

Dietary L-tryptophan alters aggression in juvenile matrinxã *Brycon amazonicus*

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Abstract This study evaluated the effect of dietary supplementation with L-tryptophan (L-TRP), a serotonin precursor, on the aggressiveness of juvenile matrinxã *Brycon amazonicus*. Fish were kept in individual aquaria for 7 days receiving the diets: D1 (control: 0.47% of TRP), D2 (0.94% of TRP), D3 (1.88% of TRP), and D4 (3.76% of TRP). After this, they were grouped with an intruder fish to establish a resident–intruder relationship during periods of 20 min. Blood cortisol, glucose, chloride, sodium and calcium; hemoglobin, hematocrit, red blood cell count and volume; liver glycogen and lipids were measured. Territoriality had significant effect on the aggressiveness of matrinxã (the residents were more aggressive than intruders, $P < 0.001$) and tryptophan significantly affected their behavior. Fish fed with the D2 diet presented a longer latency until the first attack ($P = 0.0069$) and bit the intruder fewer times ($P = 0.0136$) during the period of observation, compared to the control group. The frequency of bites and chases after the first attack was not affected by the

dietary supplementation of TRP. Physiological variables were not significantly affected by the diet, except for a moderate increase in cortisol level in fish fed with D2 diet after the fight, indicating slight activation of the hypothalamus–pituitary–interrenal axis. The results show that juvenile matrinxã have aggressive and territorial behavior and that a diet containing 9.4 g TRP kg^{-1} alter their aggressiveness, without affecting the stress-related physiological parameters.

Keywords *Brycon amazonicus* · Matrinxã · Aggressiveness · Social stress · Tryptophan

Introduction

The matrinxã (*Brycon amazonicus*, Spix and Agassiz 1829) is an Amazonian freshwater teleost (Howes 1982), with high potential for farming. This species is a migratory fish that forms shoals during the reproductive period, but during the juvenile phase present highly aggressive, territorial intraspecific behavior and establishment of social hierarchies. According to Øverli et al. (1999), fights among fish to establish social dominance are stressful, both for the winners and losers, thus leading to activation of the hypothalamus–pituitary–interrenal (HPI) axis and increased plasma cortisol levels. The stress caused by the establishment and maintenance of these social behavioral patterns

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triggers a series of physiological responses that affect growth, reproduction, and immune system and may lead the fish to death, mainly among subordinate fish (Wendelaar Bonga 1997).

Dietary supplementation with L-tryptophan (L-TRP) has been used for controlling aggressiveness and reducing stress in some fish species (Lepage et al. 2002, 2005; Hseu et al. 2003; Höglund et al. 2005). Tryptophan is the precursor of serotonin (5-hydroxytryptamine, 5-HT) and its availability is a limiting factor for the synthesis of brain 5-HT, both in mammals (Fernstrom and Wurtman 1972) and in fish (Aldegunde et al. 1998, 2000). Studies on several vertebrates have indicated that serotonin acts inhibiting aggressive behavior (Fuller 1996; Winberg et al. 2001; Hseu et al. 2003; Perreault et al. 2003; Höglund et al. 2005; Lepage et al. 2005; Summers and Winberg 2006). Animals that lose in social confrontations and become subordinates present chronic activation of the serotonergic system, while those that become dominant present transitory activation, with rapid recovery of baseline levels of serotonin (Øverli et al. 1999).

Relationship between the serotonergic system and the HPI axis in fish has been shown (Øverli et al. 1999). In mammals, serotonin has a stimulatory effect on the HPA axis, with increased release of corticosteroids. At least two subtypes of 5-HT receptors may mediate this increase: the 5-HT₂ receptor (probably 5-HT_{2A}) and the 5-HT_{1A} receptor (Fuller 1996). In fish, the effects of serotonin on the HPI axis are still contradictory. Evidences indicate that 5-HT may stimulate or inhibit the cortisol release and that these effects are context dependent (Winberg et al. 1997; Höglund et al. 2002; Lepage et al. 2002, 2005). According to Øverli et al. (1999), the monoaminergic systems (serotonin and noradrenalin) influence the release of cortisol under physiological conditions or under acute stress, but this response is not seen under chronic stress or recovery from stressful conditions. The present study investigated whether feed supplemented with L-TRP might affect the aggressive behavior and interfere with stress indicators of juveniles of *B. amazonicus* subjected to social interaction. Knowledge of the behavior of matrinxã and alternatives for controlling its aggressiveness could become useful tools for reducing the production losses and might improve the development of technology for rearing this species.

Materials and methods

Fish and experimental diets

Fish (64.1 ± 6.03 g) were kept in 500-l tanks for acclimatization with a continuous water flow system for 7 days. The water was continuously aerated with compressed air diffused through air stones. A commercial diet containing 4.6 g L-TRP kg⁻¹ was used for preparing the experimental diets and as the control diet (D1). Three supplemented diets were prepared by the sprinkle method, containing 9.4 g L-TRP kg⁻¹ (D2), 18.8 g L-TRP kg⁻¹ (D3), and 37.6 g L-TRP kg⁻¹ (D4). The L-TRP of each treatment was weighed, dissolved in hydrochloric acid 10% plus ethanol 96%GL, and sprinkled on the commercial diet. After this, diets were dried in a ventilated oven at 35°C and stored at -20°C. To avoid palatability effect, D1 was sprinkled with the acid plus ethanol solution without addition of tryptophan.

Experimental protocol

The experiment was conducted in 96-l aquaria (60 cm × 40 cm × 40 cm), with constant water aeration and controlled temperature (28°C). The sides of the aquaria were covered to avoid fish to see the neighboring aquaria. The back wall of each aquarium was covered with paper marked out with a square grid pattern that divided the areas into nine equal rectangles for use in the behavioral analysis. A total of 64 fish were weighed and measured and then randomly separated into two groups (residents and intruders). Weight ($P = 0.3772$) and length ($P = 0.8915$) did not differ between residents and intruder fish. The resident fish (eight per treatment) were tagged and kept in individual aquaria, receiving the experimental diets twice a day (9:00 a.m. and 5:00 p.m.), for 7 days. Feed consumption was individually measured by counting the pellets consumed. Intruder fish (eight per treatment) were tagged and kept in the 500-l tanks for 7 days, receiving commercial feed. After this, one intruder fish with similar weight and length (less than 10% of difference) was placed into the aquarium of each resident, to establish a resident/intruder relationship. Aquaria were recorded for 20 min immediately after the fish grouping, to analyze the fish behavior. Following this, fish were anesthetized (benzocaine,

100 mg ml⁻¹), blood was drawn, and liver was removed for biochemical and hormonal analyses. All samplings were performed between 8:00 and 11:00 a.m.

A group of 32 fish was weighed, measured, and kept in the same conditions of the resident fish, receiving the experimental diets twice a day, for 7 days. After this, fish were anesthetized and blood was drawn to determine control level of cortisol for each group (D1, D2, D3, and D4).

Behavioral, biochemical, and hormonal analysis

Agonistic behavioral patterns were analyzed by observing the 20-min recording. The method used for quantifying data was the interaction matrix (Deag 1981). Latency before the first attack was timed, and the aggressive attacks were counted. The aggressive behavior was presented as the number of acts during entire 20 min of observation, in addition to the frequency of acts performed after the first attack. Two attack categories were considered: biting, characterized by a bite on the other fish, on any part of the body; and chasing, characterized by an explosion of locomotor activity in which one fish turned against the other without body contact. A dominance index (DI) was calculated to determine the social rank of residents and intruders, through the following formula: $DI = \text{Agg}^+ / (\text{Agg}^+ + \text{Agg}^-)$ (Winberg et al. 1992), where Agg^+ is the number of aggressive acts performed and Agg^- is the number of aggressive acts received by each fish. Fish with DI higher than 0.5 were considered winners, while fish with DI smaller than 0.5 were considered losers.

Plasma glucose levels (Labtest kit) and serum sodium, potassium, and calcium levels (Drake Iselab ion selector), serum chloride levels (Labtest kit) and serum cortisol levels (radioimmunoassay; DPC kit, Diagnostic Products Corporation) were evaluated. Hemoglobin concentration, hematocrit and red blood cell count, and mean corpuscular volume (Celm DA-500 apparatus) were determined in whole blood. Liver was weighed to calculate the hepatosomatic index ($100 \times (\text{tissue weight (g)} \div \text{body weight (g)})$) and one fragment was separated to measure the total lipids (Bligh and Dyer 1959) and glycogen (Moon et al. 1989) concentration.

Statistical analysis

The experiment was set up using a completely randomized block (CRB) design (two blocks) with four treatments and four replications. Differences between treatments and the groups (residents and intruders) were assessed using one-way ANOVA. Means were compared using Tukey's test ($P < 0.05$).

Results

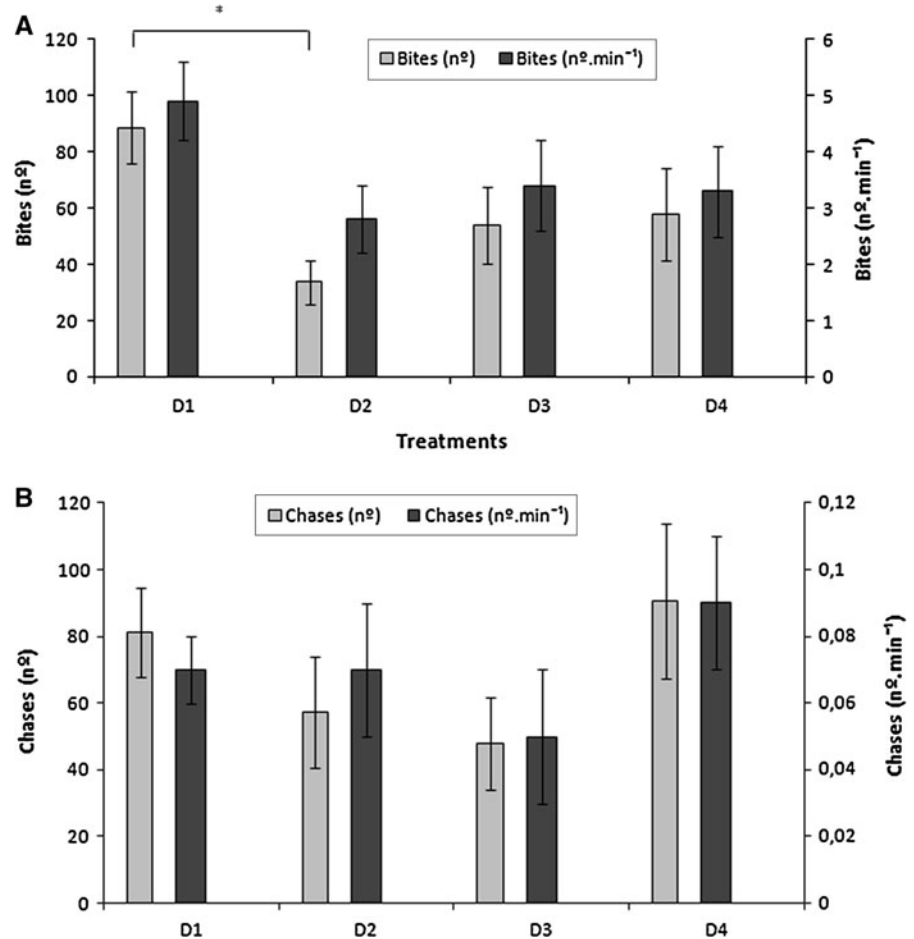
Fish fed with the D4 diet presented higher consumption compared to those of the other treatments ($P = 0.0022$) (D1: 3.74 ± 0.19 ; D2: 3.86 ± 0.09 ; D3: 3.74 ± 0.17 ; D4: 4.37 ± 0.09 g of feed/7 days). TRP consumption differed among treatments ($P < 0.0001$). Fish that received the D4 diet presented higher TRP consumption, followed by D3, D2, and D1 diets, respectively (D1: 17.6 ± 0.87 ; D2: 36.3 ± 0.87 ; D3: 70.3 ± 3.13 ; D4: 164.4 ± 3.29 mg of TRP/7 days).

Aggressive behavior

Intruder fish performed fewer attacks than the residents ($P < 0.0001$) (residents: 127 attacks; intruders: nine attacks), independent of the treatment and had DI significantly lower ($P < 0.0001$) (residents: 0.90; intruder: 0.01). Furthermore, residents were the first to attack in most of the interactions ($P = 0.0145$) (residents: 65.6%; intruders: 34.4%). Residents also presented a behavioral pattern of escorting the intruder, i.e., following it everywhere and preventing it from remaining alone in any part of the aquarium.

Dietary supplementation of TRP influenced fish behavior. Fish fed with D2 diet bit the intruder significantly less than the control fish during the entire period of observation (D1) (33.7 ± 7.9 and 88.5 ± 12.7 bites, respectively; $P = 0.0136$). Fish fed with D3 and D4 diets also bit the intruder less than the control group (53.7 ± 13.5 and 57.8 ± 16.2 bites, respectively), but without significant difference (Fig. 1a). Dietary TRP supplementation did not affect the number of chases ($P = 0.1658$), although there was a tendency toward lower occurrence of this

Fig. 1 Behavior of *B. amazonicus* fed with diets supplemented with L-TRP. **a** Number of bites performed in the entire period of observation (*left axis*) and frequency of bites performed after the first attack (*right axis*); **b** number of chases performed in the entire period of observation (*left axis*) and frequency of bites performed after the first attack (*right axis*). Bars represent mean and standard error. Star indicates significant differences ($P < 0.05$)



behavior among fish fed with D2 and D3 diets (81.2 ± 13.5 ; 57.1 ± 16.6 ; 47.8 ± 13.9 ; and 90.5 ± 23.2 , in groups D1, D2, D3, and D4, respectively) (Fig. 1b). The frequency of bites (4.9 ± 0.7 ; 2.8 ± 0.6 ; 3.4 ± 0.8 ; and 3.3 ± 0.8 bites min^{-1} , in groups D1, D2, D3, and D4, respectively) (Fig. 1a) and chases (0.07 ± 0.01 ; 0.07 ± 0.02 ; 0.05 ± 0.02 ; and 0.09 ± 0.02 chases min^{-1} , in groups D1, D2, D3, and D4, respectively) (Fig. 1b) after the first attack were not affected by the dietary supplementation of TRP ($P = 0.256$ and $P = 0.502$, respectively).

TRP supplementation affected the latency to the first attack. Fish fed with D2 diet presented a longer latency, compared to those of the control group (471.1 ± 99.7 and 103.1 ± 40.9 s, respectively; $P = 0.0069$). Fish fed with D3 and D4 diets presented longer latencies than the control fish, but without significant difference (266.2 ± 61.0 and 187.3 ± 50.2 s, respectively) (Fig. 2).

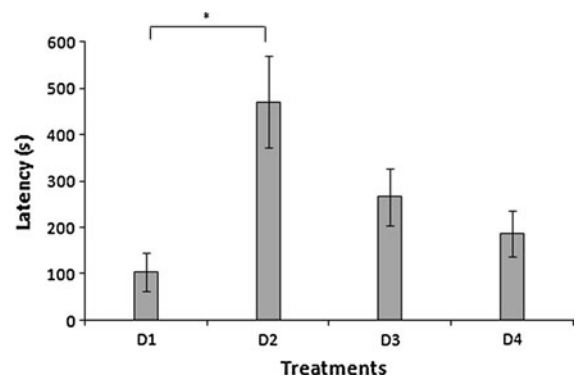


Fig. 2 Latency until first attack of *B. amazonicus* fed with diets supplemented with L-TRP. Bars represent mean and standard error. Star indicates significant differences ($P < 0.05$)

Physiological parameters

Supplementation of dietary TRP did not influence the serum cortisol of fish that were not grouped with an

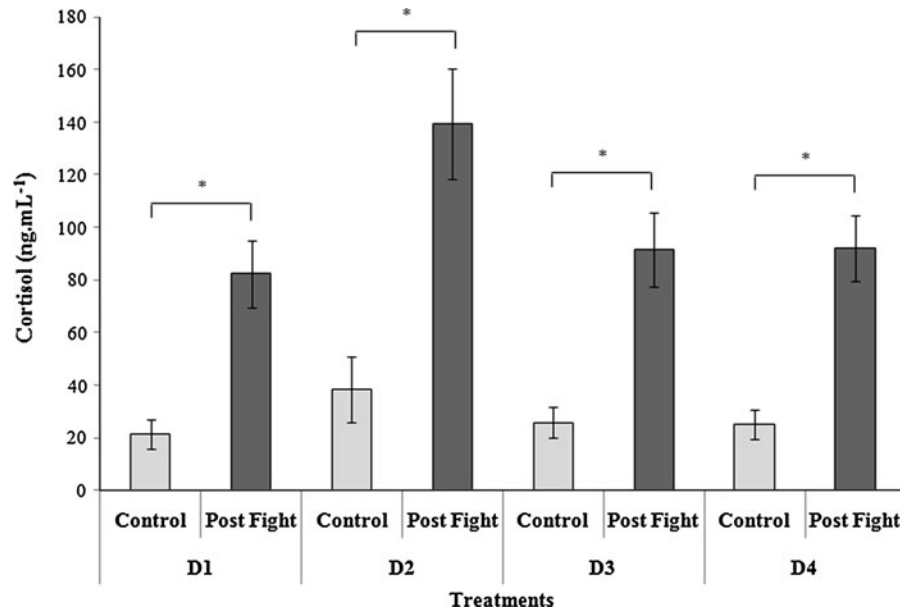


Fig. 3 Serum cortisol of *B. amazonicus* fed with diets supplemented with L-TRP. The *control* group represents the fish that were not grouped with an intruder. The *post-fight* group

represents the fish that were grouped with an intruder. Bars represent mean and standard error. Star indicates significant differences ($P < 0.05$)

intruder (control) (21.4 ± 5.5 ; 38.3 ± 12.6 ; 25.8 ± 5.8 ; and 25.1 ± 5.3 ng ml⁻¹, in groups D1, D2, D3, and D4, respectively) ($P = 0.477$). There was a trend to increase the serum cortisol in resident fed with the D2 diet and grouped with an intruder (139.4 ± 21.0 ng ml⁻¹; $P = 0.0691$) compared to D1, D3, and D4 groups (82.3 ± 12.6 , 91.7 ± 14.1 , and 92.1 ± 12.4 ng ml⁻¹, respectively). The encounter with an intruder increased the cortisol levels independent of the treatment ($P < 0.001$) (Fig. 3).

Metabolic, ionic, and hematological parameters are presented in Table 1. Supplementation with TRP did not influence the blood glucose ($P = 0.2780$), liver glycogen ($P = 0.2085$), and liver lipid levels ($P = 0.2533$) or the hepatosomatic index ($P = 0.8432$), as well as the sodium ($P = 0.7076$) and calcium levels ($P = 0.6632$), while the chloride levels were lower in fish fed with D3 feed, compared to control group ($P = 0.0366$) and the potassium levels were lower in D1 group than in D4 group ($P = 0.0076$). Supplementary TRP did not affect the hematocrit ($P = 0.5164$), hemoglobin concentration ($P = 0.7851$), red blood cell count ($P = 0.7342$), and mean corpuscular volume ($P = 0.6013$).

Discussion

The resident specimens of *B. amazonicus* performed more attacks and had a higher DI than the intruders during the interactions, indicating the establishment of territoriality, in which the resident fish won the fight and reacted aggressively to the presence of the intruder. The resident was shown to be more active, often preventing the intruder from moving around, while the latter was trying to escape. In a previous study on rainbow trout (*Oncorhynchus mykiss*), the winner fish presented higher locomotor activity and aggressive behavior, while the loser presented lower activity and were often prevented from feeding by the dominant fish (Pottinger and Carrick 2001). The aggressive behavior of the resident seems to represent an attempt to defend the invaded territory and was also described in brown trout (*Salmo trutta*) (Sundström et al. 2003) and white seabream (*Diplodus sargus cadenati*) (Caballero and Castro 1999).

Another behavioral pattern that seems to characterize defense of the territory is the resident behavior of following the intruder, preventing this from occupying space and remaining alone in the aquarium. This

Table 1 Physiological parameters of *B. amazonicus* fed with L-TRP supplemented diet

Parameters	Diets*			
	D1	D2	D3	D4
Metabolic				
Blood glucose (mg dl ⁻¹)	64.7 ± 1.7**	62.3 ± 3.2	63.8 ± 2.2	56.5 ± 2.8
Liver glycogen (%)	7.2 ± 0.4	5.9 ± 0.8	6.4 ± 0.4	5.6 ± 0.5
Liver lipid (%)	0.39 ± 0.03	0.36 ± 0.01	0.51 ± 0.08	0.46 ± 0.03
Hepatosomatic index (%)	1.18 ± 0.05	1.18 ± 0.04	1.12 ± 0.07	1.12 ± 0.05
Hematological				
Red blood cell count (10 ⁶ mm ⁻³)	2.89 ± 0.08	2.7 ± 0.13	2.88 ± 0.11	2.79 ± 0.16
Hematocrit (%)	33.4 ± 0.9	28.3 ± 1.98	28.6 ± 1.5	27.0 ± 1.9
Mean corpuscular volume (μm ³)	116.6 ± 1.9	104.5 ± 4.4	99.1 ± 3.8	96.1 ± 1.7
Hemoglobin (g dl ⁻¹)	10.5 ± 0.2	9.9 ± 0.3	10.2 ± 0.4	10.4 ± 0.5
Ionic				
Chloride (mEq l ⁻¹)	131.9 A ± 2.3	127.8 AB ± 0.8	125.9 B ± 2.8	127.9 AB ± 1.7
Sodium (mEq l ⁻¹)	146.2 ± 1.3	146.4 ± 0.8	145.1 ± 0.9	145.8 ± 1.1
Potassium (mEq l ⁻¹)	1.31 B ± 0.08	1.42 AB ± 0.14	1.36 AB ± 0.09	1.71 A ± 0.06
Calcium (mEq l ⁻¹)	0.73 ± 0.05	0.8 ± 0.06	0.72 ± 0.02	0.73 ± 0.05

Different letters in the same line indicate significant differences ($P < 0.05$)

* D1: 4.6 g L-TRP kg⁻¹; D2: 9.4 g L-TRP kg⁻¹; D3: 18.8 g L-TRP kg⁻¹; D4: 37.6 g L-TRP kg⁻¹

** Mean ± standard error

escort activity was often characterized by a “circling” movement, in which the fish swims in circles, one following the other. This behavior has been described in *Diplodus sargus cadenati*, characterized as swimming in circles with the dorsal fin extended and the body shaking (Caballero and Castro 1999). During active chases and biting episodes, the matrinxã intruder remained in the corners of the aquarium, mainly at the surface but sometimes at the bottom, while the resident fish remained mostly in the middle of the aquarium. According to Pottinger and Carrick (2001), in rainbow trout, the winner fish was characterized as maintaining a position in the middle of the water column, with extensive movement around the tank, biting and/or chasing the loser. On the other hand, the loser fish remained at the bottom of the aquarium, often adjacent to the wall, exhibiting little swimming activity and no direct acts against the winner fish.

In the present study, higher feed consumption was verified in fish fed with D4 feed. This results goes against the general consensus that 5-HT inhibits food consumption (Leibowitz and Alexander 1998). Central and oral administration of 5-HT was shown to have an anorexic effect in goldfish (De Pedro et al.

1998) and in European sea bass (Rubio et al. 2006), respectively. Studies using dietary supplementation of TRP in undisturbed fish did not detect change in feed consumption (Winberg et al. 2001; Lepage et al. 2002; Höglund et al. 2007). Furthermore, the effects of tryptophan on feeding behavior in fish seem to be context dependent. Anorexia induced by stress in brown trout can be attenuated by 7 days of pretreatment with dietary TRP (Höglund et al. 2007).

Dietary supplementation with TRP reduced the number of bites of juveniles of *B. amazonicus* during the total observation period. Fish fed with diets containing 0.94% of TRP performed fewer bites and took longer to react to the intruder. This result suggests that the supplementation of feed with TRP altered the behavior of matrinxã. In the same way, supplementation of feed with TRP reduced the aggressiveness of Atlantic cod (*Gadus morhua*) (Höglund et al. 2005) and rainbow trout (Winberg et al. 2001; Lepage et al. 2005), besides to reduce cannibalism and increase survival of grouper larvae (*Epinephelus coioides*) (Hseu et al. 2003). It is widely known that availability of TRP for the brain increases serotonergic synthesis in vertebrates (Aldegunde et al. 2000; Winberg et al. 2001; Höglund et al. 2005). Studies have supported

that increased serotonergic function is related to reduced aggressiveness in mammals (Fuller 1996; Popova 2006), in reptiles (Summers et al. 2005), and in fish (Winberg et al. 2001; Perreault et al. 2003; Höglund et al. 2005; Lepage et al. 2005). Although the present study did not measure the blood and brain concentrations of serotonin, feed supplementation with TRP may have raised its plasma and brain concentration and the serotonin production, with consequent alteration in aggressiveness in *B. amazonicus*.

The effects of TRP on *B. amazonicus* behavior did not increase as the concentration of dietary TRP increased. The most notable alteration of aggressiveness was observed in the lowest supplementation of L-TRP (0.94% of TRP). It is possible that in fish receiving 1.88 and 3.76% of TRP, increased serotonin levels might promote negative feedback through the serotonergic autoreceptors (5-HT_{1A} and 5-HT_{1B}) diminishing release of serotonin with consequent inhibition of the aggressiveness of matrixã. In mammals, the stimulation of the presynaptic autoreceptors promoted autoinhibition of the serotonergic system through the feedback mechanism (Popova 2006). According to Winberg and Nilsson (1996), little is known about the receptor subtypes in non-mammal animals. However, the serotonergic system is markedly stable through the vertebrate evolution, what may indicate similarity between mammal and non-mammal receptors (Parent 1984). Furthermore, receptors with the same characteristics shown by the 5-HT_{1A} receptors have been described in *Salvelinus alpinus* (Winberg and Nilsson 1996; Höglund et al. 2002), suggesting that the mechanisms that promote self-regulation of the serotonergic system through negative feedback may resemble those of the mammals receptors.

Despite the reduction of aggressiveness observed in D2 treatment when the total period of observation was analyzed, the frequency of bites and chases, after the first attack, were not significantly affected by the treatments, showing that the increase in latency was the major effect of TRP. The fish fed with D2 feed were more hesitant to perform a new behavior when the intruder was placed in the aquarium. Besides a decrease in aggressiveness, this result may indicate that the supplementation with TRP had an anxiogenic effect. The treatment of rats with TRP did not alter anxiety-like behavior, while the fluoxetine (a selective serotonin reuptake inhibitor) treatment showed

anxiogenic effect (Hansen et al. 2011). In fish, there is no study evaluating the effect of TRP in anxiety-like behavior; however, studies with zebrafish (*Danio rerio*) show that fluoxetine and buspirone (5-HT_{1A} partial agonist) produced an anxiolytic effect (Bencan et al. 2009; Maximino et al. 2011). Considering that the methodological approach of the present study is not the more adequate to determine the anxiety-like behavior, further studies utilizing specific anxiety tests are needed to evaluate the effects of TRP in the anxiety-like behavior.

Serotonin may function as a modulator of the production of other neurotransmitters and hormones. Relationship between the serotonergic system and the production of corticosteroids through the HPI axis has been shown (Winberg et al. 1997; Höglund et al. 2002; Lepage et al. 2002, 2005). The present study did not show difference between the treatments in fish that were not grouped with an intruder. However, there was a tendency to increase the serum cortisol levels after the fight in fish that received diet with 0.94% of TRP, contrarily to that found by Lepage et al. (2005), who reported that rainbow trout receiving additional TRP and serotonin uptake inhibitors (citalopram) presented reduced activity of the HPI axis. Other studies with rainbow trout have shown that the increased serotonin induced by tryptophan-enriched feed has context-dependent effects on the endocrine response to stress. In undisturbed fish, 7 days of tryptophan treatment induced a moderate increase in cortisol release, while fish exposed to an acute stressor showed decreased release of this hormone (Lepage et al. 2002). Differently, in matrixã, activation of the HPI axis was apparently detected in fish that were subjected to social interaction, a stressful situation. These results also go against those of studies using agonists of the 5-HT_{1A} receptor of serotonin [8-hydroxy-2-(di-n-propylamine)-tetralin (8-OH-DPAT)] that showed that among undisturbed fish, the agonist had a stimulatory effect on the HPI axis (Winberg et al. 1997; Höglund et al. 2002), while among stressed fish, the stress-induced elevation of cortisol was suppressed (Höglund et al. 2002).

No metabolic changes were observed in resident fish fed with TRP, after the contact with the intruder, differently to what has been described for the European sea bass (*Dicentrarchus labrax*) and rainbow trout, when increased tryptophan intake led to amino acids imbalance and changes in liver and body lipids

content (Papoutsoglou et al. 2005a, b). Trout that received diet supplemented with TRP (2.0% of TRP) for 11 weeks presented liver 4.7 times bigger than those of fish that received non-supplemented diet (Papoutsoglou et al. 2005a, b). The difference in relation to the present study may lie on the fact that TRP was administered for only 1 week, while in the other studies, the administration took longer. Supplementary TRP also did not affect the hematological and ionic parameters of fish.

The nutritional requirements of *B. amazonicus* have not yet been established. The tryptophan requirements for various fish species present large differences, ranging from 0.12% for the channel catfish (*Ictalurus punctatus*) (Wilson et al. 1978) to 0.40% for Indian major carp (*Labeo rohita*) (Murthy and Varghese 1997) and African catfish (*Clarias gariepinus*) (Fagbenro and Nwanna 1999).

The inclusion level of TRP used in the present study caused an increase over the 5-HT activity in other studies (Hseu et al. 2003; Lepage et al. 2002; Winberg et al. 2001). Diet supplemented with 2.47% of TRP for 11 weeks, for trout, was associated with reduced growth, increased consumption, increased food conversion, and reduced liver lipids (Papoutsoglou et al. 2005a, b); however, in the present study, no changes were observed that would indicate any harmful effect of this amino acid.

Conclusion

This study demonstrated that feeding diets containing 0.94% of TRP for 7 days alters aggressiveness in juvenile *B. amazonicus*, without affecting stress indicators. Controlling aggressiveness may contribute to improve the technology of matrinxã farming.

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