

Influence of Tryptophan supplemented diets on self-balancing, food intake and growth performance of juvenile and adult Nile tilapia

ARTÍCULO DE INVESTIGACIÓN

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ABSTRACT: This study aimed to test the effect of the ingestion of tryptophan on the self-balancing of the portion and the productive performance of Nile tilapia in the juvenile and adult stages. For this purpose, tryptophan supplemented diets were prepared as follows: RC (3,2g amino acid kg⁻¹, control), R2 - 6,40g kg⁻¹, R4 - 12,80g kg⁻¹, R6 - 19,60g kg⁻¹ and R8 - 25,60g kg⁻¹, offering two portions for the self-balancing tests. The results showed that Nile Tilapia self-balanced daily consumption of tryptophan, improving feed intake, weight gain, standard length and feed conversion. The results indicated that juvenile and adult Nile Tilapia is capable of regulating the amino acid consumption at 8,40g kg⁻¹ and 8,20g kg⁻¹ of the diet, respectively.

Key words: fish, *Oreochromis* sp, Tryptophan, serotonin, growing

Influencia de dietas suplementadas con triptófano sobre el autobalanceo, consumo de alimento y desempeño productivo en ejemplares juveniles y adultos de tilapia Nilótica

RESUMEN: este estudio tuvo por objetivo probar el efecto de la ingestión de triptófano sobre el autobalanceamiento de la ración y el desempeño productivo de la tilapia Nilótica en las fases juvenil y adulta. Para ello fueron ofrecidas dietas suplementadas con triptófano, siendo: RC (3,2g aminoácido kg⁻¹, control), R2 - 6,40g kg⁻¹, R4 - 12,80g kg⁻¹, R6 - 19,60g kg⁻¹ and R8 - 25,60g kg⁻¹ en test de autobalanceo, ofreciendo dos raciones. Se obtuvieron como resultados que la tilapia Nilótica autobalanceó el consumo diario de triptófano beneficiando el consumo de alimento, la ganancia de peso, la longitud estándar y la conversión alimenticia. Concluyendo así que

la tilapia Nilótica en las fases juvenil y adulta regula el consumo de este aminoácido en 0,84% y 0,82% de la dieta, respectivamente.

Palabras clave: peces, *Oreochromis* sp, triptófano, serotonina, crecimiento

Introduction

Most animals obtain the nutrients necessary for their metabolism from diverse food sources. Additionally, the availability of food items in the environment does not always coincide with the physiological needs of the various development stages (Schmidt-Nielsen, 2002). Therefore, diet composition rarely represents a sample proportional to the food types available in the environment (Kaiser & Hughnes, 1993).

The feeding behavior response in fish is linked to the hierarchy, pheromones production, presence of predators, among others (probably flagged by chemical communication), and this influences food search, location and capture (Botero, 2004). Hypothalamic centers are likely involved in the eating behavior and satiety and can be stimulated by either intestinal satiety or metabolic factors such as levels of metabolites in the blood or temperature changes (Botero, 2004). Furthermore, it has been demonstrated that fish prefer certain pellet formats, textures, smells and flavors (Botero, 2004). Fish were also found capable of regulating the intake of certain nutrients and ingredients (protein levels, different oils and protein sources, among others) in self-balancing and food selection studies, bringing us ever closer to their behavior, feeding preferences and nutritional requirements (Pereira-da-Silva & Pezzato, 1999, 2000; Pereira-da-Silva et al., 2004; Bordinhon, 2008).

This type of study of evaluating feeding habits and ingestion consists of offering a control diet and a diet supplemented with the nutrient or ingredient to be tested, which may be an amino acid, energy or protein, among others. Feed intake is then observed for several days between the control and the tested diet to evaluate the parameter to be tested, which is, usually, productive performance parameter in the juvenile phase (Pereira-da-Silva et al., 2004; Bordinhon, 2008).

Tryptophan (Trp), the main focus of this study, is an essential amino acid that acts as a precursor to serotonin, aids normal growth, protein synthesis and fundamental physiological mechanisms in fish and mammals, such as the release of certain hormones, sleep regulation, and interactions in fish, among others. The level of supplementation in the diet 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). According to the National Research

Council —NRC— (2011), the amino acid requirement for Nile tilapia (*Oreochromis niloticus*) is 3,20g Trp kg⁻¹ in the diet.

Tryptophan has potential to be used in fish farming. In fish, the serotonin synthesis and levels in some brain regions depend on the amount of Trp ingested in the diet and feeding time (Wolkers et al., 2014), with consequent improvement of feed conversion, group homogeneity and growth rate (Lepage et al., 2003; Rossi & Tirapegui, 2004; Feijo et al., 2011). Therefore, the productive performance responses of juvenile Nile tilapia to Trp supplemented diet can be tested since there is a response to chemical signaling caused by the increase of Trp in the diet.

There are few reports in the literature on the effects of nutrient supplementation in fish diet, especially amino acids, and Nile tilapia (*Oreochromis niloticus*) characteristics make it suitable as a model species for research on nutrition and behaviour. Therefore, this study tested the effect of Tryptophan (Trp) supplemented diet on Nile tilapia in two experiments: Experiment 1-juvenile fish by assessing self-balancing, food intake and growth performance; and, Experiment 2-adult fish by determining self-balancing and food intake.

Methods and Materials

Experiment 1: This experiment was conducted at the Laboratory of Animal Behaviour and Physiology, Department of Physiology, UNESP in Botucatu, Brazil. A total of 30 juvenile Nile tilapia weighing initially between 39,69 and 40,96g on average were distributed in individual 23L tanks, equipped with foam biofilters to maintain water quality. The water temperature was kept between 26 and 28°C, and pH between 7 and 8.

Prior to the experimental period, the fish were acclimatized during 15 days. They were kept in 500-L plastic tanks, containing 20 fish. During this period, the animals were fed freely twice daily with pelleted standard diet containing 320g kg⁻¹ CP and 13,40 MJ DE kg⁻¹ diet and 3,20g Tryptophan kg⁻¹, meeting the nutritional requirements of the species according to the NRC (2011) recommendations.

Subsequently, the fish were anesthetized using MS 222 (100mg L⁻¹) (Sigma Aldrich, St. Louis, MO, USA) anesthetic and submitted to the initial biometrics. This anesthetic was selected for being less toxic and less stressful to the fish.

Experiment 2: This experiment was conducted in the Laboratory of Ichthyology and Ornamental Fish of the FMVZ, Universidad Nacional de Colombia, in Bogotá. A total of 24 adult Nile tilapia weighing initially between 232,53 and 246,56g on average were

distributed in individual 90L tank, equipped with biofilters to maintain water quality. The water temperature was maintained between 26 and 28°C, and pH between 7 and 8.

The fish were also acclimatized during 15 days, kept in individual 90 L tanks, fed twice daily freely with pelleted standard diet containing 320g CP kg⁻¹ and 13,40 MJ DEkg⁻¹ diet and 3,20g Tryptophan kg⁻¹, meeting the nutritional requirements of the species according to the NRC (2011) recommendations. Subsequently, the fish were anesthetized using MS222 (100mg L⁻¹) anesthetic.

Experimental diets: Table 1 show the experimental diets, which were formulated according to the requirements proposed by the NRC (2011) for Nile tilapia. The diets were formulated using the Excel spreadsheet and manufactured in the Food Processing Unit of FMVZ, UNAL, in Bogotá. The experimental diets were: RC (control diet 3,2g Trp kg⁻¹); R2 (6,4g Trp kg⁻¹); R4 (12,80g Trp kg⁻¹); R6 (19,20g Trp kg⁻¹); and R8 (25,60g Trpkg⁻¹). The last four diets were supplemented with L-Tryptophan 98% (Ajinomoto, Limeira, SP, Brazil). The diets were isonitrogenous and isocaloric 320g CP kg⁻¹ with 13,40 MJ DE kg⁻¹ diet, varying only the percentage of Trp supplemented (table 1).

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 (g kg ⁻¹)				
			3,20	6,40	12,80	19,20	25,60
Crude Protein (CP)	g kg ⁻¹	320	320	320	320	320	320
Digestible Energy (DE)*	MJ kg ⁻¹	13,40	13,40	13,40	13,40	13,40	13,40
Crude Fiber (FB)	g kg ⁻¹	60,00	50,60	47,50	44,40	41,40	38,30
Ether extract (EE)	g kg ⁻¹	60,00	51,70	51,70	51,70	51,70	51,70
Starch	g kg ⁻¹	200,00	247,80	247,80	247,80	247,80	247,80
Calcium (Ca)	g kg ⁻¹	14,00	16,60	16,60	16,60	16,60	16,60
P Available	g kg ⁻¹	7,00	9,30	9,30	9,30	9,30	9,30
Sodium	g kg ⁻¹	3,00	2,00	2,00	2,00	2,00	2,00
Linoleic acid (n6)	g kg ⁻¹	10,00	10,30	10,30	10,30	10,30	10,30
Linolenic acid (n3)	g kg ⁻¹	5,00	5,00	5,00	5,00	5,00	5,00
Lysine	g kg ⁻¹	16,40	16,40	16,40	16,40	16,40	16,40
Arginine	g kg ⁻¹	13,40	20,10	20,10	20,10	20,10	20,10
Histidine	g kg ⁻¹	5,50	7,50	7,50	7,50	7,50	7,50
Isoleucine	g kg ⁻¹	10,00	13,00	13,00	13,00	13,00	13,00
Leucine	g kg ⁻¹	10,80	30,60	30,60	30,60	30,60	30,60
Methionine	g kg ⁻¹	8,60	8,60	8,60	8,60	8,60	8,60
Phenylalanine	g kg ⁻¹	12,00	16,00	16,00	16,00	16,00	16,00
Threonine	g kg ⁻¹	12,00	12,00	12,00	12,00	12,00	12,00
Tryptophan	g kg⁻¹	3,20	3,20	6,40	12,80	19,20	25,60
Valine	g kg ⁻¹	9,00	14,50	14,50	14,50	14,50	14,50
Ascorbic acid	mg kg ⁻¹	300	300	300	300	300	300
Choline	mg kg ⁻¹	400	400	400	400	400	400

Experimental design Experiment 1: The experimental design was completely randomized with two treatments (RCXR4 and RCXR8) and 15 replicates, totaling 30 fish, in which half of the fish were fed RCXR4 (treatment 1) and the other half RCXR8 (treatment 2). The feed was provided in handmade feeder sat the bottom, at the ratio of 2% body weight (BW) in grams, each diet equivalent to 1% BW. The number of pellets per gram of feed was counted in order to determine both supplied and consumed units. After 20 minutes of feeding, the feeders were removed and the remaining amount of feed pellets was determined. The amounts of ingested food, as well as Tryptophan, were determined for each fish by subtracting the leftover pellets from the initial amount supplied. After 17 days, new biometrics was performed to assess growth performance parameters.

Experimental design Experiment 2: The experimental design was completely randomized with four treatments (RCXR2, RCXR4, RCXR6 and R2XR4) and 6 replicates, totaling 24 fish. Feed was provided in handmade bottom feeders, containing

2% body weight (BW) in grams, each diet equivalent to 1% BW. The number of pellets per gram of feed was counted in order to determine both supplied and consumed units. After 20 minutes of feeding, the feeders were removed and the remaining amount of feed pellets was determined. The amounts of ingested food, as well as of Tryptophan, were determined for each fish by subtracting the leftover pellets from the initial amount supplied. After 10 days, new biometrics was performed to assess feed self-balancing and performance parameters.

Statistical Analysis: The results were analyzed using the Statistical Analysis System version 9.2 software (SAS, 2008). All values are presented as mean \pm standard deviation.

In experiment 1, the results for weight gain and growth rate, feed conversion, total feed intake and Tryptophan daily intake were subjected to one-way ANOVA ($P < 0,05$) and, when significant, paired t test was applied to determine statistical significance between means.

In Experiment 2, the results for weight gain, total feed intake and daily intake of Tryptophan were subjected to one-way ANOVA ($P < 0,05$) and, when significant, Duncan's multiple range test ($P < 0,05$) was applied to determine the difference between the means and grouping of similarities.

Results and Discussion

During the experimental phase the water parameters were stable, following the guidelines for optimal development and comfort of the species (Kubitza, 2000; Galvis et al., 2006; Rossi & Vidal, 2008; NRC, 2011; Quintero-Pinto et al., 2011; Coldevella et al., 2012). The mean temperature was kept at $27 \pm 1^\circ\text{C}$ and pH at $7,5 \pm 0,5$.

Experiment 1: Feed intake was not significantly different between treatments ($P > 0,05$). However, the Trp daily consumption was significantly different, equivalent to 8,40 and $13,50 \text{ g kg}^{-1}$ of the total amino acid consumed in the feed of treatments 1 and 2, respectively. Weight gain was also significantly different ($p = 0,006$) and higher for treatment 1. Feed conversion was also better ($p = 0,002$) for treatment 1 (table 2).

Table 2. Tryptophan intake, growth and feed performance of juvenile Nile tilapia.

Variable / Treatment	Treatment 1: RC (3,2 g Trp kg ⁻¹) vs. R4 (12,80 g Trp kg ⁻¹)		Treatment 2 RC (3,20 g Trp kg ⁻¹) vs. R8 (25,60 g Trp kg ⁻¹)		Statistical Significance P <0,05
	Mean	SD	Mean	SD	
Initial weight (g)	39,69	6,88	40,96	9,16	
Weight gain (g)	8,25	2,24	7,66	3,02	0,020*
Length gain (cm)	1,03	0,52	0,65	0,42	0,006*
Control diet intake (g)	5,09	1,95	6,93	2,06	0,204
Test diet intake (g)	6,04	1,60	5,93	2,44	0,719
Total intake (g)	11,13	2,58	12,86	4,20	0,556
Feed Conversion	1,47	0,63	1,89	1,07	0,002*
Trp intake (mg day ⁻¹)	5,51	1,28	10,24	3,98	0,005*

* (P<0,05) indicates significant difference between treatments (n=15, paired t test).

Experiment 2: The results presented in Table 3 show that adult Nile tilapia was able to balance Trp intake in the diet, consuming a certain amount of each supplied diet. The amount of Trp ingested ranged from 15,83 to 35,15 mg in the treatments. Daily consumption of Trp in treatment 1 was statistically different (P<0,05) compared to the other treatments, which did not differ (table 3).

Table 3. Tryptophan intake, growth and feed performance of adult Nile tilapia

Variable /Treatment	Treatment 1: RC (3,20 g Trp kg ⁻¹) vs. R2 (6,40 g Trp kg ⁻¹)	Treatment 2: RC (3,20 g Trp kg ⁻¹) vs. R4 (12,80 g Trp kg ⁻¹)	Treatment 3: RC (3,20 g Trp kg ⁻¹) vs. R6 (19,20 g Trp kg ⁻¹)	Treatment 4: R2 (6,40 g Trp kg ⁻¹) vs. R4 (12,80 g Trp kg ⁻¹)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Initial weight (g)	246,56 ± 67,50	233,88 ± 60,44	237,40 ± 38,20	232,53 ± 45,10
Final weight (g)	257,00 ± 66,10	245,42 ± 56,83	243,17 ± 41,30	238,08 ± 46,48
Weight gain (g)	10,40 ± 12,03	11,54 ± 7,87	6,73 ± 4,18	5,55 ± 4,75
Control diet intake (g)	15,05 ± 6,24	15,21 ± 8,17	14,98 ± 0,53	14,00 ± 3,16
Treatment diet intake (g)	17,20 ± 7,71	17,54 ± 7,54	15,81 ± 2,01	14,87 ± 2,84
Total consumption (g)	32,26 ± 6,75	32,76 ± 15,44	30,79 ± 2,45	28,88 ± 5,26
Trp total consumption (g)	0,16 ± 0,04 b	0,27 ± 0,12 a	0,35 ± 0,04 a	0,28 ± 0,05 a
Trp intake (mg day ⁻¹)	15,83 ± 4,18 b	27,33 ± 12,12 a	35,15 ± 4,00 a	28,00 ± 5,01 a

Values with different letter in the same row are statistically significant (n=6; Duncan, P<0,05).

Both juvenile and adult Nile tilapias have the ability to distinguish and choose between two diets with different levels of Trp. They were also able to regulate consumption and even to ingest it through free choice.

This results corroborates by Pereira-da-Silva et al. (2004) and Bordinhon (2008) regarding juvenile Nile tilapia ability to regulate protein intake. Pereira-da-Silva (1999) has shown that the fish can select the food to be consumed. Nile tilapia can select during feed intake with different ingredients (Pereira-da-Silva, 2000). Almada-Pagan et al. (2006) determined that sharpsnout seabream (*Diplodus puntazzo*) is also able to self-balance and regulate the consumption of proteins, carbohydrates and lipids while Fortes-Silva et al. (2010) detected preference for diets with different types of oil for Nile tilapia.

The comparison of Tryptophan supplemented feed consumption shows that in Experiment 1, feed R8 of treatment 2, the fish slightly decreased feed consumption compared to R4 in treatment 1 while subtly increasing the ingestion of control diet to self-balance amino acid intake. However not significant, it suggests and agrees with the literature regarding decreasing food intake for Trp supplemented diet since Trp increases the levels of serotonin in the brain, signaling satiety in fish (Rossi & Tirapegui, 2004; Feijo et al., 2011; Lillesaar, 2011). This response is also related to less weight and length gain for treatment 2, which had higher Trp levels in diet R8.

In Experiment 2, Tryptophan consumption was regulated by fish, consuming either more or less of the supplemented ration. Feed intake was lower and statistically different in treatment 1 ($P < 0,05$) compared to the other treatments. From the amount supplied in treatment 2, where Trp consumption was 0,27 per day (equivalent to 8,20 g Trp kg^{-1} of feed ingested), it does not pay off to supplement the diet with the amino acid since the animal regulates ingestion and does not increase it significantly. Although not significantly different, decreasing weight gain and feed intake were observed in treatment 4 compared to treatment 2, which corroborates Feijo et al. (2011) for food intake and satiety related to increase in brain serotonin.

Studies in pigs (Dapoza, 2011) during lactation showed positive effects of Trp supplementation on feed intake, resulting in higher feed intake, increased growth, improved feed conversion and lower weight loss. Moreover, Freitas-Pinheiro et al. (2008) demonstrated that increasing Trp levels in the diet of quails at rate of 2,10g kg^{-1} per day, produces a feeling of well-being and increases feed intake, determining also that for every 10 g Trp kg^{-1} added to the diet up to a maximum of 2,10g kg^{-1} , egg production increased by 21,16%. Trp supplementation had a significant effect on feed intake of laying hens, which increased when the amino acid were proportionately increased, but not excessively (Peganova et al., 2003; Peganova & Eder, 2003).

Despite the fact that there are no studies regarding Trp self-balance for Nile tilapia, the results of this study show that juvenile and adult Nile tilapia can regulate the intake of

this amino acid in $8,40\text{g kg}^{-1}$ and $8,20\text{g kg}^{-1}$ of the feed, and similar to other species, it also improved productive performance in this phase, showing better weight gain and feed conversion.

The results also suggest that Trp supplementation in the feed should not be excessive, since weight gain starts decreasing while feed production cost is increasing. Tryptophan is the most expensive synthetic amino acid available in the market. There is an optimal level of each nutrient that results in the best fish performance and in this work, among the options studied; the recommended values are $8,40\text{g Trp kg}^{-1}$ and $8,20\text{g Trp kg}^{-1}$ in the diet for juveniles and adults, respectively.

Considering these values, stimulating the responses of self-balancing the ration should still see some of the effects of amino acid on performance. Are suggested research from physiological variables related to animal welfare (cortisol and glucose, among others) aimed at an improvement in the production parameters in fish. The limitations that are now are the high cost of the amino acid Tryptophan, as well as the lack of infrastructure for the experiments on laboratories.

Conclusions

In general, it can be concluded that juvenile and adult Nile tilapia have the ability to self-balance the consumption of Tryptophan to $8,40\text{g kg}^{-1}$ and $8,20\text{g kg}^{-1}$ in the feed, respectively, showing that this amino acid supplemented at these levels benefits fish growth performance.

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