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Short Communication

Compelling palatability of flavoring Atractus AQVA® for Nile tilapia juveniles

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ABSTRACT. This study determined the compelling palatability of Atractus Aqva® flavoring for Nile tilapia juveniles (*Oreochromis niloticus*). Five isoproteic (40% crude protein) and isoenergetic (3,420 kcal kg^{-1}) experimental diets were elaborated containing 0.25 (A25), 0.50 (A50) and 0.75% (A75) flavoring inclusion, a positive control diet with 10% fishmeal (FPE) and a negative control without fishmeal (SPE). Five juvenile individuals (2.58 ± 0.27 g) were distributed in five 10 L tanks and were fed four times a day with one of the diets, randomly raffled. The same number of pellets was offered, and the following behaviors were observed: time to capture the first pellet, number of pellet rejections, and number of approximations without capture and consumed pellets in each feeding event, using three-minute recordings with a digital camera. A significant effect ($P < 0.05$) was found regarding the number of approximations without capturing the pellet, as well as a higher palatability index for A75, followed by A50, A25, SPE and FPE. Therefore, it was concluded that A75 provided the highest compelling palatability of all diets by increasing the palatability index by 10.49% and displaying a 23.13% reduced rejection of pellets, besides presenting a 3.3 fold reduction in the number of approximations without capturing pellets with diet FPE.

Keywords: *Oreochromis niloticus*; tilapia; fish farm; feeding preference; nutrition

Although more than 70 species of tilapia are known, the Nile tilapia (*Oreochromis niloticus*) stands out by its great rearing potential, easy reproduction and adaptability to handlings, besides its acceptance to several feeding sources and the high quality of the meat, which is greatly accepted by the final consumer (Higuchi *et al*., 2013; Brito *et al*., 2017). This species is the fourth most produced in the world (FAO, 2018) and the most produced species in Brazil (IBGE, 2017).

In this sense, seeking and utilizing high-quality ingredients in artificial diets are of great interest, in order to meet the animal's demand for nutrients (Decarli *et al*., 2016). As well as the intensification of feeding and its use, this is determinant for the development of any aquaculture enterprise. Feeds are the main economic burdens of the productive fish chain, with diets accounting for more than 40 to 70% of the operational cost of fish farming (Boscolo *et al*., 2005; Zho & Yue, 2012). Artificial diets with ingredients of animal origin considerably increase the cost of commercial feeds (Tacon & Metian, 2008). One alternative to reduce costs with commercial feeds would be to reduce or completely replace fishmeal as a protein source, but a major obstacle for this alternative is the acceptability by the fish, as diets rich in fishmeal usually has high palatability indexes (Broggi *et al*., 2017; Silva *et al*., 2017).

Dietary attractiveness is responsible for the initial detection of feed by fish, which uses its vision and chemoreceptors to find, reach and consume the diet. Regardless of the sensorial organs involved in feeding, palatability is responsible for the final selection of feeds and defines if the fish accepts the diet. Additionally, during the retention time of the feed inside the fish's mouth, they can detect and recognize tasteful substances, to decide swallowing or rejection (Pereira

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Table 1. Chemical composition of flavoring and experimental diets used for evaluating the compelling palatability in Nile tilapia juveniles *Oreochromis niloticus* (based on the dry matter).

Parameters	Diets					
	Atractus Aqva® flavoring	Fishmeal (control)	Without fishmeal	Scent 0.25%	Scent 0.50%	Scent 0.75%
Crude protein (%)	50.58	41.37	41.68	41.94	43.05	42.42
Lipids (%)	0.65	4.29	4.27	3.97	4.30	4.81
Dry matter (%)	95.87	94.25	94.66	94.50	95.21	94.51
Ash (%)	16.14	8.66	8.66	7.77	7.94	7.76
Crude energy (kcal kg^{-1})	3.928	4.430	4.411	4.463	4.447	4.430

da Silva & Pezzato, 2000; Lokkeborg *et al*., 2014; Olsen & Lundh, 2016).

In this sense, nutritional additives that serve as food stimulants may be an alternative to compelling palatability for aquatic animals (Srichanun *et al*., 2014). Based on the abovementioned, this study aimed to determine the compelling palatability of diets containing the inclusion of a flavoring as a feed stimulant in the preferences of the feed offered to Nile tilapia juveniles.

The experiment was conducted in the Laboratory of Aquaculture of the Aquaculture Management Study Group-GEMAq, Universidade Estadual do Oeste do Paraná (UNIOESTE), Toledo, Paraná, southern Brazil, during May 2018. The procedures presented in this study were approved by the Ethics Committee on Animal Use (CEUA) of the Universidade Estadual do Oeste do Paraná, according to an Experimental Certificate of Animal Use Nº09/18.

The flavoring used in this study was the Atractus Aqva®, supplied by the company Safeeds. Both the flavoring and test diets were analyzed for crude protein, lipids, dry matter, ashes and energy, according to the methodology described by the Instituto Adolfo Lutz (2004), except for crude energy, which was determined with the aid of a calorimetric pump (IKA® C2000) (Table 1).

All analyses were made at the Laboratory of Food Quality (LQA) of the GEMAq. The feed's amino acid composition was assessed by the method MA-009 and MA-010 (Lucas & Sotelo, 1980; White *et al*., 1986; Hagen *et al*., 1989) at a commercial laboratory (CBO Laboratory Analysis Ltda., Valinhos-SP) (Table 2).

Five experimental diets were elaborated: a diet containing 0.25 (A25), 0.50 (A50) and 0.75% (A75) of flavoring, a control diet with fishmeal (FPE), and another without fishmeal (SPE). These experimental diets were made to be isoproteic and isoenergetic, according to the recommendations of Furuya (2010) (Tables 3-4).

Table 2. Total and free amino acids present in the flavoring used for the evaluation of compelling palatability in Nile tilapia juveniles *Oreochromis niloticus* (based on the dry matter).

Chemical composition	Atractus Aqva® flavoring	
	Total amino acids (%)	Free amino acids (%)
Aspartic acid	0.10	Not detected
Glutamic acid	0.12	Not detected
Threonine	5.12	5.67
Tyrosine	0.10	Not detected
Methionine	0.07	Not detected
Cystine	0.44	Not detected
Phenylalanine	0.16	Not detected
Tryptophan	3.01	Not detected
Total	9.12	5.67

Feed extrusions were carried out in the feed factory of the GEMAq. Ingredients were initially milled with a 0.3 mm sieve in a hammer type mill. For the processing of feeds, the blend was homogenized and placed for 15 min in a mechanical blender type "Y." Diets were moistened with 20% of water and extruded (1.0 mm diameter). Upon the extrusion process, diets were dried in an air-circulating oven (55°C) for 24 h.

Five Nile tilapia juveniles (2.58 ± 0.27 g) were distributed in five 10 L tanks coated with a latex-based waterproofing material, individually equipped with aeration and temperature control systems, using a 15 W thermostat.

In order to monitor water quality, three analyses were performed at the beginning, middle and end of the experimental period. Water temperature, pH and dissolved oxygen were assessed daily, with the aid of a multiparameter model YSI® Professional Series. Luminosity within the tanks followed the natural oscillations of the circadian cycle and both physical and chemical water characteristics were similar among tanks, with mean temperature of 26.10 ± 1.18°C, pH 7.5 ± 0.42 and dissolved oxygen 4.35 ± 0.53 mg L^{-1}, consi-

Table 3. Ingredients of the experimental diets used to assess compelling palatability in Nile tilapia juveniles *Oreochromis niloticus* (based on dry matter). [1]Crude protein content. [2]Guarantee levels per kilogram of the product: vit. A: 500,000 UI; vit. D_3: 200,000 UI; vit. E: 5,000 mg; vit. K_3: 1,000 mg; vit. B_1: 1,500 mg; vit. B_2: 1,500 mg; vit. B_6: 1,500 mg; vit. B_{12}: 4,000 mg; folic acid: 500 mg; calcium pantothenate: 4,000 mg; vit. C: 15,000 mg; biotin: 50 mg; inositol: 10,000 mg; nicotinamide: 7,000 mg; choline: 40,000 mg; cobalto: 10 mg; copper: 500 mg; iron: 5,000 mg; iodine: 50 mg; manganese: 1,500 mg; selenium: 10 mg; zinc: 5,000 mg. [3]Butylhydroxytoluene (BHT).

Ingredient	Diet composition (%)				
	Fishmeal (control)	Without fishmeal	Scent 0.25%	Scent 0.50%	Scent 0.75%
Soy protein concentrate (60%)[1]	16.58	26.06	26.13	26.20	26.27
Corn meal	26.99	24.85	24.40	23.97	23.50
Rice grits	9.98	10.00	10.00	10.00	10.00
Poultry viscera meal	10.00	10.00	10.00	10.00	10.00
Fishmeal (55%)[1]	10.00	0.00	0.00	0.00	0.00
Flavoring	0.00	0.00	0.25	0.50	0.75
Feather meal	6.79	6.80	6.80	6.80	6.80
Corn gluten meal (60%)[1]	5.00	5.00	5.00	5.00	5.00
Blood meal	5.00	5.00	5.00	5.00	5.00
Soybean oil	2.39	3.12	3.25	3.37	3.49
Distilled alcohol yeast	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	0.76	1.95	1.95	1.95	1.96
Wheat gluten	1.00	1.00	1.00	1.00	1.00
Mineral-vitamin supplement[2]	1.00	1.00	1.00	1.00	1.00
Calcitic lime	0.00	0.72	0.72	0.71	0.72
L-lysine HCL	0.63	0.58	0.58	0.58	0.59
L-threonine	0.56	0.55	0.55	0.55	0.55
Salt	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.35	0.40	0.40	0.40	0.40
Vitamin C (35%)	0.20	0.20	0.20	0.20	0.20
Choline chloride	0.15	0.15	0.15	0.15	0.15
Antifungal (calcium propionate)	0.10	0.10	0.10	0.10	0.10
Antioxidant (BHT)[3]	0.02	0.02	0.02	0.02	0.02
Total	100.00	100.00	100.00	100.00	100.00

dered adequate for the species development (Ridha & Cruz, 2001). A polystyrene barrier was placed in the tanks' surroundings, isolating them from the laboratory routine, thus minimizing possible effects on the animals' behavior.

Before the beginning of the experiment, the fish underwent an adaptation and training eight days, in order to adapt to the human presence in the room, which occurred during the registers of feeding behavior when fish were fed, as well as during the time needed to quantify the number of pellets until apparent satiety. At this time, fish were fed with a commercial feed (40% crude protein and pellet size of 1 mm). Upon the adaptation period, the compelling palatability essay was started. Fish were fed four times a day at 08:00, 11:00, 14:00 and 17:00 h. Daily, all tanks were siphoned for solids removal, such as excreta and feed remain, by the system of water renewal. Thirty pellets were provided per fish in each feeding.

The experimental design was completely randomized, with four replicates per day. A treatment draw was made for each fish daily. A three-minute filming period was set for each feeding with a GoPro® Hero 5 Black 12MP 4K camera, by the exact time the diet was added in the observation tanks. The essay lasted for eight days, and 160 footages were obtained (5 fish × 4 feedings × 20 trials a day). For each tested diet, 32 feeding trials were made.

The compelling palatability assay was performed according to the methodology developed by Kasumyan (1997) (Kasumyan & Morsi, 1996; Kasumyan & Doving, 2003; Kasumyan & Sidorov, 2012). These authors proposed the palatability index as an estimate of the gustative preference (in percentage), using the formula: $PI = ((R-C)/(R+C)) \times 100$, in which PI is the palatability index; R is the consumption of pellets of the diet tested; and C is the pellet's consumption of the control diet.

Table 4. Calculated composition of experimental diets used to evaluate compelling palatability for Nile tilapia juveniles *Oreochromis niloticus.*

Ingredient	Diet composition (%)				
	Fishmeal (control)	Without fishmeal	Scent 0.25%	Scent 0.50%	Scent 0.75%
Starch	25.34	24.00	23.72	23.44	23.17
Total arginine	2.48	2.61	2.61	2.62	2.62
Calcium	1.31	1.31	1.31	1.31	1.31
Digestible energy (kcal kg^{-1})	3,420	3,420	3,420	3,420	3,420
Total phenylalanine	1.95	2.05	2.05	2.05	2.05
Crude fiber	1.07	1.29	1.29	1.28	1.28
Available phosphorus	0.90	0.76	0.76	0.76	0.76
Total phosphorus	1.00	1.00	1.00	1.00	1.00
Fat	6.65	6.16	6.26	6.37	6.48
Total histidine	1.02	1.05	1.05	1.05	1.05
Total isoleucine	1.56	1.61	1.61	1.61	1.61
Total leucine	3.38	3.41	3.41	3.41	3.41
Total lysine	2.60	2.60	2.60	2.60	2.60
Total methionine	1.00	1.00	1.00	1.00	1.00
Crude protein	40.00	40.00	40.00	40.00	40.00
Fish digestible protein	33.80	34.45	34.46	34.46	34.48
Total threonine	2.20	2.20	2.20	2.20	2.20
Total tryptophan	0.40	0.43	0.43	0.43	0.43
Total valine	2.23	2.23	2.23	2.23	2.23

Table 5. Mean values of the compelling palatability test using different flavorings in comparison to fishmeal (positive control) in Nile tilapia juveniles *Oreochromis niloticus*. Means followed by distinct superscript letters in the columns indicate significant differences by the Tukey's test ($P < 0.05$).

Treatment	Palatability index (%)	Rejections after capture	Approximations without capture	Time to capture first pellet(s)
Fishmeal (control)	0	1.60 ± 0.51	1.62 ± 0.53^b	4.41 ± 2.23
Flavoring 0.75%	10.49	1.23 ± 0.49	0.49 ± 0.44^a	5.13 ± 2.40
Flavoring 0.50%	7.09	1.63 ± 0.62	0.64 ± 0.21^a	6.98 ± 2.91
Flavoring 0.25%	5.04	1.29 ± 0.49	0.54 ± 0.24^a	4.48 ± 1.56
Without fishmeal	1.02	1.77 ± 0.89	0.51 ± 0.27^a	6.20 ± 3.03

Upon data collection, the obtained footages were analyzed regarding the following feeding behaviors (Alves *et al*., 2019): time of capture of the first pellet (seconds), number of rejections after capture and number of approximations without capturing the pellet and consumed pellets. Then, the palatability index was calculated for each treatment. All data were submitted to parametric variance analysis (ANOVA), and in case of significant effect, the multiple comparison test of means test of Tukey was performed at a 5% significance level. Before the analyses, the normality of errors was verified (Shapiro-Wilk's test), as well as the homogeneity of variances (Levene's test). All analyses were made with the aid of the Statistic 7.1 (2005) software.

The higher palatability index was verified in treatment A75 (10.49%), in comparison to A50 (7.09%), A25 (5.04%), and SPE (1.02%), in comparison to FPE (Table 5).

Significant differences were observed regarding the number of approximations without capturing pellets ($P < 0.05$). The diet FPE had the highest value, with 1.62 approximations without capturing. No differences were verified amongst the remaining diets. However, it is noteworthy that in A75, the number of approximations without capturing pellets was 3.3 times lower in comparison to FPE. No statistical difference was found regarding the parameter of time to capture the first pellet and for the number of rejections after capture.

Although no statistical differences were found ($P > 0.05$) regarding number of rejections after capture, it is noteworthy that the lowest mean value of rejection number were found in A75, with 1.23 pellets, followed by A25 with 1.29, FPE with 1.60 pellets, A50 with 1.13 pellets and SPE with the highest rejection of 1.77 pellets. The data present in the study highlights that the diet A75 presented 23.13% less rejection concerning FPE.

Fish feeding behavior is stimulated by substances of low molecular weight, including amino acids, peptides and nitrogen compounds. Amino acids are responsible for chemical signals received by the gustatory system of fish, classified as stimulants, may act alone or in combination and may differentiate the attractiveness of a feed (Kasumyan, 1997; Hara, 2011; NRC, 2011; Suresh *et al*., 2011; Olsen & Lundh, 2016; Siikavuopio *et al*., 2017; Alves *et al*., 2019).

Despite the absence of a significant effect, the lower rejection of pellets, as well as a lower number of approximations without capturing the pellets and the differences found for the palatability index may be associated to the compound formed by the flavoring amino acids, with total and free amino acids present regard 9.12 and 5.67%, respectively.

Alves *et al*. (2019) observed that Nile tilapia juveniles fed with poultry, feather and swine liver hydrolysates in replacement to fishmeal showed highlighted that the positive differences found for the palatability index, pellet consumption and number of rejections after capture for the poultry protein hydrolysate may be associated to the concentration of free amino acids.

The inclusion of 0.75% of the Atractus Aqva® flavoring increased palatability index and provided the lower rejection of pellets, as well as a lower number of approximations without capturing the pellets concerning the control diet containing fishmeal. Therefore, it can be efficiently used to stimulate the consumption of feed for Nile tilapia juveniles in replacement of fishmeal is replaced.

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