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Apparent digestibility coefficient of chitosan foam for nile tilapia

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Brazilian Journal of Development

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ABSTRACT

Chitosan foam can be used as a filter element for pollutants in Nile tilapia farming tanks to reduce the industrial chitin waste and value it. Another possibility is the inclusion of chitosan foam in fish feed. Therefore, this study aims at evaluating the apparent digestibility of nutrients and energy of chitosan foam for Nile tilapia. Apparent digestibility determination was carried out by the indirect fecal collection method, using chromic oxide as an inert indicator, a reference-diet and a test diet (70% reference-diet and 30% chitosan foam). Hence, 120 juveniles of Nile tilapia (50 ± 5 g) were used, divided into six replications. After the collection period, bromatological analyses of foam, diets and feces were carried out, as well as the determination of chromium concentration in feces. The coefficients of apparent digestibility concerning nutrients and energy were then calculated. Chitosan foam showed 83.7% digestible dry matter, 5.7% digestible protein, 7.9% digestible fat, 0.6% digestible ashes, 17.6% digestible crude fiber and 1021 kcal kg^{-1} digestible energy for juveniles of Nile tilapia. It can be concluded that chitosan foam is partially digestible for Nile tilapia and can be used mainly as a feed source of fiber and fat.

Keywords: alternative feed; biopolymers; filter element; sustainability.

Brazilian Journal of Development

1 INTRODUCTION

The formulation of fish diets should be based on knowledge of the nutritional requirements of the species, of food management, of nutritional values of food, such as composition, digestibility and energy value, in order to increase nutrient utilization efficiency and minimize waste excretion and environmental impact (Bomfim, 2013).

Currently, an economical alternative is the substitution of ingredients traditionally used in fish feed by other agro-industry products and by-products, wastes and products not intended for human consumption. However, before the substitution, the analysis of the chemical composition and the feeding tests to determine the nutritional value of a new ingredient (Santos et al. 2008) are required.

Chitin is one of the most abundant natural biopolymers and can be found in the exoskeleton of crustaceans, mollusc cartilage, insect cuticles, cell walls of microorganisms (Kyzas and Bikiaris, 2015) and fish scales (Kumari et al. 2015). The remains discarded by the processing industries of crustaceans (shrimp, prawn, crab and lobster) are a potential source of chitin, whose main components are chitin (15-40%), protein (20-40%), calcium carbonate and magnesium (20-50%), and smaller constituents, such as astaxanthin, lipids and other minerals (Queiroz et al. 2017).

Chitin (poly-β- (1 → 4) -N-acetyl-D-glucosamine) can be deacetylated to produce chitosan. Chitosan is a nitrogen-containing polysaccharide (poly-β- (1 → 4) -2-amino-2-deoxy-D-glucose), characterized as promising material due to its non-toxicity characteristics, biocompatibility, biodegradability, low cost, its potential for adsorption of dyes, heavy metals (Kyzas and Bikiaris, 2015) and aquaculture pollutants, and is used as a powder (Chung et al. 2005; Bernardi et al. 2018). Another possibility is its use as a natural additive in the food industry, due to its antioxidant activity (Ghannam et al. 2016).

Brazilian Journal of Development

The transformation of chitosan powder into foam facilitates its use in adsorption processes, because the biopolymer is structured and malleable. The semiquantitative chemical composition of the chitosan foam reveals that on the porous side of the foam there is approximately 50% carbon, 10% nitrogen, 39% oxygen, 0.3% chlorine, 0.2% potassium and 0.5% of other chemical elements (Zadinelo et al. 2018).

Previous studies have shown that chitosan foam can be used as a filter media in fish farming systems for the adsorption of aquaculture pollutants. After adsorption, its composition is little altered, because there is the presence of the adsorbed elements, and destination of the waste material would be to use as agricultural fertilizer, due to the fact that it is rich in nutrients (Zadinelo et al. 2018). Another alternative for the use of chitosan foam would be to use it as an ingredient for inclusion in feeds for aquatic organisms.

Thus, the objective of this study was to evaluate the apparent digestibility coefficient of the nutrients and the energy of the chitosan foam for Nile tilapia.

2 MATERIAL AND METHODS

The experiment was conducted in Laboratory of Productive Systems of Fish, UFPR - Sector Palotina. The coefficients of apparent digestibility of nutrients and energy of the chitosan foam for Nile tilapia (*Oreochromis niloticus*) were evaluated in diets processed in the pelletized form.

A total of 120 juveniles of Nile tilapia with a mean weight of 50±5 g, and sex reversed, were used. Fish were distributed in six circular net tanks of plastic (1.0 cm mesh) with a capacity of 50 L, contained in six 1000 L fiberglass feed tanks. The feed tanks (1,000 L each) were in a recirculation aquaculture system with mechanical filtration and biofilter (500 L tanks with biological media), and the daily renewal was on the order of ten times its volume.

For collection of feces, six cylindrical fiberglass tanks (180 L) were used, which have a conical bottom where were adapted bottles for collecting feces by sedimentation. Three tanks were used to collect feces from the reference diet, and other three tanks were used for collecting feces from feed-test (with chitosan foam). It is characterized by two treatments (reference diet and the test diet) and three replicates.

Oxygenation of water was maintained through 1/2 hp blower, in which they were connected to the pipe by plastic hoses with microporous stones. The physical-chemical variables of water, such as dissolved oxygen and temperature were monitored at 1 p.m. on alternate days, while pH, ammonia, nitrite, alkalinity and hardness were monitored weekly.

The dissolved oxygen and temperature were measured on an Alfakit AT160® oximeter, the pH on a Kasvi AI 03449® pH meter. The concentrations of ammonia were determined following the methodology proposed by Koroleff (1976), and the concentrations of nitrite by the methodology proposed by Baumgarten (1996). Alkalinity and hardness were determined by titration according to the methodology proposed by Macêdo (2003).

The mean values for water temperature were 24.33±0.82°C; the mean pH was 7.10±0.11; and dissolved oxygen of 6.11±0.17 mg L^{-1}. The mean concentrations of total ammonia, nitrite, alkalinity and water hardness were, respectively, 0.07±0.09 mg L^{-1}; 0.06±0.07 mg L^{-1}; 86.33±3.22 mg L^{-1} of $CaCO_3$; 24.92±2.13 mg L^{-1} of $CaCO_3$.

The reference and test diets (Table 1) were prepared to evaluate the apparent digestibility coefficient of the chitosan foam for Nile tilapia. The determination of the apparent digestibility was done according to the NRC (1993), by the indirect method of collecting feces using 0.1 g of chromic oxide (Cr_2O_3) as an inert indicator, a practical diet as reference and a test diet (Table 1). The test feed consisted of 70% of the reference diet and 30% of the ingredient to be tested, in this case the chitosan foam, correcting only the amount of mineral and vitamin supplement and common salt.

Table 1. Percentage composition of the reference and test diets, used to determine the coefficients of apparent digestibility of chitosan foam for Nile tilapia

Foods	Reference diet	Test diet
Soybean meal	70.22	49.50
Corn	20.20	14.14
Butyl hydroxytoluene	0.01	0.01
Dicalcium phosphate	2.90	2.03
Limestone	0.13	0.09
Soy oil	3.94	2.76
Supplement 1	2.00	2.00
salt	0.50	0.50
Chromic oxide	0.10	0.10
Food testing: Foam chitosan	0.00	29.22
Total	100.00	100.00

[1]Supplement mineral and vitamin, warranty levels per kilogram of the product: Vit. A, 1200000 IU; Vit. D3, 200000 IU; Vit. E, 12000mg; Vit. K3, 2400mg; Vit. B1, 4800mg; Vit. B2, 4800mg; Vit. B6, 4000mg; Vit. B12, 4800mg; B.C. Folic acid, 1200mg; Pantothenate Ca, 12000mg; Vit. C, 48000mg; Biotin, 48mg; Hill, 65000mg; Niacin, 24000mg; Iron, 10000mg; Copper, 6000mg; Manganese, 4000mg; Zinc, 6000mg; Iodine, 20mg; Cobalt, 2mg; Selenium, 20mg.

Source: Elaboration of the authors.

Brazilian Journal of Development

The chitosan foam was produced from commercial chitosan (Polymar Science and Nutrition S/A (Fortaleza, Brazil) using the drying process of the foam layer method. The biopolymer is composed of 50% chitosan and 50% commercial neutral emulsifier/stabilizer composed of monoglycerides of distilled fatty acids, fatty acid salt, sorbitan monostearate and polyoxyethylene sorbitan monostearate. Chitosan (5%) was dissolved in 2% acetic acid solution and kept under stirring for the foam preparation. After complete homogenization, 5% of commercial emulsifier was added to the chitosan solution and subjected to the mechanical incorporation of air in a domestic blender at full speed. The foam was standardized on metal forms with a 1.0 cm thick layer, which were kept in an oven with air circulation at 70°C until constant weight (Muniz et al. 2015).

For the production of reference diet and the test diet, the components were ground in a hammer-mill with 1.0 mm sieve. Subsequently, they were mixed according to their formulation and then processed. The pelletization was done on a trial pelletizer, after prior moistening of the mixture of ingredients with water at a temperature of about 50°C. The chitosan foam was ground into a 1.0 mm sieve mill, which presented as a cream-colored fine powder. After processing, the diets were dried in a forced ventilation oven for 24 hours.

For fish adaptation period, for each diet, fish were kept for five days, in which the fish remained inside the net tanks in the feed tank (1000 L). Fish were hand fed at apparent satiety, three times a day, at 8:00 a.m., 1:00 p.m. and 6:00 p.m. The feed was supplied through a funnel with a wide end at the opening of the net tanks, due to the fluctuation capacity of the test diet, so that the diets would be kept in a limited space and the fish could feed. During this period, the feed tanks were siphoned twice a day and there was the daily renewal of 50% of the water. At 6:30 p.m., the net tanks were transferred from the feed tanks to the digestibility tanks, for the collection of feces. The feces were discarded during the adaptation period. The next morning (8:00 a.m.), the net tanks with the fish were removed from the digestibility tanks and replaced in the feed tank, followed by the same management described above. After the withdrawal of the net tanks, the digestibility tanks were washed and the water was exchanged integrally.

After the period of adaptation to the diet, the collection of feces was carried out with management similar to that described in the previous paragraph. The feeding was performed at 8:00 am, 1:00 pm and 6:00pm. After a period of 30 minutes, after the last feed (6.30 pm), the net tanks with the fish were transferred to the tanks. In these, the fish remained until 8:00 am, then were removed and replaced in the 1000 L feed tanks. After it was observed that the

feces were sedimented in the feces collector coupled to the tanks, the water outlet valve of the tank was closed and removed the collection bottle with feces. At that time, the feed tanks (1000L) were siphoned twice a day and there was 50% daily renewal of the water. After the removal of the net tanks, the digestibility tanks were washed and their water changed integrally.

The feces collected during the experimental period were stored in identified plastic bottles in the freezer at -18°C for later analyzes. The collections were carried out until the collection of approximately 1000g of wet feces per replicate/tank was reached in both treatments.

The chitosan foam and the diets were properly milled for the analysis. The feces, however, were thawed and sieved in a 1.0 mm mesh for the removal of scales, subsequently dried in a forced ventilation oven (55ºC for 72 hours) and then milled in a ball mill.

The chemical analysis of food test, diets and feces were carried out at the Laboratory of Animal Nutrition and Feeding (LANA), UFPR - Sector Palotina. Determinations of dry matter, mineral matter, ethereal extract, crude protein, crude fiber and crude energy were carried out according to the methodology proposed by AOAC (2005).

The determination of chromium oxide concentration was carried out at the Laboratory of Instrumental Analysis of the UFPR – Sector Palotina, by flame atomic absorption spectrometry (Kimura and Miller, 1957).

The calculation of the apparent digestibility coefficients of the nutrients and energy of the food tested was performed according to the equations used by Bureau et al. (1999) and Bureau and Hua (2006). The apparent digestibility coefficients for the nutrients and energy of the test diet and the reference diet were calculated according to equation 1:

Equation 1: $ADC = 1 - ((F \div D) \times (Di \div Fi))$

Where:

ADC = Apparent digestibility coefficient;

D =% nutrient of the diet;

F =% nutrient of feces;

Di =% indicator of diet;

Fi =% indicator of feces.

Brazilian Journal of Development

The apparent digestibility coefficient of the tested ingredient (ADCi) was calculated based on the digestibility of the reference diet and of the test diet, according to equation 2:

Equation 2: $ADCi = ADCt + ((1 - s) \times Dr \div (s \times Di)) \times (\mathrm{ADCt} - \mathrm{ADCr})$

Where:

ADCi = Apparent digestibility coefficient of the test ingredient;

ADCt = apparent digestibility coefficient of the test diet;

ADCr = Apparent digestibility coefficient of the reference

diet Dr =% of nutrient of the reference diet;

Di =% gross energy of the tested ingredient;

Dt =% of nutrient of the diet tested;

s = Proportion of the ingredient tested in the test diet (0.3 in this study);

1 - s = Proportion of reference diet in the diet tested (0.7 in this study).

3 RESULTS AND DISCUSSION

Chemical composition

Chitosan foam is a fibrous material (35.7%) with a nitrogen content of 5.7% (Table 2).

Table 2. Chemical composition of chitosan foam, and reference and test diets (values expressed as 100% dry matter).

Ingredients	DM %	MM %	N %	CP %	EE %	CF %	GE (kcal kg^{-1})
Chitosan foam	85.6	1.0	5.7	35.4	18.2	35.7	5120.0
Reference diet	96.6	8.8	5.7	35.6	6.5	6.5	4626.0
Test diet	95.3	6.9	5.3	33.3	11.6	11.9	4826.0

DM: dry matter; MM: mineral matter; N: nitrogen; CP: crude protein; EE: ethereal extract; CF: Crude fiber; GE: gross energy

The test diet, with 30% chitosan foam, presented as characteristic the flotation capacity in the water, even being a processed pelleted feed. This is due to the low density of the chitosan foam (0.047 ± 0.002 g cm^3) (Zadinelo et al. 2018). It was considered a positive feature because as well as the extruded feed allows better observation and control of animal consumption.

Brazilian Journal of Development

Apparent digestibility coefficient

The apparent digestibility coefficient and the digestible nutrient values and energy of the chitosan foam in pelleted diets for Nile tilapia are shown in Table 3.

Table 3. Apparent digestibility coefficient and digestible values of chitosan foam in pelleted diets for Nile tilapia

Apparent digestibility coefficients						
Ingredients	DM %	GE %	CP %	EE %	CB %	MM %
Chitosan foam	97.71 ± 0.25	19.94 ± 1.75	15.95 ± 1.88	43.41 ± 3.73	49.51 ± 2.62	61.62 ± 9.09
Digestible values						
Ingredients	DDM %	DE (kcal kg^{-1})	DP %	DEE %	DCF %	DMM%
Chitosan foam	83.71	1021.30	5.70	7.90	17.67	0.62

DM: dry matter; GE: gross energy; CP; crude protein; EE: ether extract; CF: Crude fiber; MM: mineral matter; DDM: digestible dry matter; DE: digestible energy; DP; digestible protein; DEE: digestible ether extract; DCF: digestible Crude fiber; DMM: digestible mineral matter

The apparent digestibility coefficient of the dry matter of the chitosan foam (97.71%) for tilapia was higher than those found by Shiau and Yu (1999) for chitosan (82.34%) and Köprücü and Özdemir (2005) for exoskeleton meal of lobsters (*Astacus leptodactylus*) (75.7%) and the gammarid meal (*Gammarus kischineffensis*) (77.0%).

The coefficients of apparent digestibility of crude energy (19.94%), crude protein (15.95%), ethereal extract (43.41%), crude fiber (49.51%) and mineral matter (61.62%) found in this study were lower than the values reported in other studies, probably as a function of the emulsifier that is part of the chitosan foam composition. Shiau and Yu (1999) found apparent digestibility values of 88.01% for protein and 88.83% for lipids in a diet supplemented with 10% chitosan for Nile tilapia fingerlings. The authors state that depression of dietary nutrient digestibility by addition of chitosan may result in depression of fish growth.

Köprücü and Özdemir (2005) reported apparent digestibility coefficients of 71.0 and 75.8% for protein, 72.0 and 75.8% for lipids, 69.3 and 71.5% for fiber or chitin, and 54.8 and 65.6% for energy, in tilapia fed with the exoskeleton flour of lobsters and gamarid flour, respectively. These values were lower than for other tested ingredients (anchovy flour, corn gluten meal and soybean meal), due to the high ash content (30.0 and 27.5%) and chitin (10.2 and 6.6%) in the exoskeleton flour of lobsters and gamarid flour, respectively. The low

digestibility of the chitosan foam obtained in the present study may be due to the composition of this material.

The named portion of fiber, in animal nutrition, represents the group of compounds that are not digested by animals or that have slow digestion. Analytically, to measure the amount of fiber in the food, these are submitted to simulated conditions of the animal gastrointestinal tract (mainly chemical conditions), and subsequently their contents are quantified. In fact, the analytical part cannot truly quantify the fiber contents, which can only be measured by the animal (in vivo) (Undersander et al. 1993). However, this definition is widely used in animal nutrition.

In the case of crude fiber, the foods are first submitted to digestion with diluted acid and then to digestion with diluted base (alkali). The remaining portion after the two digests is named crude fiber, then considered as the portion of the indigestible carbohydrates by the animal (Detmann et al. 2010; Silva and Queiroz, 2002). As the majority of fiber sources consumed by the animals are from a vegetable source, the definition of crude fiber basically covers the fibers of vegetal origin: cellulose and alkali-insoluble lignin (Silva and Queiroz, 2002). However, in animal feeds, the crude fiber composition quantifies other indigestible carbohydrates that are not of vegetable origin.

The crude fiber analyzed in shrimp flour is basically composed of chitin, a structural polysaccharide composed of recurrent units of N-acetyl-D-glucosamine with β-binding, forming extended fibers undigested by non-ruminants (Boscolo et al. 2004; Lima et al. 2007). The components of dietary fiber are divided into the following groups: non-starch polysaccharides, oligosaccharides, resistant analogous carbohydrates, lignin, dietary fiber compounds (phenolic compounds, cell wall protein, oxalates, phytates, waxes, cutin and suberin) and animal origin (chitin, chitosan, collagen and chondroitin) (Giuntini and Menezes, 2011).

The inclusion of fibrous materials in the diet increases energy losses in feces, since they are poorly digested by fish (NRC, 1993). As can be seen in Table 1, the chitosan foam presents high crude fiber content (35.7%), which represents the structural carbohydrates of animal origin (chitin or derivatives), which, although having a good digestibility coefficient (49.51%), may have led to a low digestibility of other nutrients, including energy.

Chitin in the diet is known to decrease lipid absorption and induce increased water content in feces (diarrhea). Due to the low digestibility, chitin physically blocks the access of

digestive enzymes to lipids and proteins, thus affecting the utilization of these nutrients (Olsen et al. 2006).

Future studies are needed to verify the effects of chitosan foam on fish growth; however, chitosan and chitin have already been evaluated for some species of fish. Juvenile fish (*Pagrus major, Anguilla japonica* and *Seriola quinqueradiata*) fed a diet supplemented with 10% chitosan presented lower rates of growth and feed efficiency, indicating that the inclusion of chitosan in the diet inhibited the processes involved in the digestion, absorption and assimilation of basal diet (Kono et al. 1987).

In hybrid tilapia fingerlings (*O. niloticus x O. aureus*), weight gain generally decreased linearly as dietary chitin and chitosan supplementation levels increased (2, 5 and 10%) compared to a control diet without supplementation (Shiau and Yu, 1999).

Most nutrients of chitosan foam were partially digested by Nile tilapia, resulting in 1,021 kcal kg^{-1} of digestible energy. However, this aquaculture filter material may be a product with potential to be used in fish feed, mainly as a source of fiber and potentially lipids (7.9% of digestible ethereal extract).

The processing of crustaceans by the industries leads to the generation of waste, which can cause environmental contamination, when not used and discarded inappropriately. The waste generated is a renewable and abundant natural resource, which can be a direct source of nutrients, or be used to extract chitin and convert it to chitosan. Chitin and chitosan are used for the treatment of effluents (Bessa-Junior and Gonçalves, 2013). Chitosan foam is composed of 50% chitosan and 50% commercial neutral emulsifier/stabilizer (Muniz et al. 2015), and can be used in the treatment of aquaculture effluents (Zadinelo et al. 2018) and may also be included in the feeding of the fish used for cultivation, closing the productive chain in a more sustainable way.

4 CONCLUSION

Chitosan foam may be included in Nile tilapia diets, mainly as source of crude fiber and fat and is partially digestible, however, potential levels of inclusion should be studied.

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Brazilian Journal of Development

6 ETHICS STATEMENT

The experimental procedures were conducted according to the Ethical Principles of Animal Experimentation adopted by the National Council for the Control of Animal Experimentation (CONCEA), in accordance with Protocol 10/2018, approved by Ethics Committee on the Use of Animals of the Palotina Sector of UFPR (CEUA/Palotina).

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