Influence of diets supplemented with tryptophan on self-balancing, feed intake and productive performance in white cachama (Piaractus brachypomus) [1]

ARTÍCULO DE INVESTIGACIÓN

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ABSTRACT: This study evaluated the ability of juvenile white Pirapitinga to select food with different tryptophan content in order to self-balance the amino acid intake using information originated from the food source. Therefore, how Tryptophan (Trp) intake affected self-balance of food and productive performance is assessed. The experiment consisted in providing diets supplemented with Trp and a control diet to juveniles with initial weight from 46.99 to 47.59 g as follows: Rc (0.32% Trp - control ration), R2 - 0.64% Trp, R4 - 1.28% Trp and R6 - 1.92% Trp. The number of the diet corresponds to the number Trp was multiplied by in the control diet . Two hand-made feeders were used at the bottom of the aquarium in which 2% of the fish live weight was placed in each diet per day for 14 days. The results showed that the white Pirapitinga was able to self-balance the daily consumption of Trp, benefiting food consumption, weight gain and standard length, as well as food conversion. In conclusion, juvenile Pirapitinga can regulate the tryptophan consumption at a 0.80% level in the diet, equivalent to 20.69 mg/day.

Key words: feed intake, Fish, Pirapitinga, self-selection

Influencia de dietas suplementadas con triptófano sobre el auto-balanceo, consumo de alimento y desempeño productivo en cachama blanca (*Piaractus brachypomus*)

RESUMEN: Este estudio evaluó la capacidad de juveniles de cachama blanca en seleccionar alimentos con diferente contenido de triptófano con el fin de auto-balancear la ingesta de aminoácidos, usando información procedente de la fuente alimentar. Por lo tanto, se evaluó cómo afectó el consumo de triptófano (Trp) el auto-balanceo de alimento y el rendimiento productivo. El experimento consistió en el suministro de dietas suplementadas con Trp y una dieta control, a juveniles entre 46,99 e 47,59 g de peso inicial, siendo: Rc (0,32% del aminoácido – ración control), R2– 0,64%, R4– 1,28% y R6- 1,92% en test de auto-balanceo. El número de la dieta corresponde al número por el cual fue multiplicado el valor de Trp de la dieta control. Fueron usados dos alimentadores hechos a mano, colocados en la parte inferior del acuario, donde se colocó 2% de peso vivo de los peces de cada una de las dos dietas, durante 14 días. Los resultados mostraron que la cachama blanca fue capaz de auto-balancear el consumo diario de Trp, beneficiando el consumo de alimento, el aumento de peso y longitud estándar, así como la conversión alimenticia. En conclusión, la cachama blanca en fase juvenil puede regular el consumo de triptófano en un nivel de 0,80% en la dieta, equivalente a 20,69 mg/día.

Palabras clave: consumo, Peces, Pirapitinga, auto-selección

Introduction

Most animals obtain the nutrients necessary for their metabolism from diverse food sources. However, food sources available in the environment do not always coincide with the physiological needs of the various stages of development (Schmidt-Nielsen, 2002). Therefore, animal diet composition is a representative sample of the available food in the environment (Kaiser & Hughnes, 1993).

The feeding behavior in fish is linked to the hierarchy, pheromone production, presence of predators, among others (probably flagged by chemical communication), influencing food search, location and capture (Botero, 2004). The hypothalamic centers, likely involved in eating behavior and satiety, can be stimulated by either intestinal satiety or metabolic factors, like levels of metabolites in the blood or changes in temperature (Botero, 2004). Moreover, it has been shown that fish have a preference for certain pellet shapes, textures, smells and tastes (Botero, 2004), and regulate the intake of some nutrients and ingredients (protein levels, different oils and protein sources, among others) through testing of self-balancing and food selection, bringing us ever closer to their behavior, feeding preferences and nutritional requirements (Pereira-da-Silva & Pezzato, 1999; Pereira-da-Silva & Pezzato, 2000; Pereira-da-Silva et al., 2004; Bordinhon et al., 2013).

To assess this mechanism, a common experimental design is implemented, which consists of feeding the fish a control diet and diets supplemented with the nutrient or ingredient to be tested, which may be an amino acid, energy or protein, among others.

Feed intake over several days is observed and recorded, the nutrient intake in both diets (control and studied) is determined while assessing the parameter to be tested, usually productive performance in the juvenile phase (Pereira-da-Silva et al., 2004; Bordinhon et al., 2013).

Tryptophan is an essential amino acid that acts as a precursor of serotonin, contributes to normal growth, protein synthesis and fundamental physiological mechanisms in fish and mammals, such as the release of some hormones, sleep regulation, and fish interactions, among others. According to the supplemented level, tryptophan affects feed intake, improves feed conversion, growth rate, group homogeneity and influences reproduction (Lepage et al., 2003; Rossi & Tirapegui, 2004; Feijo et al., 2011; Lillesaar, 2011).

In fish, serotonin synthesis and levels in some brain regions depend on the amount of Trp present in the diet and feeding time (Wolkers et al., 2013), with consequent improvement in feed conversion rate, group homogeneity and growth rate (Lepage et al., 2003; Rossi & Tirapegui, 2004; Feijo et al., 2011).

Tryptophan has potential for use in aquaculture. Therefore, the productive performance of juvenile Pirapitinga under Trp supplemented diet can be tested since there is a response to chemical signaling caused by the increase of Trp in the diet.

Studied species

The Pirapitinga is an omnivorous species of easy cultivation, very produced in Colombia, Brazil, Venezuela, Ecuador and Panamá. It has a great productive performance and good market acceptance. According to Vasquez-Torres (2005), Pirapitinga reaches commercial weight between 180 and 365 days of cultivation, reaching productivity between 12 and 30 kg/Ha.

Quintero-Pinto et al. (2011) reported that a balanced diet for Pirapitinga in the juvenile stage requires 32% CP and 3200 kcal DE/kg diet. According to NRC (2011), the amino acid requirement in the diet fed to freshwater fish species (common carp, Nile tilapia, channel catfish, rainbow trout, among others) ranges from 0.30 to 0.40%, values used for feed formulation in this species.

This study evaluates the ability of Pirapitinga juveniles to select feed with different Tryptophan levels, using information coming from the food source, testing the effect of this amino acid intake on feed self-balancing and productive performance.

Methods and Materials

Facilities, experimental animals and acclimation period:

This experiment was conducted in the Laboratory of Ichthyology and Ornamental Fish of FMVZ, Universidad Nacional de Colombia, UNAL, in Bogota. A total of 28 Pirapitinga juveniles with average initial weight varying from 46.99 to 47.59 g were distributed in individual fish tanks of 90 L each, equipped with foam biofilters to maintain water quality. The water temperature was maintained between 26 and 28ºC, and pH between 7 and 8.

Prior to the experimental period, the fish were acclimatized during 15 days in individual fish tanks of 90 L each. In this period, the animals were fed freely, twice daily, a pelleted standard diet containing 32% CP, 3200 kcal DE/kg and 0.32% tryptophan. Subsequently, the fish were anesthetized with 100 ppm tricaine methane sulfonate - MS222 (Sigma Aldrich, USA) and submitted to the initial biometrics.

Experimental diets:

Table 1 shows the composition of the experimental diets, which were formulated in an Excel spreadsheet and manufactured in the Food Processing Unit (UPA) of the FMVZ, UNAL, in Bogotá. The experimental diets were: Rc (control diet, 0.32% Trp), R2 (0.64% Trp), R4 (1.28% Trp), and R6 (1.92% Trp), the last three were supplemented with L-Tryptophan 98% of Ajinomoto. The diets were isonitrogenous and isocaloric with 32% CP and 3200 kcal DE/kg, varying only the percentage of Trp supplemented.

Table 1. Chemical composition of experimental diets (based on natural matter) with increasing levels of Tryptophan.

Ingredient/Nutrient	Unit	Requirement	Tryptophan levels in the diet			
			0.32%	**0.64%**	**1.28%**	**1.92%**
Crude Protein (CP)	%	**32.00**	**32.00**	**32.00**	**32.00**	**32.00**
Digestible Energy (DE)*	Kcal / kg	**3200**	**3200**	**3200**	**3200**	**3200**
Crude Fiber (CF)	%	6.00	5.06	4.75	4.44	4.14
Ether extract (EE)	%	6.00	5.17	5.17	5.17	5.17
Starch	%	20.00	24.78	24.78	24.78	24.78
Calcium (Ca)	%	1.40	1.66	1.66	1.66	1.66
P Available	%	0.70	0.93	0.93	0.93	0.93
Sodium	%	0.30	0.20	0.20	0.20	0.20
Mineral matter	%	12.00	8.40	8.40	8.40	8.40
Linoleic acid (n6)	%	1.00	1.03	1.03	1.03	1.03
Linolenic acid (n3)	%	0.50	0.50	0.50	0.50	0.50
Lysine	%	1. 64	1. 64	1. 64	1. 64	1. 64
Arginine	%	1.34	2.01	2.01	2.01	2.01
Histidine	%	0.55	0.75	0.75	0.75	0.75
Isoleucine	%	1.00	1.30	1.30	1.30	1.30
Leucine	%	1.08	3.06	3.06	3.06	3.06
Methionine	%	0.86	0.86	0.86	0.86	0.86
Cystine	%	0.17	0.45	0.45	0.45	0.45
Phenylalanine	%	1.20	1.60	1.60	1.60	1.60
Threonine	%	1.20	1.20	1.20	1.20	1.20
Tryptophan	**%**	**0.32**	**0.32**	**0.64**	**1.28**	**1.92**
Valine	%	0.90	1.45	1.45	1.45	1.45
Vitamin C	%	0.03	0.03	0.03	0.03	0.03
Vitamin Choline	%	0.04	0.04	0.04	0.04	0.04

Experimental design:

The experimental design was completely randomized with four (4) treatments (RcXRc; RcXR2; RcXR4 and RcXR6) and 7 replicates each, totaling 28 fish. The ration was provided in handmade feeders at the tank bottom, 2cm tall and 10 cm in diameter, containing 4% of fish body weight (BW) in grams, each ration in separated feeder, with ration equivalent to 2% BW. The number of pellets per gram of feed was counted in order to determine both supplied and consumed units. After 20 minutes, the feeders were removed from the tanks, the amount of food consumed was given by the difference between the pellets offered and the remainder and, therefore, Tryptophan intake was determined for each diet and fish. After counts the number of consumed pellets, tryptophan consumption was calculated from the concentration of that nutrient in the food. It was not added to the dye feed, only this was offered in separate feeders. In addition, the pellets were cut to the same size and the same weight. After 14 days, a new biometrics was performed to assess fish growth performance.

Statistical analysis:

The results were analyzed using the Statistical Analysis System software, SAS version 9.2 (SAS, 2008). All values are presented as mean ± standard deviation.
The parameters weight gain, total feed intake, feed conversion and daily intake of tryptophan were compared with one-way ANOVA ($P<0.05$) and, when significant, Duncan's multiple range test ($P<0.05$) was used to determine the difference between the means and grouping of similarities.

Results and discussion

During the experimental phase the water parameters were stable and followed the guidelines for optimal development and comfort of the species (Rossi & Vidal, 2008 NRC, 2011; Quintero-Pinto et al., 2011; Coldevella et al., 2012). The mean temperature was maintained at 27 ± 1°C and pH at 7.5 ± 0.5.

The results show no statistically significant difference for initial and final weight, 140 weight gain, feed intake (control, treatment and total) (Table 2). The feed conversion rates between treatments 1 and 4, and between treatments 1, 2 and 3 were not significantly different from each other; however, treatment 4 had the worst feed conversion ($P<0.05$), while treatment 3 had the best ($P<0.05$).

Table 2. Tryptophan intake, growth and feed performance for Pirapitinga juveniles.

Variable/Treatment	Treatment 1: Rc (0.32% Trp) Rc vs. (0.32% Trp)	Treatment 2: Rc (0.32% Trp) R2 vs. (0.64% Trp)	Treatment 3: Rc (0.32% Trp) vs. R4 (1.28% Trp)	Treatment 4: Rc (0.32% Trp) vs. R6 (1.92% Trp)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Initial weight (g)	47.39 ± 1.39	47.17 ± 1.56	46.99 ± 1.17	47.59 ± 1.17
Final weight (g)	79.93 ± 14.31	86.31 ± 7.16	88.62 ± 8.81	76.11 ± 14.86
Weight gain (g)	32.54 ± 13.46	39.19 ± 7.50	41.63 ± 8.47	28.73 ± 15.09
Control diet intake (g)	15.17 ± 3.93	17.10 ± 1.87	18.21 ± 1.61	15.84 ± 3.66
Treatment diet intake (g)	14.98 ± 3.85	17.68 ± 1.42	18.07 ± 2.65	14.90 ± 4.40
Total intake (g)	30.15 ± 7.76	34.78 ± 3.22	36.28 ± 4.22	30.73 ± 8.01
Feed conversion	1.02 ± 0.29 ab	0.91 ± 0.15 b	0.89 ± 0.10 b	1.34 ± 0.54 a
Trp total intake (g)	0.096 ± 0.02 c	0.17 ± 0.01 b	0.29 ± 0.04 a	0.34 ± 0.10 a
Trp intake (mg/day)	6.89 ± 1.77 c	11.99 ± 1.05 b	20.69 ± 2.78 a	24.05 ± 6.86 a

Values followed by different letter in the same row are statistically significant (n = 7; Duncan, $P<0.05$).

Pirapitinga juveniles have the ability to select between two diets with different levels of Trp, and to regulate food intake even ingesting it through free choice.

Tryptophan intake was regulated by the fish, ranging between 6.89 and 24.05 mg/day. A significant difference was observed for treatments 1, 2 and 3 (P<0.05) and no difference between treatments 3 and 4 (P>0.05). Trp intake was higher for treatments 3 and 4, due to the higher content of this amino acid in the supplemented rations. These results agree with Pereira-da-Silva et al. (2004) and Bordinhon et al. (2013), regarding the ability to regulate protein intake of Nile tilapia. Almaida Pagan et al. (2006) determined that sharpsnoutseabream (*Diplodus* puntazzo) is also able to regulate and balance the intake of protein, carbohydrates and lipids, while Fortes-Silva et al. (2010) detected preference for diets with different oil types for Nile tilapia. Pereira-da-Silva & Pezzato (1999) and Pereira-da-Silva & Pezzato (2000) also reported on selection and feed intake with different ingredients in Nile tilapia.

Total food intake was not statistically significant different (P>0.05); however, the highest values were found for treatments 3 and 2 while the lowest values, for treatments 1 and 4. This behavior of decreasing feed intake can be explained by the effect of amino acid supplementation on the production of serotonin in the brain, which acts on food intake, as explained by Rossi & Tirapegui (2004), Feijo et al. (2011) and Lillesaar (2011).

The feed conversion rate was significantly different between treatments (P<0.05). Treatment 3 had the best-feed conversion treatment 4 while the worst obtained. Treatments 2 and 3 obtained absolute values of higher weight increase. However, the reasons for not presenting significant differences among the four treatments for weight gain were probably the fact that treatments 1 and 4 showed a high standard deviation and that the trial period was too short to statistically evaluate the productive performance.

Dapoza (2011) reported positive effects of dietary Trp supplementation on feed intake, growth and feed conversion, as well as diminished weight loss for lactating pigs. Freitas-Pinheiro et al. (2008) showed that increasing Trp levels up to 0.21% per day in the diet of quails produces a feeling of well-being and increases food intake. Additionally, for every 1% of additional Trp in the feed up to a maximum level of 0.21%, egg production increased by 21.16%. Trp supplementation had a significant effect on dietary intake of laying hens, which increased proportionally to the amino acid levels provided that the dosages did not increase excessively (Peganova et al., 2003 and Peganova & Eder, 2003).

The effects of Trp supplemented diet on Pirapitinga growth performance have not yet been evaluated. The results of this work show that Pirapitinga juveniles can regulate tryptophan intake at 0.80%, and as with other animal species, productive performance improves in this phase, yielding better weight gain and feed conversion.

The results suggested that the supplementation with Trp in the feed should not be excessive since fish weight gain decreases after reaching an optimal point while feed production costs increase.

Conclusions

Juvenile Pirapitinga is able to self-balance tryptophan intake at 20.69 mg/day benefiting feed conversion. At the studied increasing levels this amino acid improves the species production performance; however, up to an optimum level.

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