mg of tissue were placed in a total volume of 0.5 mL and centrifuged at 12,000× *g* at 4 °C for 15 min. The supernatant was recovered and stored at −80 °C in 30 µL aliquots. Soluble protein was determined using the Bradford assay [22]. For the quantification of acid

proteases, 0.5% hemoglobin solubilized in 100 mM glycine-HCl pH 2 buffer was used as a substrate. Alkaline proteases were quantified using 0.5% casein solubilized in 50 mM Tris-HCl and 10 mM CaCl at pH 9 [23]. In both assays, the samples were incubated at 37 °C, and the reaction was stopped using 0.5 mL of 20% trichloroacetic acid and centrifuged at 16,000× *g* for five minutes. Absorbance was read at 280 nm. The extinction coefficient (ε) to calculate the activity of acid and alkaline proteases was 0.005 mL/µM cm. To quantify trypsin activity, 1 mM BAPNA (Nα-Benzoyl-DL-Arginine-P-nitroanilide) dissolved in 50 mM Tris-HCl was used as a substrate, in pH 8 at 37 °C. Trypsin was read at 410 nm using an ε of 8800 mL/µM cm [24]. The Maroux et al. [25] method was used to determine the activity of leucine aminopeptidase, where 0.1 M leucine p-nitroanilide dissolved in DMSO with 50 mM sodium phosphate was the substrate, at pH 7.2, and incubated at 37 °C. Absorbance was measured at 410 nm with an ε of 8800 mL/µM cm. Lipase activity was determined using β-naphthyl acetate (100 mM) dissolved in 50 mM Tris-HCl at as substrate, at pH 7.5, with sodium taurocholate (100 mM) at 37 °C. The reaction was stopped with 0.72 N TCA. Fast Blue (100 mM) and a 1:1 ethanol/ethyl acetate mixture was added, and the absorbance was quantified at 540 nm using ε d 0.02 mL/µM cm [26].

The enzyme activity was determined using the following equations: units by mL (U/mL) = [Δabs × final reaction volume (mL)]/[ε × time (min) × volume extracted (mL)] − 1; specific activity (U/mg protein) = U mL/mg of soluble protein; and the molar extinction coefficient (ε).

2.8. RNA Extraction and Quantitative Reverse Transcription PCR (RT-qPCR)

Total RNA was extracted from complete larvae samples using Trizol (Invitrogen, Waltham, MA, USA), according to the manufacturer's protocol. The concentration and purity of RNA samples were assessed by the ratio between the absorbance at 260 and 280 nm in a spectrophotometer (Jenway GenovaNano, Cole-Parmer, Staffordshire, UK). RNA (1 µg) was reverse-transcribed (RT) using the SuperScript II kit (Invitrogen, Waltham, MA, USA), with a final volume of 20 µL. RT reactions were performed in a thermocycler (Mastercycler nexus GSX1, Eppendorf AG, Hamburg, Germany). The standard RT program used was as follows: 5 min at 65 °C, 10 min at 25 °C, 50 min at 42 °C (cDNA strand extension), 15 min at 70 °C (reverse transcriptase inactivation), and finally 20 min at 37 °C. Somatostatin receptor 1 (*sstr1*), tyrosine hydroxylase (*th*), histidine decarboxylase (*hdc*), corticotropin-releasing hormone (*crh*), 5-hydroxytryptamine (serotonin) receptor 1A, G protein-coupled (*htr1a*), gonadotropin releasing hormone 1 (*gnrh1*), arginine vasopressin-induced 1 (*avpi1*), and tryptophan hydroxylase 1 (*tph1*) for *A. tropicus* were designed from the species transcriptome (NCBI Accession: PRJNA395289) [27]. These genes were selected due to their influence on aggressive behaviors and interactions with specific neurotransmitters [28,29] (Table 4). The RT-qPCR was performed in a CFX96 Real-Time System (BioRad, Hercules, CA, USA) using 5 µL of EvaGreen Supermix (BioRad), 0.5 µL primers mix, and 4.5 µL of cDNA for a final volume of 10 µL. The RT-qPCR program was used at: 50 °C for 2 min, 95 °C 10 s, followed by 40 cycles at 95 °C 15 s, and 62 °C 1 min. *β-actin* was used as the reference gene [30]. The relative gene expression was calculated as the fold-change compared to the control and using the $2^{-\Delta\Delta Ct}$ formula [31].

Table 4. Oligonucleotide design for the real-time polymerase chain reaction (qPCR) of aggressive genes in *A. tropicus* larvae.

Protein	Gen	Primers (5′-3′)	Alignment Temperature (°C)
Somatostatin receptor 1	*sstr1*	FW: CCTCAGCATTGACCGCTACA RV: AATACCGCCATCCACTGACG	60
Tyrosine hydroxylase	*th*	FW: GGACCAGATGTACCAGCCAG RV: GCAGTTCATCCCTCGCAGAT	59
Histidine decarboxylase	*hdc*	FW: GCATTTCGACTGCACTGCTT RV: CTTCGGCTGAGTGGGATCTG	59
Corticotropin-releasing hormone	*crh*	FW: AACGTCAACAGGGCTTTCCA RV: TCTTCCCGTCAGGTCTTCCA	60
5-hydroxytryptamine (serotonin) receptor 1A, G protein-coupled	*htr1a*	FW: AAGCGCAGTGTGGAACCTAA RV: GCTGTCGGGGTATTAGGCAG	60
Gonadotropin-releasing hormone 1	*gnrh1*	FW: AGTCAGCACTGGTCATACGG RV: CTCACCTCCTCCGCAATGTC	59
Dopamine receptor D1	*drd1*	FW: TTTTTGGCCCTTTGGCTCATT RV: AAGTTCAAAATGGAGGCTGTGG	59
Arginine vasopressin-induced 1	*avpi1*	FW: AGGGAGGACCACTGAAGATGA RV: CCAGCAGAGGACAAGTCTGC	60
Tryptophan hydroxylase 1	*tph1*	FW: CCCCCGTATCGAGTTCACAG RV: AGGGGCAGGTTCTTGAGGTA	60

2.9. Effect of the Mitigants on the Ethology of Cannibal Larvae

We identified the effect of Trp (10 g/kg) on the cannibalistic behavior and/or ethology in the second stage experiment, comparing it to the larvae fed with the control diet (CD). Cannibal larvae were placed in 15 × 10 × 8 cm fish tanks. To reduce the effect of stress caused by the transference of the larvae to the experimental tanks, an acclimation time of 15 min was allowed. Subsequently, 15 min video recordings were made (Gopro Hero 7 Silver, Monterey, CA, USA). Two larvae per tank were evaluated under the following challenges: without shelter, with rocks, and with artificial vegetation. The videos were analyzed using Tracker 5.1.5 (Free Software Foundation, Inc., Franklin Street Boston, MA, USA) and BORIS 7.9.24 [32], which allow the identification and measurement of aggressive behavior and shelter preference (rocks and artificial vegetation). The same experiment was carried out on CD-fed larvae to determine the effects of Trp on cannibalism.

2.10. Statistics Analysis

Normality (Kolmogorov–Smirnov) and homoscedasticity (Bartlett) tests were performed. A one-way ANOVA was carried out for all the analysis, and in the case of finding differences, a posteriori test of unequal N HSD (Tukey) was used. A Student's *t*-test was used to compare among treatments where applicable. To analyze the gene expression data, non-parametric Kruskal–Wallis and Nemenyi posteriori tests were used. All tests were performed using the software Prism V. 9.0 with a significance value of 0.05.

3. Results

3.1. Growth Indexes and Survival

In the first stage, no significant differences were observed in the weight and total length between *A. tropicus* larvae fed with the Trp diets and CD (Figure 1). Likewise, no significant differences were observed in survival; however, the larvae fed with Trp presented a slight tendency of better survival (Table 5). There were no differences in AWG, SGR, FCR, PER, K, CV, and SH ($p < 0.05$). At the end of this stage, the distribution of the weights and sizes of the fish did not show significant differences among the treatments (Figure 2).

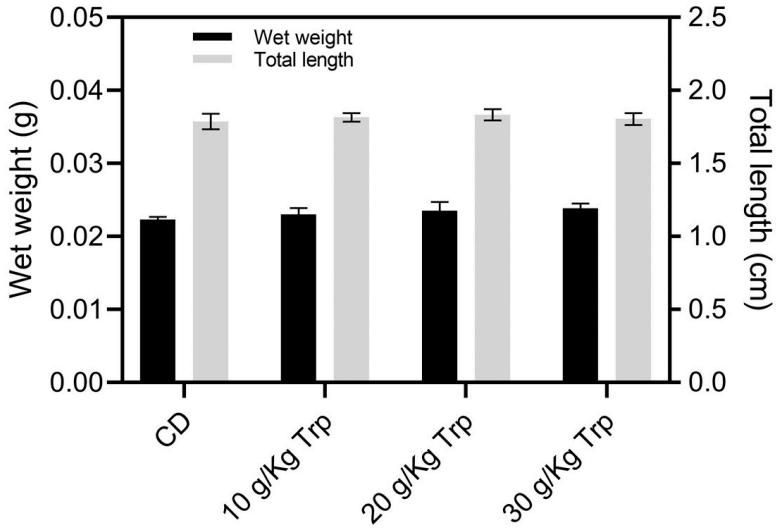

Figure 1. Growth in the weight (g) and total length (cm) of *A. tropicus* larvae fed with different Trp concentrations and the control diet (CD). Values are mean ± SD.

Table 5. Growth performance and feed utilization indexes of *A. tropicus* larve fed with different concentrations of Trp and the CD (mean ± standard deviation, SD).

	Trp (g/kg)			
	CD	**10**	**20**	**30**
Initial weight (g)	0.018 ± 0.001	0.018 ± 0.001	0.018 ± 0.001	0.018 ± 0.001
Final weight (g)	0.022 ± 0.0003	0.023 ± 0.001	0.023 ± 0.001	0.023 ± 0.001
Initial total length (cm)	1.28 ± 0.09	1.28 ± 0.09	1.28 ± 0.09	1.28 ± 0.09
Final total length (cm)	1.78 ± 0.05	1.81 ± 0.02	1.83 ± 0.03	1.80 ± 0.03
S (%)	28.25 ± 1.76	34.75 ± 5.30	28.66 ± 1.04	29.75 ± 1.76
FI (g/d)	0.031 ± 0.001	0.031 ± 0.003	0.031 ± 0.003	0.031 ± 0.001
AWG (g/fish)	0.004 ± 0.0003	0.005 ± 0.0008	0.005 ± 0.001	0.005 ± 0.00004
SGR (%/d)	5.07 ± 0.52	5.35 ± 0.29	5.53 ± 0.38	5.24 ± 0.39
FCR	7.23 ± 0.85	6.68 ± 0.53	5.77 ± 0.46	5.64 ± 0.02
PER	0.13 ± 0.01	0.15 ± 0.1	0.15 ± 0.04	0.15 ± 0.04
K	0.39 ± 0.02	0.39± 0.01	0.37± 0.01	0.38± 0.02
CV (%)	6.43 ± 2.98	8.63 ± 2.23	8.20 ± 3.05	6.95 ± 0.05
SH	0.71 ± 0.33	0.96 ± 0.25	0.91 ± 0.34	0.77 ± 0.006

Significant differences among the diets are indicated by different letters ($p < 0.05$). FI: feed intake; AWG: absolute weight gain; SGR: specific growth rate; S: survival; FCR: feed conversion rate; PER: protein efficiency rate; K: condition factor; CV: coefficient of variation; SH: size heterogeneity.

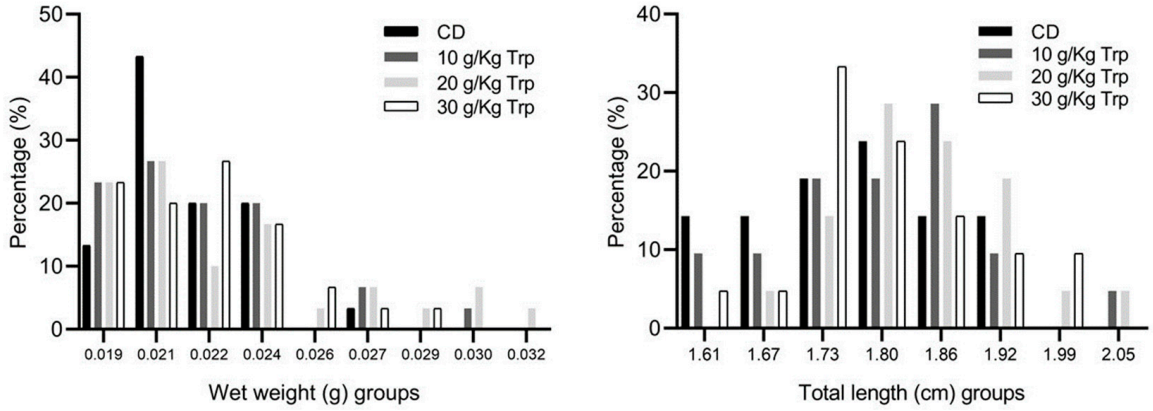

Figure 2. Wet weight (g) and total length (cm) class distribution by cannibalism effect in *A. tropicus* larvae fed with Trp and CD.

3.2. Cannibalism and Deformities

In all treatments where Trp was administered, the percentage of cannibalism was lower (56.75–64.25%) compared to that where CD was administered. The lowest percentage of cannibalism was observed for 10 g/kg Trp (56.75 ± 2.47%) and the highest value occurred in the larvae of the CD treatment (64.75 ± 1.76%), presenting a significant difference ($p < 0.05$). The percentage of fish with deformities was 5.75 ± 0.43%, and no differences were observed between treatments ($p > 0.05$) (Figure 3).

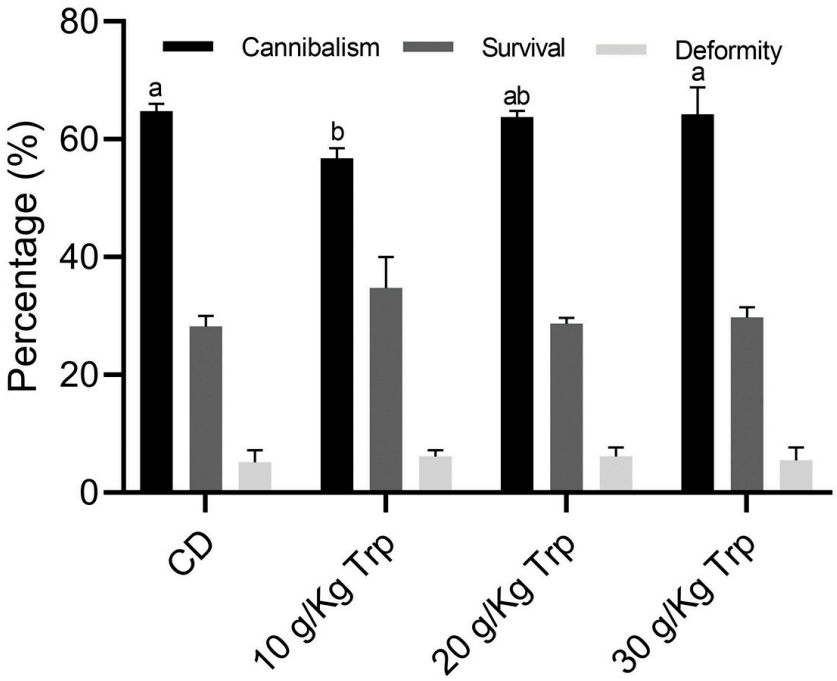

Figure 3. Cannibalism, survival, and deformity percentages in *A. tropicus* larvae fed with different Trp concentrations. Values are mean ± SD. Significant differences among the diets are indicated by different letters ($p < 0.05$).

The larvae identified as cannibals and non-cannibals had an average weight of 0.025 ± 0.002 g and 0.023 ± 0.003 g, respectively (*t*-test, $p = 0.007$). The larvae identified as cannibals had a total size of 1.83 ± 0.09 cm, and the larvae identified as non-cannibals a total size of 1.72 ± 0.10 cm (Figure 4) (t-test, $p < 0.0001$). The average difference recorded between the weight of a cannibal fish and its prey was 0.003 ± 0.003 g (16.12 ± 13.44%). In total size, the average difference between the cannibal fish and its prey was 0.15 ± 0.06 cm (8.96 ± 3.72%).

To continue to stage two, based on the results obtained, the 10 g/kg Trp diet was selected in which the lowest percentage of cannibalism and the highest survival occurred. The administration of this feed was continued only to larvae identified as cannibals for 10 more days.

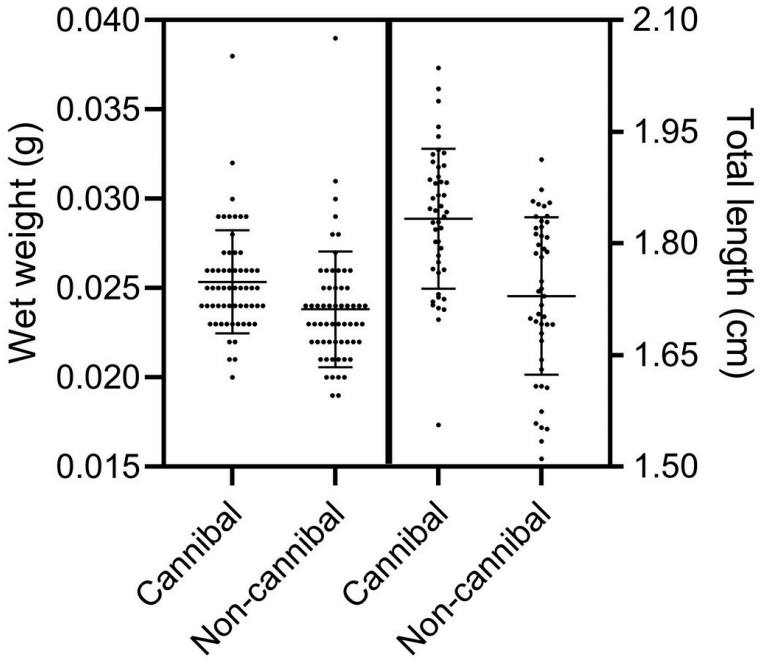

Figure 4. Wet weight (g) (n = 60) and total length (cm) (n = 45) of *A. tropicus* cannibal and non-cannibal larvae. Values are mean ± SD.

3.3. Growth Indexes and Survival of Cannibals

The cannibal larvae of the CD treatment obtained a greater final weight (0.179 ± 0.03 g), compared to those treated with 10 g/kg Trp (0.080 ± 0.01 g) (*t*-test, $p = 0.0224$). The same was observed for the total size (3.68 ± 0.34 cm in CD and 2.72 ± 0.18 cm in 10 g/kg Trp) (Figure 5) The highest survival was recorded in the 10 g/kg Trp group with 75.00 ± 7.07%, presenting significant differences compared to the CD group (30.00 ± 17. 32%) (*t*-test, $p = 0.0044$). The AWG (0.13 ± 0.03) (*t*-test, $p = 0.019$), SGR (14.62 ± 2.87) (*t*-test, $p = 0.029$), and PER (0.17 ± 0.01) (*t*-test, $p = 0.041$) were higher for CD. Also, the CD group showed a higher CV (55.64 ± 6.84) compared to the treatment of 10 g/kg Trp (26.74 ± 14.20) (*t*-test, $p = 0.015$) and SH (6.07 ± 0.75), showing significant differences compared to the 10 g/kg Trp group (*t*-test, $p = 0.012$). The FCR value for the 10 g/kg Trp treatment was 12.43 ± 1.60, showing a significant difference compared to the CD group (5.66 ± 0.62) (*t*-test, $p = 0.030$). FI and K did not show any differences (Table 6).

Table 6. Growth performance and feed utilization indexes of *A. tropicus* cannibal larvae fed with 10 g/kg Trp and CD (mean ± standard deviation, SD).

	CD	**10 g/kg Trp**
Initial weight (g)	0.049 ± 0.004	0.048 ± 0.006
Final weight (g)	0.179 ± 0.03 *	0.080 ± 0.02
Initial total length (cm)	2.16 ± 0.11	2.19 ± 0.08
Final total length (cm)	3.68 ± 0.34 *	2.72 ± 0.18
S (%)	23.33 ± 5.77	75.0 ± 7.07 *
FI (g/d)	0.72 ± 0.07	0.76 ± 0.08
AWG (g/fish)	0.13 ± 0.03 *	0.03 ± 0.01
SGR (%/d)	14.62 ± 2.87 *	5.81 ± 3.18
FCR	5.66 ± 0.62	12.43 ± 1.60 *
PER	0.17 ± 0.01 *	0.05 ± 0.03
K	0.35 ± 0.02	0.39 ± 0.01
CV (%)	55.64 ± 6.84 *	26.74 ± 14.20
SH	6.07 ± 0.75 *	1.90 ± 1.02

Significant differences among the diets are indicated by an asterisk mark ($p < 0.05$). FI: feed intake; AWG: absolute weight gain; SGR: specific growth rate; S: survival; FCR: feed conversion rate; PER: protein efficiency rate; K: condition factor; CV: coefficient of variation; SH: size heterogeneity.

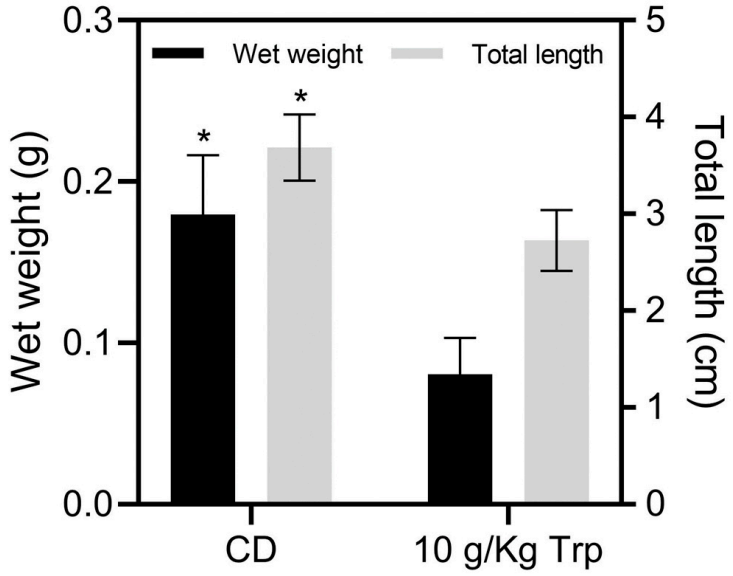

Figure 5. Growth in the weight (g) and total length (cm) of *A. tropicus* cannibal larvae fed with 10 g/kg Trp and CD. Values are mean ± SD. Significant differences among the diets are indicated by an asterisk mark ($p < 0.05$).

3.4. Cannibalism

The percentage of cannibalism was 20.0 ± 10.0% in the fish fed with 10 g/kg Trp, which was significantly different compared to the CD treatment (76.66 ± 5.77%) (*t*-test, $p = 0.0011$) (Figure 6). At the end of the bioassay, the modification of the distribution of the weights and sizes of the fish by treatment was observed, directly related to the results of cannibalism (Figure 7).

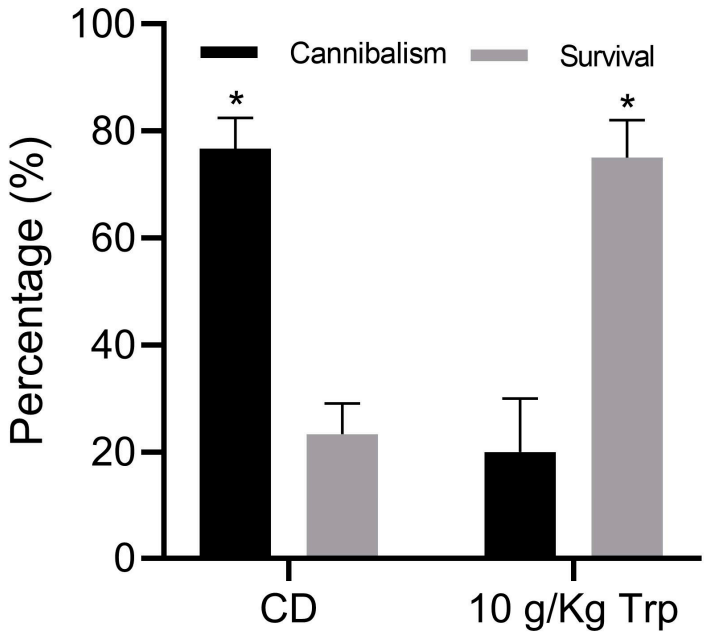

Figure 6. Survival and cannibalism of *A. tropicus* cannibal larvae fed with 10 g/kg Trp and CD. Values are mean ± SD. Significant differences among the diets are indicated by an asterisk mark ($p < 0.05$).

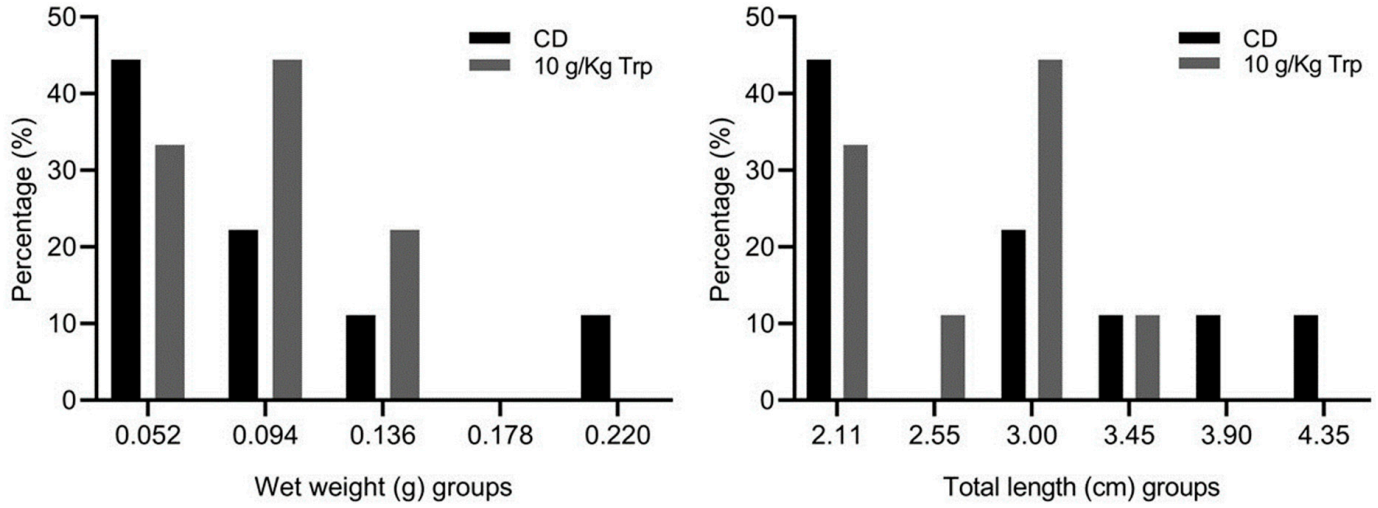

Figure 7. Wet weight (g) and total length (cm) class distribution by cannibalism effect in *A. tropicus* cannibal larvae fed 10 g/kg Trp and CD. Values are mean ± SD.

3.5. Digestive Enzyme Activity

In the first stage, larvae from the CD treatment showed a greater activity in acid protease ($p < 0.05$) compared to those from the 10 and 20 g/kg Trp groups. Also, the CD treatment obtained a greater activity for alkaline protease, with a significant difference with all Trp treatments ($p < 0.05$). For trypsin, 20 g/kg Trp ($p < 0.05$) was statistically different compared to the CD treatment. The 10 and 20 g/kg Trp treatments showed the greatest activity for lipase compared to the other treatments ($p < 0.05$). Leucine aminopeptidase did not show any difference among treatments ($p > 0.05$).

When comparing the digestive enzyme activity between cannibals and non-cannibals, the acid protease activity was greater for cannibals and non-cannibals of the 10 g/kg Trp treatment ($p < 0.05$). CD cannibal and non-cannibal larvae presented a greater alkaline protease activity ($p < 0.05$). Trypsin enzymatic activity between cannibals and non-cannibals was higher in the cannibals of the CD treatment ($p < 0.05$). All treatments with Trp led to a greater leucine aminopeptidase activity than CD; however, no difference was recorded between cannibals and non-cannibals. The cannibal larvae from the 10 g/kg Trp treatment showed the greatest activity for lipase (*t*-test, $p < 0.05$).

In the second stage, the larvae treated with Trp recorded a greater activity in acidic and alkaline proteases and leucine aminopeptidase (*t*-test, $p < 0.05$). The larvae fed with CD recorded a higher trypsin activity (*t*-test, $p < 0.05$). No statistical differences were observed in lipase activity for CD and the 10 g/kg Trp treatment (Table 7).

Table 7. Digestive enzymatic activities (mean ± standard deviation, SD) of *A. tropicus* larvae fed with different concentrations of Trp and CD.

	Activities		**Trp (g/kg)**		
	(u/mg Protein)	CD	10	20	30
First stage	Acid protease	12.233 ±0.924 ^{a}	2.505 ± 2.272 ^{b}	3.546 ± 1.207 ^{b}	7.645 ± 0.792 ^{a,b}
	Alkaline protease	17.374 ± 1.550 ^{a}	8.160 ± 2.257 ^{b}	7.388 ± 0.852 ^{b}	7.784 ± 0.859 ^{b}
	Trypsin	1.334 ± 0.088 ^{a}	0.682 ± 0.067 ^{a,b}	0.640 ± 0.018 ^{b}	0.645 ± 0.236 ^{a,b}
	Leucine aminopeptidase	0.366 ± 0.014	0.290 ± 0.007	0.317 ± 0.057	0.337 ± 0.107
	Lipase	1.711 ± 0.654 ^{b}	4.529 ± 0.318 ^{a}	4.514 ± 0.099 ^{a}	2.413 ± 0.079 ^{b}

Table 7. *Cont.*

	Activities		Trp (g/kg)			
			CD	10		
Second stage	Acid protease		20.492 ± 4.162	35.869 ± 3.670 * (0.033)		
	Alkaline protease		7.043 ± 0.835	14.395 ± 2.342 * (0.028)		
	Trypsin		0.755 ± 0.079	1.032 ± 0.086 * (0.0151)		
	Leucine aminopeptidase		0.432 ± 0.017	1.333 ± 0.203 * (0.0167)		
	Lipase		3.178 ± 0.640	3.072 ± 0.400		
			CD	10	20	30
Cannibals vs. Non-cannibals	Acid protease	C	28.261 ± 0.028 ^{b,*}	43.110 ± 0.045 ^{a,*}	18.780 ± 0.102 ^{d}	25.451 ± 0.017 ^{c,*}
		N	23.576 ± 0.062 ^{b}	39.280 ± 0.049 ^{a}	19.469 ± 0.031 ^{d,*}	23.228 ± 0.020 ^{d}
	Alkaline protease	C	15.887 ± 0.016 ^{a}	10.241 ± 0.218 ^{c,*}	14.263 ± 0.035 ^{b,*}	9.963 ± 0.072 ^{c,*}
		N	18.021 ± 0.128 ^{a,*}	9.722 ± 0.186 ^{b}	8.393 ± 0.166 ^{c}	9.762 ± 0.016 ^{b}
	Trypsin	C	1.919 ± 0.318 ^{a,*}	1.491 ± 0.143 ^{b,*}	1.203 ± 0.082 ^{b,*}	1.154 ± 0.144 ^{b,*}
		N	1.493 ± 0.232 ^{a}	1.170 ± 0.078 ^{b}	0.851 ± 0.087 ^{c}	0.863 ± 0.069 ^{c}
	Leucine aminopeptidase	C	0.284 ± 0.044 ^{c}	0.527 ± 0.079 ^{a}	0.684 ± 0.164 ^{a,b}	0.382 ± 0.051 ^{b,c}
		N	0.184 ± 0.086 ^{c}	0.494 ± 0.045 ^{a,b}	0.514 ± 0.053 ^{a}	0.379 ± 0.042 ^{b}
	Lipase	C	0.900 ± 0.011 ^{b}	3.288 ± 0.038 ^{a}	0.481 ± 0.025 ^{c}	0.588 ± 0.032 ^{c}
		N	3.324 ± 0.019 ^{b,*}	3.889 ± 0.001 ^{a,*}	1.759 ± 0.016 ^{c,*}	1.502 ± 0.012 ^{d,*}

Significant differences among the diets are indicated by different letters ($p < 0.05$). Significant differences between cannibals and non-cannibals are indicated by * ($p < 0.05$).

3.6. Gene Expression

When comparing cannibal and non-cannibal larvae, all cannibals in the 20 g/kg Trp treatment showed overexpression in all genes, followed by 30 g/kg Trp with overexpression only in *avpi1*, *crh*, *htr1a*, *sstr1*, *th*, and t *ph1* genes. In non-cannibal larvae, the 10 g/kg Trp treatment showed a greater expression, with statistical differences compared to the other treatments and with cannibal larvae from the same treatment (*avpi1*, *crh*, *hdc*, *htr1a*, *sstr1*, and *th*). *tph1* was overexpressed in all treatments and was significantly different compared to non-cannibals (Figure 8).

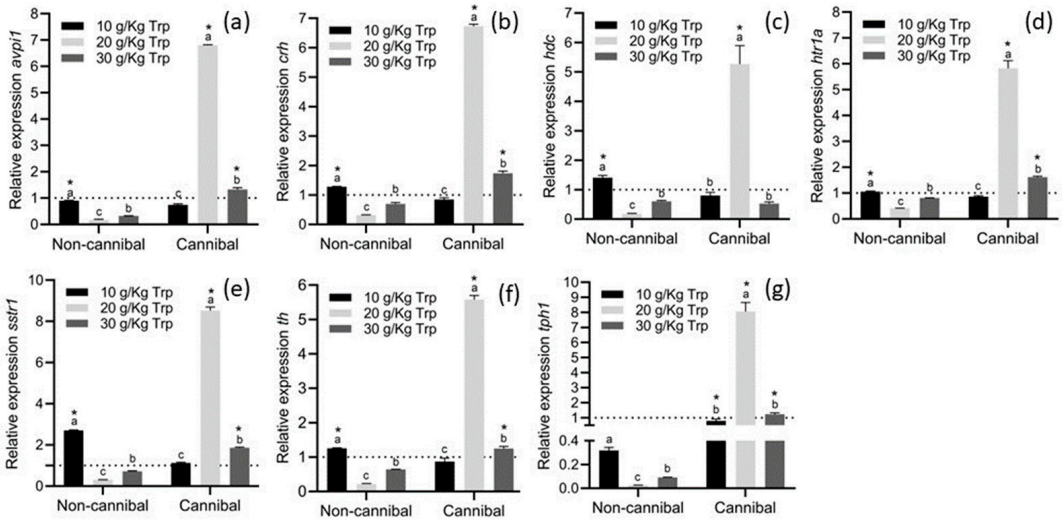

Figure 8. Relative gene expression of *avpi1* (**a**), *crh* (**b**), *hdc* (**c**), *htr1a* (**d**), *sstr1* (**e**), *th* (**f**), and *tph1* (**g**) in *A. tropicus* larvae fed with different Trp concentrations. Relative mRNA levels of the indicated genes were measured by RT-qPCR using β-actin as the reference gene. Data are presented as fold-changes in the mRNA levels, in comparison to the sample with CD (dotted line) (n = 3, mean ± SD). Significant differences with respect to the CD group (dotted line) are indicated by different letters ($p < 0.05$) Significant differences between the cannibal and non-cannibal larvae are indicated by * ($p < 0.05$).

In the second stage, larvae from the 10 g/kg group showed overexpression of *avpi1*, *crh*, and *htr1a* genes ($p < 0.05$) compared to those from the CD group. The same was observed for *gnrh* ($p > 0.05$). A subexpression was observed in the *tph1*, *th*, *sstr1*, and *hdc* genes ($p < 0.05$) of larvae fed with 10 g/kg Trp (Figure 9).

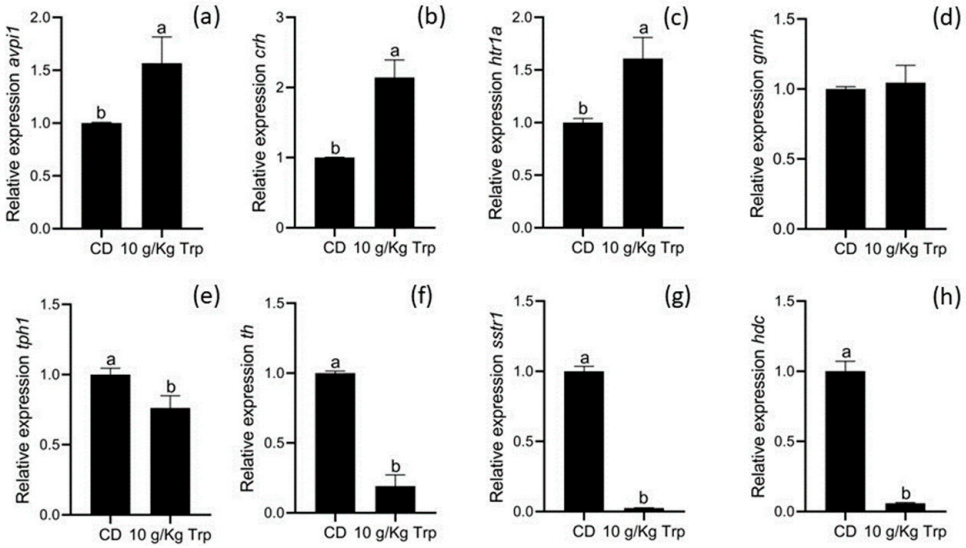

Figure 9. Relative gene expression of *avpi1* (**a**), *crh* (**b**), *htr1a* (**c**), *gnrh* (**d**), *tph1* (**e**), *th* (**f**) *sstr1* (**g**), and *hdc* (**h**) in *A. tropicus* cannibal larvae fed with 10 g/kg Trp. Relative mRNA levels of the indicated genes were measured by RT-qPCR using β-actin as the reference gene. Data are presented as fold-changes in the mRNA levels, in comparison to the sample with CD (n = 3, mean ± SD). Significant differences with respect to the CD group are indicated by different letters ($p < 0.05$).

3.7. Behavior

In total, 12 videos were obtained (185.8 min), and a total of 12 events were recorded, of which 9 were defensive behaviors (escapes), where there was no contact between fish. The remaining three events were direct attacks (with contact). The organisms with rocks as refuge were present in three events, followed by without refuge in six events; finally, only three events were recorded for the organisms with vegetation as refuge. Regarding the effect of diet, larvae fed with 10 g/kg Trp displayed 25% of aggressive behaviors and those fed with CD 75%. Regarding the number of attacks, cannibals fed with 10 g/kg Trp did not display aggressive behaviors, unlike those fed with CD. The use of artificial vegetation also led to zero aggressive behaviors in both treatments (Table 8).

Table 8. Attack types and cannibalism behavior of *A. tropicus* larvae fed with 10 g/kg Trp and CD in combination with different shelters.

		Lateral Attack			Frontal Attack		
	Escape	**Head**	**Middle**	**Tail**	**Head**	**Middle**	**Total**
Without shelter							
CD	2	2	0	0	0	0	4
10 g/kg Trp	2	0	0	0	0	0	2
Total	4	2	0	0	0	0	6
Rocks							
CD	2	0	1	0	0	0	3
10 g/kg Trp	0	0	0	0	0	0	0
Total	2	0	1	0	0	0	3

Table 8. *Cont.*

	Escape	Lateral Attack			Frontal Attack		Total
		Head	Middle	Tail	Head	Middle	
			Artificial Vegetation				
CD	2	0	0	0	0	0	2
10 g/kg Trp	1	0	0	0	0	0	1
Total	3	0	0	0	0	0	3
SUMA total	9	2	1	0	0	0	12

4. Discussion

4.1. Growth

Trp is an essential amino acid (AA) in fish; due to this, it is necessary to know the requirement in important species (*Salmo gairdneri*, *Oncorhynchus mykiss*, and *Rhamdia quelen*). This value ranges from around 0.1% to 0.5% [33]. In this sense, the formulation of the CD diet used contained 1.06% of Trp according to the total amino acid analysis carried out. The relationship of Trp functions in the development of fish larvae has been well studied; it has been demonstrated that a low Trp administration is related to the presence of deformities (scoliosis, lordosis, and eye cataracts) [34]. Furthermore, its administration has been related to the modulation of aggressiveness, feed intake, immune system, stress, oxidative damage, and feed efficiency ratio [35–38]. Administering a high concentration of Trp can activate the kynurenine pathway, thus increasing kynurenic acid concentrations, which have been linked to negative physiological effects, such as increased stress and a decreased immune system [39]. In coho salmon (*Oncorhynchus kisutch*), exposure to 50 µL/mg of Trp compared to 5 µL/mg for 3 h significantly increases the expression of kynurenine aminotransferase 2 (*KIAT 2*) [40]. Therefore, the administration of its optimal level must be sought for each species.

The effect of Trp administration on growth has been reported for Asian seabass juveniles (*Lates calcarifer*), in which Trp reduced growth and feed intake, although it increased serotonin levels in the brain [41]. In grouper juveniles (*Epinephelus coioides*), administering 0.25%, 0.5%, and 1% of tryptophan in the diet resulted in a lower height and weight than those in the control [37]. This same effect was observed in pikeperch (*Sander lucioperca*) larvae: using 5, 10, and 20 g Trp per kg, the weight and height were lower than those in the control treatment [42]; and in Pabda (*Ompok bimaculatus*) fry, the fish growth with Trp (2, 4, and 6 ppm) was lower than that in the control [17]. A similar result but only with high levels of Trp was found in Indian major carp (*Cirrhinus mrigala*), and in Indian catfish (*Heteropneustes fossilis*), a high concentration of Trp in the diet decreased growth [43,44]. The *A. tropicus* larvae did not show a significant difference in growth in the first stage of this study, only a slight tendency of improved growth in weight and size compared to those fed with CD. However, in the second stage, the cannibal larvae fed with Trp (10 g/kg) had a lower growth and final size than the CD larvae. This difference in growth between the two stages of the study can be attributed to cannibalism, due to the "jumper" effect. This effect consists of a rapid gain in weight and size when a cannibal larva consumes another larva [45]. This can lead to an increase in the differences between sizes and weights. This statement was proved in our study, in which the values of CV and SH were higher in the CD group, proving that the high cannibalism of this treatment (76.66 ± 5.77%) results in a low survival (23.33 ± 5.77%) and generating a great heterogeneity in size and weight of the fish fed with CD, unlike the larvae fed with Trp (cannibalism: 20.0 ± 10.0%; survival: 75.0 ± 7.07%). Similar results have been reported in Asian seabass larvae (*Lates calcarifer*), where the coefficient of size variation (%) and size heterogeneity decreased with the increase in the level of Trp supplementation [46]. This heterogeneity in size may also be a trigger for cannibalism, for example, in the Atlantic cod (*Gadus morhua*), African catfish (*Heterobranchus longifili*), giant grouper (*Epinephelus lanceolatus*), and black rockfish (*Sebastes schlegelii*) [47–50].

4.2. Cannibalism

Although a decreasing trend in cannibalism due to the administration of Trp was seen in the first stage, it was in the second where it was significantly clearer. These results coincide with those for Asian seabass (*Lates calcarifer*) fry, which when using 0.5, 1.0, 1.5, and 2% of Trp displayed a decreased cannibalism and increased survival compared to the control, with the lowest percentage (0.5) being the best treatment [46]. In grouper (*Epinephelus coioides*) juveniles, the use of 0.25%, 0.5%, and 1% of tryptophan in the diet decreased cannibalism, increasing the serotonin concentration (5-HT) in the brain [37]. In pikeperch (*Sander lucioperca*) larvae fed with 5, 10, and 20 g Trp per kilogram, the levels of 5-HT in fish tissue increased and cannibalism levels decreased [42]. Also, in Pabda (*Ompok bimaculatus*) fry, Trp (2, 4, and 6 ppm) decreased cannibalism and increased survival compared to the control treatment [17]. In Atlantic cod (*Gadus morhua*), Trp (28 g/kg) supplemented to juveniles decreased aggressivity [51]. In rainbow trout (*Oncorhynchus mykiss*), the increase in the concentration of Trp in the plasma and brain decreased the aggressive behavior in dominant fish [52,53], which is related to the action of the 5-HT neurotransmitter. Serotonin (5-HT) is a neurotransmitter that has been related to behaviors such as aggression, reaction to stress, feeding, maturation, and sexual behavior [54].

The synthesis of 5-HT takes place in serotonergic neurons, where Trp serves as a precursor. The enzyme tryptophan hydrolase hydroxylates tryptophan into L-5-hydroxytryptophan, which is subsequently decarboxylated by the enzyme L-amino acid decarboxylase, generating 5-hydroxytryptamine (5-HT). Continuing the process, 5-HT is degraded by the enzyme monoamine oxidase, transforming it into 5-hydroxyindole acetaldehyde. At the end of the reaction, the enzyme aldehyde dehydrogenase produces 5-hydroxyindoleacetic acid (5-HIAA) [54–56]. The bioavailability of Trp in the brain of organisms is important as it allows 5-HT synthesis to take place. In this sense, Trp competes with other amino acids (AAs) (valine, isoleucine, leucine, tyrosine, phenylalanine, and methionine) to enter the brain of organisms, which makes the balance between Trp, the other AAs, and carbohydrates important (since carbohydrates promote the uptake of AAs, except for Trp) and thus generates adequate concentrations of AAs in the plasma, allowing the flow of Trp to the brain [55] and thereby the synthesis of 5-HT. The use of Trp as a mitigator of cannibalism in fish is based on the principle of increasing the bioavailability of this amino acid, which functions as an essential substrate for the synthesis of 5-HT and, in turn, reduces aggressiveness in fish, promoting the reduction in cannibalism. On the other hand, the differences between the total weight and size of a cannibal fish and its prey were 16.39 $\pm$ 10.864% and 15.23 $\pm$ 5.68%, respectively, similar percentages to those reported by Sepúlveda-Quiroz et al. [57].

4.3. Digestive Enzymes

The interaction between Trp and digestive enzymes has been studied in several works; however, this interaction related to cannibalism has not yet been addressed. For *A. tropicus* larvae, it has been reported that the functionality of its digestive system and the differentiation of its organs are completely developed at 9 DAH [58] and, in addition to their anatomy (mouth width and depth, lengths of upper and lower jaw, and mouth depth angle), allow them to capture and ingest their own conspecifics (intracohort cannibalism) [57]. With the administration of Trp, the enzymes acid and alkaline proteases, trypsin, and leucine aminopeptidase registered a lower activity than with that of CD, except for lipases. It has been shown in vitro that Trp can be an activator of amylase, lipase, and trypsin [59]. An increase in the enzymatic activity is reflected in a greater hydrolysis of macronutrients, releasing a greater number of micronutrients [60], which are used by the body, generating a greater absorption of these microelements, resulting in optimal growth in fish [61]. In our study, cannibal and non-cannibal larvae presented significant differences in enzymatic activity; trypsin activity was higher in all cannibals, contrasting with the activity of lipase enzymes, which were more expressed in non-cannibal larvae. The function of trypsin is to hydrolyze proteins by breaking peptide bonds [62]. In turn, lipases promote the digestion

of lipids, participating in the denaturation of triacylglycerol to diacylglycerol, subsequently converting it to monoacylglycerol [63].

In the second stage, cannibal larvae fed with Trp (10 g/kg) showed a greater enzymatic activity (acid and alkaline proteases and leucine aminopeptidase) than the CD cannibal larvae. The activity of trypsin was greater in the cannibal larvae of the CD treatment. In juveniles of Jian carp (*Cyprinus carpio*), when fed with a concentration of 3.8 g/kg of Trp in the diet, the organisms increased their growth and obtained a greater activity of digestive enzymes (trypsin, lipase, and α-amylase) and brush border enzymes and an increase in the height of the intestinal folds [64]. On the other hand, Trp has been considered as a component that can improve digestive enzymatic activity through two components, melatonin (a Trp metabolite) and cholecystokinin (a regulatory hormone), both of which act on the secretion of pancreatic enzymes [65,66], such as trypsin, chymotrypsin, lipase, and amylase [67]. In the case of the administration of Trp in juvenile silver catfish (*Rhamdia quelen*), there was no difference in trypsin and chymotrypsin; however, by performing a polynomial regression, an increase in acid protease activity was identified with respect to the increase in Trp (1–3.1 g/kg) [68]. Changes in the physiology of two salmon species (*Salmo salar* and *Oncorhynchus kisutch*) have been observed following Trp inclusion, with modifications in digestive enzymatic activity, increased 5-HT concentrations, and decreased cortisol levels [69,70].

4.4. Gene Expression

The gene expression results show a significant difference between the cannibal and non-cannibal larvae, where cannibals exposed to a high Trp treatment have a greater overexpression. Trp functions as a substrate in the synthesis of 5-HT, a neurotransmitter that regulates aggressive behavior, among other aspects [54,56]. In zebra fish (*Danio rerio*), seven neurological pathways were identified (hypothalamo-neurohypophysial system (HNS), serotonin (5-HT), somatostatin, dopamine, hypothalamo-pituitary-interrenal (HPI), hypothalamo-pituitary-gonadal (HPG), and histamine) in relation to the expressed genes for aggressiveness [28]. Among the genes that participate in these metabolic pathways are those used in this study (*sstr1*, *th*, *hdc*, *crh*, *htr1a*, *gnrh1*, *avpi1*, and *tph1*) and whose relation to aggressive behavior has already been reported in humans, mice, and fish [29].

In the second stage, the cannibal larvae of *A. tropicus* fed with Trp showed an overexpression of *avpi1* (HNS), *crh* (HPI), and *htr1a* (5-HT), as well as a subexpression of *tph1*(5-HT), *th* (dopamine), *sstr1* (somatostatin), and *hdc* (histamine) with respect to the cannibal larvae fed with CD. The expression of *avpil* (arginine vasopressin-like) is related to behaviors such as aggression and social interactions; in particular, overexpression has been detected in dominant male zebra fish (*Danio rerio*) [28,71]. On the other hand, the HPI pathway is responsible for different processes, such as stress response [39]; in this sense, the corticotropin-releasing hormone (CRH, or corticotropin-releasing factor (CRF)) works as an activator of this pathway [72]. In rainbow trout (*Oncorhynchus mykiss*), the use of CRF through injections decreased the number of attacks, increased locomotion and head movements, and increased the concentrations of serotonin, 5-HIAA, and dopamine [73,74]. When comparing dominant and subordinate males of *Astatotilapia burtoni*, the relative abundance of mRNA of 5-HT transporters, such as *htr1a* and *htr2a*, in the telencephalon was higher in subordinate than in dominant males, and was related to an increase in 5-HT production [75]. In this sense, the use of a specific agonist (8-OH-DPAT) in HTR1A receptors decreases aggression in fighting fish (*Betta splendens*) [76]. In male zebra fish (*Danio rerio*), a differentiation between dominants and subordinates was identified with respect to the genes involved in sexual behavior (*cyp19a1b*, *cyp17*, *hsd11b2*, *hsd17b3*, and *ar*) and aggressiveness (*avplr1b*, *tph1b*, *htr1a*, *sst1*, *sstr1*, *th*, and *slc6a3*), with the dominant males being the ones with the highest expression [77]. Although the social components have not yet been studied in *A. tropicus* larvae, the results obtained indicate that the HNS, 5-HT, and HPI pathways were modified using Trp, and thus a decrease in cannibalism was observed.

4.5. Behavior

The effect of Trp administration can be seen in the absence of aggressive behavior in the larvae of *A. tropicus* under the influence of refuge. Regardless of the presence or absence of any type of shelter (rocks and artificial vegetation), the larvae fed with Trp (10 g/kg) did not attack, unlike the larvae fed with CD. Of the three scenarios analyzed, the artificial vegetation did not lead to attacks among the larvae fed with CD, as reported by Sepúlveda-Quiroz et al. [57]. The use of enriched environments attempts to replicate the conditions of the natural environment in the culture ponds, improving stress levels by reducing aggression, cannibalism, energy expenditure, injuries, and diseases [78–81].

5. Conclusions

The use of 10 g/kg of Trp in the diet of *A. tropicus* larvae reduces cannibalism, improving survival, specifically proven in larvae with cannibalistic behavior. Both cannibal and non-cannibal larvae showed a difference in digestive enzyme activity and expression of aggressiveness genes. The inclusion of tryptophan generates the activation of the HNS, 5-HT, and HPI pathways, demonstrated by the overexpression of the *avpi1*, *crh*, and *htr1a* genes. Furthermore, cannibal behaviors did not occur with the use of Trp, regardless of the type of shelter, although artificial vegetation was better than other shelters. It is recommended that studies continue to focus on explaining other effects of Trp in *A. tropicus*.

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