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Digestibilidade aparente em juvenis de cioba e atividade digestiva das enzimas

Apparent digestibility in marine mutton snapper juveniles and digestive activity of enzymes

Digestibilidad aparente en juveniles de cioba marino y actividad digestiva de enzimas

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Leon Lima Leal
ORCID: https://orcid.org/ 0000-0000-0002-9103-7372
Universidade Estadual de Santa Cruz, Brasil
E-mail: leonleal@bol.com.br

Ana Paula de Souza Ramos
ORCID: https://orcid.org/ 0000-0002-5805-9596
Universidade Estadual do Sudoeste da Bahia, Brasil
E-mail: aninharamos.bio@gmail.com

Marcel José Martins Dos Santos
ORCID: https://orcid.org/ 0000-0001-7035-5534
Universidade Estadual de Santa Cruz, Brasil
E-mail: marceljmsantos@gmail.com

Francisco Oliveira de Magalhães Júnior
ORCID: https://orcid.org/ 0000-0000-0002-4479-9075
Universidade Estadual de Santa Cruz, Brasil
E-mail: jr.oliveira@zootecnista.com.br

José Fernando Bibiano Melo
ORCID: https://orcid.org/ 0000-0003-2068-4641
Universidade Federal do Vale do São Francisco, Brasil
E-mail: melojfb@yahoo.com.br

Marianne Schorer
ORCID: https://orcid.org/ 0000-0002-4931-3265
Universidade Estadual de Santa Cruz, Brasil
E-mail: marianne.schorer@gmail.com

Luis Gustavo Tavares Braga
ORCID: https://orcid.org/ 0000-0002-8989-5845
Universidade Estadual de Santa Cruz, Brasil
E-mail: lgtbraga@gmail.com

Resumo

A espécie marinha de cioba, *Lutjanus analis*, tem hábitos alimentares carnívoros e com potencial para o cultivo. O objetivo deste estudo foi avaliar o coeficiente de digestibilidade aparente (CDA) do cioba, e avaliar seu perfil enzimático após alimentação com fonte proteica vegetal e animal. O CDA foi o método indireto de coleta fecal, utilizando o óxido crômico como marcador biológico. Oitocentos peixes, com peso médio de 28,0 ± 2,58 g, foram aclimatados por 15 dias em tanque-rede (2 m³), e instalados no mesmo ambiente de coleta. A aclimatação de 54 peixes foi realizada em aquários de digestibilidade (200 L), sendo iniciada a coleta de fezes. Para o cioba, a oferta da farinha de polvo melhora (P <0,05) a digestibilidade da matéria seca (CDAMS, 67,17%), proteína bruta (CDAPB, 90,9%) e energia bruta (CDAGE, 78,8%). Em relação à digestibilidade dos lipídios (CDAL), a farinha de anchova possui mais (P <0,05) lipídios digestíveis (78,1%), vindo em seguida dos ingredientes testados, farinha de peixe (72,4%) e farinha de polvo (69,7%). A digestibilidade aparente da matéria seca, proteína bruta e energia bruta foram baixas (P <0,05) para farelo de resíduo de camarão e farelo de soja. O cioba alimentado com farinha de polvo apresenta maiores valores de coeficientes de digestibilidade, sugerindo este ingrediente para as espécies. Todas as dietas causaram baixa atividade da amilase nos juvenis, sendo que as atividades da lipase e da protease alcalina foram maiores com a inclusão na dieta da farinha de anchova banda larga e farinha de polvo, respectivamente.

Palavras-chave: Alimentação em cativeiro; Atividade de enzimas; Espécies carnívoras; Fontes de proteína; *Lutjanus analis*; Teste de digestibilidade; Valor nutritivo.

Abstract

The marine species of snapper, *Lutjanus analis*, has carnivorous eating habits and with potential for cultivation. The aim of this study was to evaluate the apparent digestibility coefficient (CDA) of mutton snapper, and to evaluate its enzymatic profile after feeding with vegetable and animal protein sources. CDA was the indirect method of fecal collection, using chromic oxide as a biological marker. Eight hundred fish, with an average weight of 28.0 ± 2.58 g, were acclimated for 15 days in a net tank (2 m³), and installed in the same collection environment. The acclimatization of 54 fish was carried out in digestibility aquariums (200 L), and the collection of feces started. For mutton snapper, the supply of octopus flour improves (P <0.05) the digestibility of dry matter (CDAMS, 67.17%), crude protein (CDAPB, 90.9%) and crude energy (CDAGE, 78, 8%). Regarding the digestibility of lipids (CDAL), anchovy flour has more (P <0.05) digestible lipids (78.1%), followed by the tested ingredients, fish meal (72.4%) and flour octopus (69.7%). Apparent digestibility of dry matter, crude protein and crude energy were low ($P < 0.05$) for shrimp meal and soybean meal. Mutton snapper fed with octopus flour has higher digestibility coefficients, suggesting this ingredient for species. All diets caused low amylase activity in juveniles, and lipase and alkaline protease activities were higher with the inclusion of broadband anchovy flour and octopus flour, respectively.

Keywords: Captive feeding; Carnivorous species; Digestibility test; Enzymes activity; *Lutjanus analis*; Protein sources; Nutritive value.

Resumen

La especie marina de cioba, *Lutjanus analis*, tiene hábitos alimenticios carnívoros y con potencial de cultivo. El objetivo de este estudio fue evaluar el coeficiente de digestibilidad aparente (CDA) del pargo y evaluar su perfil enzimático después de la alimentación con fuentes de proteínas vegetales y animales. El CDA fue el método indirecto de recolección de heces, utilizando óxido crómico como marcador biológico. Ochocientos peces, con un peso promedio de 28.0 ± 2.58 g, fueron aclimatados durante 15 días en un tanque (2 m^3), e instalados en el mismo ambiente de recolección. Se realizó la aclimatación de 54 peces en acuarios de digestibilidad (200 L) y se inició la recolección de heces. Para la cioba, el suministro de harina de pulpo mejora ($P < 0.05$) la digestibilidad de la materia seca (CDAMS, 67.17%), proteína cruda (CDAPB, 90.9%) y energía bruta (CDAGE, 78, 8%). En cuanto a la digestibilidad de los lípidos (CDAL), la harina de anchoa tiene más ($P < 0.05$) lípidos digestibles (78.1%), seguida de los ingredientes probados, harina de pescado (72.4%) y harina de pulpo (69.7%). La digestibilidad aparente de la materia seca, la proteína cruda y la energía bruta fueron bajas ($P < 0.05$) para la harina de camarón y de soja. La cioba alimentada con harina de pulpo tiene coeficientes de digestibilidad más altos, lo que sugiere este ingrediente para las especies. Todas las dietas causaron una baja actividad de amilasa en los juveniles, y la actividad de la lipasa y la proteasa alcalina fue mayor con la inclusión de harina de anchoa de anchoa y harina de pulpo, respectivamente.

Palabras clave: Actividad enzimática; Alimentación en cautividad; Especies carnívoras; Fuentes proteicas; *Lutjanus analis*; Prueba de digestibilidad; Valor nutricional.

1. Introduction

The *Lutjanus* sp. genus is a marine species of snapper native to the Atlantic coastal waters of the Americas from Massachusetts to southern Brazil with potential for captive culture, with great productive performance, demand for the consumer market and high market value (Freitas, et al., 2011; Aguilar-Betancourt, et al., 2017). Thus, the development of the marine aquaculture productive chain, highlighting the mutton snapper *Lutjanus analis* culture, reduces extractive fishing and preserves the natural stocks, and provides a tool for protecting this significant group of reef fish (Sanches, et al., 2007).

In the literature, there is no information of the digestibility of ingredients for the mutton snapper, and the measurement of the digestibility of different protein sources occurs through the indirect method, with the partial collection of feces, and the addition of an inert marker in the diet (Kitajima & Fracalossi, 2010). All methods have advantages and disadvantages of fecal contamination with endogenous components and required to estimate the absorption of ingredients and reduce feed costs.

In literature several studies with the digestibility of original sources of feeds for freshwater fish, as the soybean meal, fish meal or shrimp residue meal (Gonçalves & Carneiro, 2003; Portz & Cyrino, 2004; Oliveira & Fracalossi 2006; Braga, et al., 2008; Ribeiro, et al., 2011; Montoya-Mejía, et al., 2017), but for marine species few works (Gaylord & Gatlin, 1996; Zhou, et al., 2004; Oujifard, et al., 2012) represents this area of carnivorous fish nutrition.

Using vegetable sources influence marine carnivorous species feeding, such as mutton snapper, and may influence on performance and enzymatic activity during digestion. The profile of digestive enzyme activities (proteases, carbohydrases, and lipases), already studied for carnivorous marine fish (Lamarre, et al., 2007; Candiotto, et al., 2018; Egerton, et al., 2018; Hani, et al., 2018), except for mutton snapper.

The close relationship among the enzymes present in the fish's digestive tract and the use of dietary nutrients, allows us to understand the nutritional requirement of the diet. Thus, this study aimed to evaluate the apparent digestibility coefficient of the mutton snapper, *Lutjanus analis*, and characterize its enzymatic activity after feeding with protein, carbohydrates and lipids of vegetable or animal protein source.

2. Material and Methods

The conduction of the research was at the State University of Santa Cruz - UESC, at the Aquanut–Laboratory of Fish Nutrition, in the municipality of Ilhéus, Bahia, Brazil (latitude 14º 47' 20" S, longitude 39º 02' 58" W). The collection of the mutton snapper juveniles were in an estuary area in the village of Cajaíba in Bahia de Camamu (SISBIO no. 42117-1), Bahia (latitude 13° 57′ 23,92″ S, longitude 39° 2′ 0,58″ W) with the help of local fishermen, using slingshot trawling to decrease morbidity (98% survival). Fish were caught and acclimatized for 15 days in a net-tank (2 m^3), installed in the same collection environment.

The *Lutjanus analis* juveniles received fresh fish fillet minced three times a day during this adaptation period and then transferred to the Aquanut Laboratory using a transport box (500 L) with forced oxygenation.

The distribution of juveniles were in three polyethylene tanks (300 L) at the density of 1 fish per 10 L^{-1}. Mortality recorded in this period was less than 10%. All tanks were part to the water recirculation system, and connected to a matured biological filter composed of expanded clay, oyster shell, hoses and shingle as a substrate for the development of nitrifying bacteria.

The salinity was every day monitored and, when necessary, the addition of saltwater of the Rio Cachoeira estuary (Ilhéus, BA) to the system, previously filtered and stored in the Laboratory. Weekly we measure the dissolved oxygen concentrations (OD monitored the water quality), temperature (ºC), pH and salinity using YSI digital multiparameter probe, with a mean value of 6.92 mg L^{-1} OD; 27.4°C; 6.85; 35 respectively, remaining within the recommended range for genus *Lutjanus* in marine aquaculture (Oliveira et al., 2018).

To adapt juveniles to a commercial diet (42% crude protein and 4235 Kcal Kg^{-1} crude energy), juveniles received macerated fish fillet for five days, and gradually we added the diet to the fish fillet pieces. After 15 days, the *adaptation of animals* to commercial feed (pellets with 2.5 mm granulometry - Guabi Aqua–Pirá, for carnivorous fish) occurs. Nine fish (28.0 ± 2.58 g) were weighed and distributed in each of the 25 digestibility aquariums (200 L), with conical shape in water recirculation system with bio-filter already matured. The scheme was in a randomized experimental design with five treatments and three replications.

Collector tubes (200 mL) at the bottom ends of the digestibility aquariums, had a tapered shape, and the fish feces decanted, according to the modified Guelph system (Portz and Cyrino 2004). One hour after of the last feeding, water was exchange (40%) to eliminate leftovers. Subsequently, the collector tubes were kept under refrigeration at 0°C to reduce biological changes in fish feces. The collection of feces happened for six days and every day. At 8:00 am, feces were collected, and centrifuged for 10 min at 5000 rpm. The supernatant was discarded, and the precipitated material was lyophilized. Feces were stored in a refrigerator for later chemical composition analysis.

Five protein ingredients from a different animal source, with five repetitions were formulated: octopus meal (*Octopus sp*.), broadband anchovy (*Anchoviella lepidentostole*), shrimp industry residue (*Xiphopeneaus kroyeri*), commercial fish meal, and soybean meal (Table 1). To get octopus meal, broadband anchovy meal and shrimp residue meal, the dry of the raw materials (whole products) were in a forced ventilation oven at 50 ºC for 48 hours and individually processed in a knife-type mill (0.5 mm mesh).

Table 1. Chemical composition of the ingredients used in the experimental diets based on dry matter for *Lutjanus analis.*

Ingredient	DM (%)	CP (%)	Ash (%)	GE (kcal kg^{-1})	L (%)
Soybean meal	88.61	43.74	6.83	4620	1.84
Fish meal	93.54	55.06	20.17	4775	2.81
Octopus meal	17.60	65.97	9.78	4979	1.99
Broadband anchovy meal	25.61	48.60	13.61	5795	6.08
Shrimp residue meal	23.09	49.03	32.83	3266	4.49

DM – Dry matter; CP – Crude protein; Ash – Ashes content, GE – Gross energy; L - lipids. Source:Authors.

Based on the nutritional information of the European sea bass (*Dicentrarchus labrax*), five feeds were prepared, and except for the control diet, feeds comprised 70% of the reference diet and 30% inclusion of the evaluated ingredient. The ingredients were carefully milled in knife mills (0.5 mm sieve), mixed with premix vitamin/ mineral and chromium oxide (Cr_2O_3), which an external marker to evaluate the digestibility of the diets and ingredients (Table 2).

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Table 2. Food composition and nutritional levels of the diet for *Lutjanus analis*.

		Treatments (g Kg^{-1})				
Ingredients	RefD	SBM	FM	OM	BAM	SRM
Fish meal – 55%	455.00	315.81	315.81	315.81	315.81	315.81
Soybean meal – 45%	350.00	242.93	242.93	242.93	242.93	242.93
Corn gluten meal – 22%	49.70	34.50	34.50	34.50	34.50	34.50
Wheat bran	40.00	27.76	27.76	27.76	27.76	27.76
Corn meal	26.00	18.05	18.05	18.05	18.05	18.05
Maize starch	10.00	6.94	6.94	6.94	6.94	6.94
Fish oil	50.00	34.70	34.70	34.70	34.70	34.70
Soybean meal	0.00	300.00	0.00	0.00	0.00	0.00
Fish meal	0.00	0.00	300.00	0.00	0.00	0.00
Octopus meal	0.00	0.00	0.00	300.00	0.00	0.00
Broadband anchovy meal	0.00	0.00	0.00	0.00	300.00	0.00
Shrimp residue meal	0.00	0.00	0.00	0.00	0.00	300.00
Premix vitamin-mineral[1]	10.00	10.00	10.00	10.00	10.00	10.00
Common salt	5.00	5.00	5.00	5.00	5.00	5.00
DL-Methionine	4.00	4.00	4.00	4.00	4.00	4.00
Antioxidant	0.20	0.20	0.20	0.20	0.20	0.20
Chromium oxide III	0.10	0.10	0.10	0.10	0.10	0.10

[1]Mixture mineral and vitamin (Composition kg^{-1} of product): vit. A = 6.000.000 UI; vit. D3 = 2.250.000 UI; vit. E = 75.000 mg; vit. K3 = 3.000 mg; vit. thiamine = 5.000 mg; riboflavin = 10.000 mg; vit. pyridoxine = 8.000 mg; biotin = 2.000 mg; vit. C = 192.500 mg; niacin = 30.000 mg; folic acid = 3.000 mg; Fe = 100.000 mg; Cu = 600 mg; Mn = 60.000 mg; Zn = 150.000 mg; I = 4.500 mg; Cu = 15.000 mg; Co = 2.000 mg; Se = 400 mg.
RefD – Reference diet; SBM – Soybean meal; FM – Fish meal; OM – Octopus meal; BAM - Broadband anchovy meal; SRM - Shrimp residue meal. Source:Authors.

For preparing the experimental diets, after the homogeneous mixing of the dry ingredients, there was the manual inclusion of fish oil and warm water (35 °C), and the pellets produced in a meat grinder (2 mm matrix) equipped with reverser. Pellets were dried at 50 °C for 24 hours in a forced ventilation oven, identified and stored in plastic bags and kept in a refrigerator (4 °C). The fish were fed until apparently satiety, five times a day between 08:00 and 16:00.

We based the calculation of the apparent digestibility coefficient of the ingredients on the chromium oxide content and the chemical composition of the diets and feces. The laboratory analyzes for crude protein (CP), dry matter (DM), gross energy (CE) and lipids (L)

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of the ingredients, diets, and feces, and performing the chromium concentration according to the method described by AOAC (2012). The calculation of the apparent digestibility coefficients of the diets (ADC) was according to Maynard and Loosli (1969), using the formula:

ADC (%) = 100 - 100 x [(%Cr_2O_3 in diet /% Cr_2O_3 in feces) x (% nutrient in feces /% nutrient in diet)].

We performed the determination of the apparent digestibility coefficients of the tested ingredients using the following relation (Bureau et al., 1999):

$$ADC_i=[(ADC_{dt}xD_t-((ADC_{dr}x0.7D_r)/(0.7DM_{dr}+0.3DM_i))/(0.3D_t)/(0.7DM_{dr}+0.3DM_i)]$$

Where ADC_{dt} = coefficient of apparent digestibility of the test-diet; D_t =% of the nutrient (or kcal kg^{-1} of gross energy) of the test-diet (dry matter basis); $ADCd_{rd}$ = coefficient of apparent digestibility of the reference diet; DM_{dr} = dry matter content of the reference diet; DM_i = dry matter content of test ingredient; D_r =% nutrient (or kcal kg^{-1} gross energy) of the reference diet; D_i =% of the nutrient (or kcal kg^{-1} of gross energy) of the test-ingredient.

After the fecal collection period, the fish continued to receive the experimental diets for three days. Randomly, six mutton snapper from each digestibility aquarium were collected, anesthetized and laparotomies occur for removal of the anterior portion of the intestine. After identification of samples, they were immediately frozen and kept at low temperature (4 °C) for further analysis of enzymatic activity. For determination of digestive enzyme activities, the tissues weighing between 80 and 100 mg, and homogenized in buffer (10 mM phosphate/ 20 mM tris-pH 7.0) for 3 minutes (4 °C) using bench-top homogenizer (Marconi), and then centrifuged at 3000 rpm to get the supernatant. The supernatant was used in the enzymatic assays from lipase, amylase, specific alkaline protease, and tissue proteins evaluation.

The determination of the amylase activity was according to the method proposed by Bernfeld (1955) and changed by Hidalgo et al. (1999). Also, the glucose concentration was measured according to Park and Johnson (1949) method.

For measurement of the alkaline proteolytic activity, 1% casein solution as a substrate of the reaction, and according to Walter (1984). The determination of non-specific lipase activity was according to the method described by Gawlicka et al. (2000). The total protein analysis was according to Melo et al. (2006) method.

All tested data for normality and homoscedasticity, and afterward were submitted to variance analysis (one-way ANOVA) to verify the relation among the treatments, and when necessary were submitted to the Tukey´s test with 5% probability. Statistical analyses were performed using R-br software.

3. Results and Discussion

For the mutton snapper, the offer of the octopus meal improves ($P < 0.05$) apparent digestibility of the dry matter (ADC_{DM}, 67.17%), crude protein (ADC_{CP}, 90.9%), and gross energy (ADC_{GE}, 78.8%) (Table 3). Regarding the digestibility of the lipids (ADC_{L}), the broadband anchovy meal has more ($P < 0.05$) digestible lipids (78.1%), coming next from the testing ingredients, fish meal (72.4%), and octopus meal (69.7%). The apparent digestibility of the dry matter, crude protein, and gross energy were low ($P < 0.05$) for shrimp residue meal and soybean meal.

Table 3. Apparent digestibility coefficient of dry matter (ADC_{DM}), crude protein (ADC_{CP}), gross energy (ADC_{GE}) and lipids (ADC_{L}) of protein ingredients for *Lutjanus analis*.

	Apparent digestibility coefficient (%)			
Treatments	Dry matter (%)	Crude protein (%)	Gross energy (%)	Lipids (%)
Soybean meal	30.98b	67.83b	26.19b	44.54c
Fish meal	52.53ab	64.90b	68.73a	72.35ab
Octopus meal	67.17a	90.97a	78.80a	69.69ab
Broadband anchovy meal	45.16ab	60.48b	65.52a	78.10a
Shrimp residue meal	29.19c	65.76b	36.66b	53.12bc
Coefficient of variation (%)	8.47	8.87	9.98	13.67

Means followed by different letters in the same column were significantly different according to Tukey’s test (0.05). Source:Authors.

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The higher digestibility of the dry matter (ADC_{DM}) of octopus meal (67.17%), not yet reported in aquaculture. The mutton snapper evidence a low digestibility of the dry matter (52.53%) of fishmeal, which was above the values observed for the silver mojarra, *Diapterus rhombeus* (49.60%) (Magalhães Jr., et al., 2016), but below to those observed in *Arapaima gigas* (89.2%) (Cipriano, et al., 2016). It relates the fishmeal composition to fish species, age, time of year, stocking conditions, among others, and may vary constantly (Moghaddam, et al., 2007), being difficult to compare digestibility values with other fish species. The fish meal inclusion in diets for aquaculture is a common practice, but it presents a setback for its long-term sustainability as a food ingredient. There are serious financial pressures to reduce the amount of fishmeal in diets to minimize dietary costs and potentially improve profitability.

The low ADC_{DM} on shrimp meal (29.19%) to the other ingredients was by the amount of ash (30.9%) and chitin present in the shrimp shell making up between 15-20% of its weight, being a natural fiber more abundant after the cellulose (Shahidi, et al., 1999). The low dry matter digestibility of soybean meal (30.98%) by mutton snapper, values below to those observed in pirarucus (76.7%) (Cipriano, et al. 2016) and mojarra (67.45%) (Magalhães Jr., et al., 2016).

The mutton snapper require diets with high protein value because they have short intestine (210 to 460 mm), and have a particular preference of brachyuran crustaceans, fishes (Teleostei), stomatopods and shrimps (Duarte & García, 1999). Also, the greater digestibility of the crude protein (ADC_{CP}) (90.9%) of octopus meal occurs because of the large number of amino acids in its composition, and easily absorbed by aquatic organisms (Valverde, et al., 2013). This high digestibility efficiency is because of the abundance of the essential amino acids (arginine, leucine and lysine), and the non-essential amino acids (glutamate and aspartate) that make up these protein sources (Valverde, et al., 2013). The spotted catfish *Pseudoplatystoma coruscans* (Gonçalves & Carneiro, 2003), the grouper *Cromileptes altivelis* (Laining, et al., 2003), and the pirarucu *Arapaima gigas* (Cipriano, et al., 2016), had lower (84.1%, 82.4% and 89%, respectively) digestibility after using fish meal as one ingredient in the diet, than the octopus meal (90.9%) in this study, but have a greater digestibility of crude protein than the mutton snapper after fed with fish meal (64.90%).

The mutton snapper ADC_{CP} was low for fish meal (64.90%), broadband anchovy meal (60.48%), shrimp residue meal (65.76%), and soybean meal (67.83%), also showing the low enzymatic action of alkaline protease during digestibility of these ingredients, except for soybean meal.

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The carnivorous marine fish Silver mojarra (*Diapterus Rhombeus*) also show greater ADC_{CP} (92.97%) of the fish meal included in the diet, as Red drum (*Sciaenopolis ocellatus*) (95.87%), Black salmon (*Rachycentrun cabadum*) (96.27%), European seabass (*Dicentrachus* L.) (93.50%), Golden fish (*Sparus aurata*) (87.50%), and Halibut (*Scophthalmus maximus*) (82.80%) (McCoogun & Reigh, 1996; Zhou, et al., 2004; Davies, et al., 2009). The Atlantic cod *Gadus morhua* shows higher ADC_{CP} (86.4%) of the broadband anchovy meal (Tibetts, et al. 2006), below to the value observed in this experiment.

The shrimp residue meal showed very low ADC_{CP} for the mutton snapper. Diets for shrimp (*Penaeus monodon*) showed high protein digestibility of the residue with 88.7% crude protein digestibility (Chen, et al., 2016).

Several fish species had a low digestibility of soybean meal crude protein, reported for Red drum, Coho salmon *Oncorhynchus kisutch*, Rainbow trout, Sea bass, Haddock *Melanogrammus aglefinus*, Largemouth bass *Micropterus salmoides*, Gilthead, Atlantic cod, and Surubim *Pseudoplatystoma reticulatum* (Sullivan & Reigh, 1995; McGoogan & Reigh, 1996; Lin, et al., 2004; Portz & Cyrino, 2004; Tibbetts, et al., 2006; Wu, et al., 2006; Thompson, et al., 2008; Silva, et al., 2012). Among the ingredients of vegetable proteins, soybean meal is the most nutritious source of vegetable protein; however, the high concentration of anti-nutritional factors (eg. lectins, saponin) can limit the levels of inclusion of soybean meal in aquatic food. Compared to fish meal, soy meal had the advantage of being more digestible than the fish meal in this study. But this is because the fish meal does not always have the same quality standard. In this study, fishmeal presented 20% mineral matter in its composition, a fact that reduces the digestibility of this ingredient for the mutton snapper.

The gross energy digestibility (ADC_{GE}) of the mutton snapper was greater in the octopus meal (78.80%), fish meal (68.73%) and broadband anchovy meal (65.52%), and was very low for shrimp residue meal (36.66%) and soybean meal (26.19%). As expected, the gross energy had the highest direct effect on energy digestibility. In the literature, the gross energy values were well above those observed for soybean meal for Silver mojarra (*Diapterur rhombeus*), Tambaqui (*Colossoma macropomum*), Black salmon (*Rachycentrun cabadum*), and Seabass (*Centropomus parallelus*), 65.23%, 76.82%, 77.90% and 82.66%, respectively (Barroso, et al. 2002; Zhou, et al. 2004; Magalhaes Jr., et al. 2016; Buzzolo, et al., 2018).

The digestibility of the lipids (ADC_L) on mutton snapper was better after fed with broadband anchovy meal, fish meal and octopus meal (78.10%, 72.35% and 69.69%, respectively), already expected because of the high lipid composition of these ingredients

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(Khan, et al., 2012; Morillo-Velarde, et al., 2015). For the "striped bass" hybrid (*Morone saxatilis* x *Morone chrysops*) and goldfish (*Salminus brasiliensis*) the higher ADC_L values (88.2% and 93.9%, respectively) happened after fed with the fish meal (Sullivan & Reigh, 1995; Braga, et al., 2008). According to Gaylord & Gatlin (1996) lower values of ADCL (66.5%) for red drum (*Sciaenops ocellatus*) after feeding with meat-and-bone meal. The high digestion efficiency of carnivorous fish to digest lipid makes it the key source of energy, saving the protein for growth (NRC, 2011).

Soybean meal and shrimp residue meal have the lowest ADC_L in mutton snapper. The soybean meal showed low lipid digestibility value (44.5%) because of the low lipid content in its composition (1.8%), being the only vegetable protein source tested. For the matrinxã (*Brycon cephalus*), after feeding with soybean meal, h_{ave} also low values of ADCL (47.1%) (Sallum, et al., 2002). For the surubim, the digestibility of lipids was satisfactory with ADC_L mean values of 83.47% (Teixeira, et al., 2010).

The digestive enzyme activity of the mutton snapper has differences (P < 0.05) according to the tested ingredients. The lipase activity varied proportionally with the amount of ethereal extract in the diet of each ingredient, and the highest (P < 0.05) activity of this enzyme occurred in the diet with broadband anchovy meal, and the lowest values attributed to the octopus meal, soybean meal and fishmeal with an average value of 2.54 UI mg^{-1}. The shrimp residue meal has an intermediate value to the other ingredients, also differing statistically (Table 4).

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Table 4. Activity of digestive enzymes (UI mg^{-1} protein) of the *Lutjanus analis* fed with different protein sources.

	ested feed					
Enzymatic activity	SM	OM	BAM	SRM	FM	CV (%)
Total intestine						
Amylase	0.26c	0.50ab	0.41bc	0.33c	0.58a	21.62
Lipase	2.18c	1.85c	9.18a	5.75b	3.59c	24.08
Alkaline protease[1]	24.36a	25.45a	15.81b	17.91b	19.64b	12.92

SM – Soybean meal; OM – Octopus meal; BAM – Broadband anchovy meal; SRM – Shrimp residue meal; FM – Fish meal. [1]Non-specific Enzyme. Source:Authors.

The activity of amylase had the highest indexes in mutton snapper juvenile when fed with fish meal and octopus meal. Broadband anchovy, shrimp residue meal and soybean meal have lower ($P < 0.05$) activity when compared with fish meal.

The lipase activity in mutton snapper was greater ($P < 0.05$) in treatment with broadband anchovy meal, different from other treatments.

The alkaline protease activity of juvenile was greater ($P < 0.05$) using the soybean meal and the octopus meal, and the lowest activity were for the broadband anchovy, shrimp and fish meal.

The enzymatic activity of amylase was low in the mutton snapper, similarly reported by other species of carnivorous fish, as *Hoplias malabaricus*, *Paralichthys orbignyanus* and *Arapaima gigas* (Cipriano, et al., 2016; Gioda, et al., 2017; Candiotto, et al., 2018). According to Hidalgo, et al. (1999), the activity of digestive amylase can reveal the capacity of different species to use protein and carbohydrates. The reduced amylase activities were in all tested feed for the mutton snapper and was expected since diets are not sources of carbohydrates. Another aspect is that the mutton snapper has a short gastrointestinal tract, hindering the absorption of carbohydrates and influencing the enzymatic activity of amylase.

The broadband anchovy meal in diets showed the highest lipase activity in the mutton snapper, and according to Melo, et al. (2012) this change of the enzymatic pattern activity depends on the amount of fat in the intestinal contents, and this comes with the number of lipids in the diet. According to Cahu, et al. (2009), fish larvae have altered lipase activity according to the lipid and phospholipid content. Also, carbohydrate content may influence lipase activity when fat proportions including in the diet for *Larmichthys crocea* (Zhou, et al., 2016). The mutton snapper fed with the broadband anchovy meal, shrimp and fish meal has a superior activity of the lipase, and it occurred because of its nutritional composition, amount of lipids, which shows greater availability, and utilization of proteins and lipids as energy sources for mutton snapper.

Mutton snapper fed with octopus meal or soybean meal has the highest alkaline protease activity with average values of 24.36 and 25.45 UI mg^{-1}, respectively. Probably because of the protein concentration of this ingredients. The *P. mesopotamicus* and *Rhamdia quelen,* species with omnivorous feeding habits, when fed with increasing levels of protein in diets improved the proteolytic activity in its intestines (Moraes & Bidinotto, 2004; Melo, et al., 2012). Including soybean meal and octopus meal in the diet caused in juvenile greater protease activities. Mutton snapper's digestive tract provides rapid digestion and gastric emptying; this time of nutrient transit can present modification in the responses of the activities in the digestive enzymes.

4. Final Considerations

The mutton snapper fed with the octopus meal has greater digestibility coefficients values, suggesting that this ingredient is favorable for this species. All diets provided a low amylase activity in the juvenile mutton snapper. The lipase and alkaline protease activities were greater in diets with the broadband anchovy meal and octopus meal, respectively. Evaluating different sources of protein for fish is important to understand the digestibility and feed efficiency of marine species.

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Percentage of contribution of each author in the manuscript

Leon Lima Leal – 20%

Ana Paula de Souza Ramos – 10%

Marcel José Martins Dos Santos – 10%

Francisco Oliveira de Magalhães Júnior – 10%

José Fernando Bibiano Melo – 10%

Marianne Schorer – 10%

Luis Gustavo Tavares Braga – 30%