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Mesquite bean and cassava leaf in diets for Nile tilapia in growth

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ABSTRACT. This work evaluated the inclusion of mesquite bean bran (*Prosopis juliflora*) and cassava leaf bran (*Manihot esculenta*) in diets for Nile tilapia (85.22 ± 3.13 g). Three hundred and thirty-six fish were distributed in 28 fiberglass tanks (120 L) in a 2 × 4 factorial scheme for two sources of oil and four levels of bran (0, 5, 10 and 20%) (n = 4). After 60 days, growth performance (feed intake, weight gain, apparent feed conversion and survival rate) and fish body composition were evaluated. Heights and density of villi were measured for morphometric analysis of the intestinal mucosa. Animal performance, body composition and villi density were not affected ($p > 0.05$) by the source and level of inclusion of bran. There was a significant effect of the level of inclusion of bran on villi height, with a linear trend, indicating that the higher the inclusion levels of bran, the lower the height of the villi. The bran studied can be used in diets for Nile tilapia up to 20% without compromising growth performance and body composition change, but the presence of these by-products can result in a deleterious effect on fish villi.

Keywords: alternative feeds, performance, intestinal mucosa, *Oreochromis niloticus*.

Farelos da vagem da algaroba e da folha da mandioca em rações para tilápia do Nilo em crescimento

RESUMO. Avaliou-se a inclusão dos farelos da vagem da algaroba (*Prosopis juliflora*) e folha da mandioca (*Manihot esculenta*) em rações para tilápia do Nilo (85,22 ± 3,13 g). Foram utilizados 336 peixes, distribuídos em 28 tanques (120 L), em esquema fatorial 2 x 4, duas fontes de óleo e quatro níveis de farelo (0, 5, 10 e 20%) (n = 4). Ao final de 60 dias, foram avaliados o desempenho zootécnico (consumo de ração, ganho de peso, conversão alimentar aparente e taxa de sobrevivência) e a composição da carcaça dos peixes. Para análise da histologia intestinal, foram mensuradas a altura e a densidade das vilosidades. O desempenho zootécnico, a composição da carcaça e a densidade das vilosidades intestinais não foram afetados ($p > 0,05$) pela fonte e nível de inclusão de farelo. Houve efeito significativo do nível de inclusão dos farelos sobre a altura das vilosidades intestinais, com comportamento linear decrescente, indicando que quanto maior o nível de inclusão de farelo, menor a altura das vilosidades. Os farelos estudados podem ser incluídos em até 20% em rações de tilápias do Nilo sem comprometer o desempenho zootécnico e alterar a composição corporal, no entanto, a presença destes coprodutos em rações pode resultar em efeito deletério sobre as vilosidades intestinais dos peixes.

Palavras-chave: alimentos alternativos, desempenho, mucosa intestinal, *Oreochromis niloticus*.

Introduction

The Nile tilapia (*Oreochromis niloticus*) is an important species in intensive aquaculture for presenting fast growth and excellent performance in intensive production systems (SCORVO FILHO et al., 2010), for being omnivorous and easily accepting feeds, from the post-larva to the finishing phases (BOSCOLO et al., 2001).

In intensive aquaculture, production costs related to feeding can reach 70% of total production cost (GUIMARÃES et al., 2008), a fact that has been stimulating studies about alternative feeds that meet the nutritional requirements of animals, without changing the quality of feed (SOUZA et al., 2004). Thus, studies that involve the exploitation of alternative foods such as components of feeds, in part or totally replacing the traditional foods, are becoming increasingly frequent (CHENG; HARDY, 2002; GUIMARÃES et al., 2008; KÖPRÜCÜ; ÖZDEMIR, 2005; LOPES et al., 2010; RICHTER et al., 2003; SANTOS et al., 2009).

The mesquite is a leguminous tree native to arid and semiarid regions of the world (HARRIS

et al., 2003), and concentrates its nutritional value in the beans, being a rich source of carbohydrates and proteins with gross energy value comparable to corn. The Brazilian production is concentrated in the Northeast region and studies have been conducted for inclusion of the mesquite bean in feed of cattle, sheep, pigs and birds to minimize the costs of animal production (STEIN et al., 2005).

Mesquite bean bran is obtained from the drying of beans at 60 to 80°C and subsequent grinding. The use of bran is recommended because during processing, besides of incorporating all components of the bean, there is the possibility to control possible thermolabile antinutritional factors (SILVA et al., 2002).

Cassava is a common food in tropical and semitropical countries, and is known by various names, such as cassava, tapioca, manioc, manihot, among others. It has high potential in animal feed being a rich source of energy. Its co-products can be used as ingredients in animal feed (VIOLA et al., 1988). Among these co-products the bran from cassava leaves stands out for its high nutritional value and excellent acceptability by animals (LEONEL, 2001).

The objective of this study was to evaluate the zootechnical performance, chemical composition of carcass and intestinal histology of juvenile Nile tilapia (*Oreochromis niloticus*) fed with feeds containing mesquite bean and leaves of cassava.

Material and methods

The experiment was carried out for 60 days at Laboratory of Fish Nutrition and Feeding (AQUANUT) of State University of Santa Cruz (UESC). In this experiment 336 male juvenile Nile tilapia (*Oreochromis niloticus*), sexually reverted, with initial weight of 85.22 ± 3.13 g were used, distributed in a completely randomized design in a 2 x 4 factorial scheme (four food and two levels of inclusion) with four replications. The fish distributed in 28 cylindrical fiberglass tanks with a working volume of 120 L. A tank with 12 fish was considered an experimental unit.

The tanks were connected to a water recirculation system equipped with biological filter and flow rate of 1.4 L $min.^{-1}$ in each tank, maintained through a water pump (¾ hp). Each tank was individually aerated through porous stone powered by a 1 hp blower.

Throughout the experiment the temperature, pH and dissolved oxygen daily were measured daily, morning and afternoon.

Treatments consisted of three diets with inclusion of 5, 10 and 20% of the mesquite bean bran and three other diets with addition of 5, 10 and 20% of cassava leaf bran, in addition to control diet, free of the products mentioned. The isocaloric and isonitrogenous formulations were made according to the requirements for the species, according to Furuya et al. (2010), and apparent digestibility of co-products obtained by Braga et al. (2010) and other ingredients by Boscolo et al. (2002) (Table 1).

Table 1. Composition of experimental feeds, in percentages of natural matter.

Ingredient (%)	Level of inclusion of by-products[1]						
	Control	M5	M10	M20	C5	C10	C20
Cassava leaf bran	0.00	0.00	0.00	0.00	5.00	10.00	20.00
Mesquite bean bran	0.00	5.00	10.00	20.00	0.00	0.00	0.00
Fish meal	32.00	32.00	32.00	32.00	32.00	32.00	32.00
Cotton meal	22.78	13.99	5.20	5.00	14.66	6.53	5.00
Soybean meal	18.13	22.20	26.28	19.43	22.17	26.22	21.79
Starch	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Corn meal	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Soybean oil	4.57	4.31	4.04	3.55	3.74	2.90	1.19
Wheat bran	4.00	4.00	4.00	4.00	4.00	4.00	4.00
L-lysine HCl	2.49	2.47	2.45	0.00	2.41	2.33	0.00
Mineral and vitamin premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Antioxidant BHT	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	Calculate value						
Humidity (%)	89.23	88.59	89.62	89.02	89.86	88.93	89.15
Digestible protein (%)	25.92	25.48	25.17	25.74	25.48	25.32	25.55
Digestible energy (kcal kg^{-1})	3.077	3.068	3.000	3.000	3.010	3.010	3.000
Crude fiber (%)	5.12	5.12	5.42	6.00	5.23	5.72	5.81
Ether extract (%)	4.30	4.30	4.30	4.30	4.30	4.30	4.30
Lysine (%)	1.60	1.60	1.60	1.60	1.60	1.60	1.60

[1]M: Mesquite bean bran; C: Cassava leaf bran; [2]Composition kg^{-1}: Mg: 2.600 mg; Zn: 14.000 mg; Fe: 10.000 mg; Cu: 1.400 mg; Co: 20 mg; I: 60 mg; Se: 60 mg; Vit. A: 1.000.000 UI; Vit. D3: 400.00 UI; Vit. E: 10.000 mg; Vit. K3: 500 mg; Vit. B1: 2,500 mg; Vit.B2: 2.500 mg; Vit.B6: 2.500 mg; Vit. B12: 3.000 mcg; Vit. C: 35.000 mg; Ac. Folic: 500 mg; Ac. Pantothenic: 5.000 mg; Niacin: 10.000 mg; Biotin: 80.000 mcg; Colin: 200.000 mg; Methionine: 130 g; Inositol: 5.000 mg; Etoxiquin: 15.000 mg.

For the preparation of feeds the foods were processed individually in a knife mill with 0.5 mm sieve and subsequently mixed according to the formulation of each feed, moistened with water to be pelleted and dried in an oven at 55°C for 48h.

The feeds were ground to present a particle size adjusted to the size of the fish's mouth and fed to apparent satiation, twice daily, always at 8 and 4 pm.

At 60 days, weighing was carried out of all fish in each experimental unit. Four specimens of each repetition were removed to determine body composition and two specimens were removed for the analysis of intestinal histology.

Feed intake, weight gain [(final biomass - initial biomass of fish)/experimental period], apparent feed conversion (feed intake/weight gain) and survival rate [(dead individuals/individuals living) x 100] were determined, as evaluation of production performance.

For the analysis of body composition of tilapia the samples were dried in a forced air sterilizer (55°C) for 72h and ground for later determination of dry matter, crude protein, ether extract and ash, according to the methodology described by Silva and Queiroz (2002).

Regarding the analysis of the histology of the intestinal mucosa, the middle portion of the intestine was removed and the samples were placed in a Styrofoam plate and opened lengthwise. Each sample was fixed in Bouin's solution for 12h and the material was processed with routine histological technique. The 7 micrometers thick cross-sections were stained using Hematoxylin-Eosin (H-E). The histological slides were analyzed according to the length of the villi, using an optical microscope and image analyzer (Image Pro Plus - Version 5.2).

Specifically for the analysis in scanning electron microscopy, samples were washed with 0.9% saline solution, immersed in Karnovisk's fixative solution for 2 hours, cut and stored in the same final solution. Subsequently, the samples were transported to the Laboratory of Electron Microscopy and Ultra Structural Analysis of the Department of Plant Pathology of Federal University of Lavras (UFLA).

In the laboratory, samples were placed in 30% glycerol for 30 min., then immersed in liquid nitrogen and cut on a metal surface cooled with liquid nitrogen using a scalpel. The fragments were then transferred to a solution of 1% osmium tetroxide for 3 hours, washed with distilled water three times and dehydrated in acetone series (25, 50, 75, 90 and 100% three times each). After dehydration, samples were taken to the critical point apparatus (Balzers CPD 030) for replacement of acetone for CO_2 for complete drying. Specimens obtained were mounted on aluminum stubs with carbon ribbon on a film of aluminum foil and coated with gold in the evaporator (Balzers SCD 050) for observation in a scanning electron microscope LEO EVO 40 (ALVES, 2004). Images were recorded digitally and the most representative were recorded on Corel Draw Photopaint software. The best images of each sample were selected for visual analysis of the villi.

In addition to this evaluation, measurements were made of the number of villi in different parts of each sample. After checking the scale of the photography, the site of observation was measured, the area was determined and the density of villi was calculated.

The data obtained were subjected to two-factor analysis of variance at 5% level of probability and polynomial regression using the statistical program Statistical Analysis System (SAS, 2003).

Results and discussion

The temperature, pH and dissolved oxygen observed during the experimental period were 26.2 ± 1.1°C, 6.6 ± 0.1 and 4.8 ± 1.8 mg L^{-1}, respectively, remaining within the range recommended for fish (BALDISSEROTTO, 2002).

By means of two-factor analysis of variance there was no effect ($p > 0.05$) of the interaction between source of bran and level of inclusion in the feeds on the zootechnical performance, chemical composition of carcass and intestinal histology.

For the variables feed intake, weight gain, apparent feed conversion and survival rate there were no differences between the tilapia that received feeds with mesquite bean bran or cassava leaves bran, regardless of the level of inclusion, i.e., the growth of fish fed diets with up to 20% of any of the brans (mesquite or cassava) is equivalent (Table 2).

Table 2. Growth of Nile tilapia according to the source and level of inclusion of the mesquite bean bran and cassava leaf bran.

Treatment	Variable[1]				
	IW (g)	FC (g)	WG (g)	AFC	SUR (%)
Mesquite bean bran	85.2	135.3	83.1	1.66	93.1
Cassava leaf bran	85.2	138.5	82.8	1.69	95.1
0%	83.4	131.7	76.4	1.74	92.9
5%	85.9	135.5	85.1	1.61	94.5
10%	85.7	137.5	85.9	1.65	92.4
20%	85.8	143.0	83.8	1.72	96.5
CV[2](%)	3.67	9.59	19.53	13.17	5.59
Level	0.3445	0.3916	0.6153	0.6348	0.3012
Source	0.9938	0.5002	0.9535	0.7399	0.4102
Level x Source	0.9997	0.2900	0.9834	0.9559	0.4739

[1]IW: initial weight; FC: feed consumption; WG: Weight gain; AFC: apparent feed conversion; SUR: survival rate; [2]Coefficient of variation.

At the end of the experimental period, the mean values of feed intake, weight gain, apparent feed conversion and survival rate of tilapia fed mesquite bean and cassava leaves did not differ ($p > 0.05$) from those obtained with control feed (0% bran inclusion).

The results of this study are consistent with Bohnenberger et al. (2010) that evaluated the effect of different levels (0, 5, 10, 15 and 20%) of protein concentrate from cassava leaves on the production performance of Nile tilapia in the process of sex reversal.

In contrast to the results obtained in this study, Ng and Wee (1989), working with inclusion levels of 20, 40, 60 and 100% of cassava shoots bran, found a linear decrease in the performance of Nile tilapia with increase in the level of inclusion. However, it should be noted that the co-product used in that study differs from the composition used in this study, and the bran contained stems and leaves (leaf blade and

petiole). According to Leonel (2001), the chemical composition and, consequently, the nutritional value may be affected by the cut system and portion of the shoot.

The nutritional value of mesquite for tilapia was already highlighted by Pezzato et al. (2004), who reported the apparent digestibility of crude protein of 81.9% and 3210 kcal kg^{-1} of digestible energy, indicating the possibility of its use in commercial feeds.

A common difficulty observed when alternative food sources are used in fish feed is the acceptability, which is related to palatability (AZAZA et al., 2009). The differences may be related to the presence of fishmeal in the formulation of rations in this experiment, which in addition to provide better amino acid balance may have made the feed more palatable.

Regardless of the inclusion level, there was no effect of source of bran (mesquite or cassava) on dry matter, crude protein, ether extract and carcass ash of juvenile Nile tilapia (Table 3).

Table 3. Body composition (%) of Nile tilapia according to the source and level of inclusion of mesquite bean bran and cassava leaf bran.

Treatment	Variable			
	Humidity	Crude protein	Ether extract	Mineral matter
Mesquite bean bran	74.68	41.33	28.42	10.11
Cassava leaf bran	74.47	41.31	27.61	9.93
0%	74.51	41.07	28.01	10.73
5%	74.75	41.26	29.16	9.55
10%	74.33	41.64	28.01	10.14
20%	74.72	41.32	26.89	9.67
CV[1](%)	1.17	3.06	9.55	11.64
Level	0.6640	0.8331	0.4294	0.1928
Source	0.4377	0.9604	0.4037	0.6636
Level x Source	0.2265	0.8036	0.8636	0.9397

[1]Coefficient of variation.

Bohnenberger et al. (2010) found no influence of the levels of cassava leaves protein concentrate on the variables of chemical composition of Nile tilapia in the process of sex reversal. The absence of differences in body composition of fish is important and may indicate a similar or appropriate nutritional balance of feeds (BOSCOLO et al., 2002; VEIVERBERG et al., 2010).

Histological analysis in optical microscopy of the small intestine of juvenile Nile tilapia fed diets containing different levels of inclusion of mesquite bean and cassava leaves bran showed normal appearance, having no morphological characteristics of certain pathologies (Figure 1).

The small intestine of tilapia is formed by a villiform mucous layer consisting of a simple cylindrical epithelium which cells are known as enterocytes (SILVA et al., 2005). The enterocytes of tilapia were well preserved and no morphological changes were observed in this study. According to Kuperman and Kuzmina (1994), the intestinal mucosa is of great importance in the digestive, absorptive and metabolic processes in teleost fish.

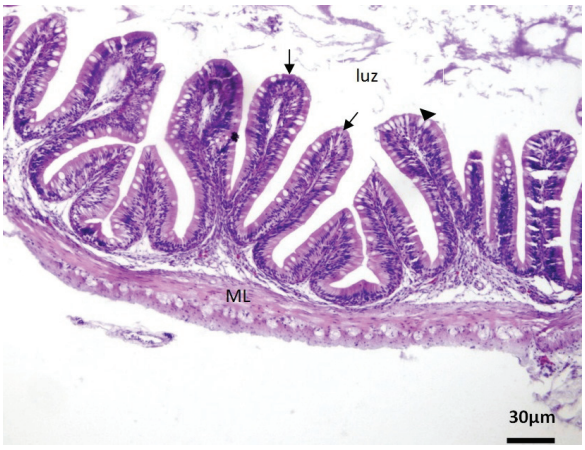

Figure 1. Photomicrograph of the small intestine of O. niloticus. Cross section of the small intestine of tilapia showing the general organization, with the mucus layer in the form of villi (arrows). Presence of goblet cells (arrowhead) between the simple cylindrical epithelium, and the smooth muscle layer (ML) developed.

Among enterocytes it was possible to identify the presence of mucin-producing goblet cells, which were faintly stained as these cells do not have affinity with the staining method used in this study (H-E).

We did not observe the presence of intestinal crypts, suggesting that the enterocytes are proliferating at the base of the villi. Teleost fish do not have crypts as mammals and the cell proliferation function of the villi epithelium is made by undifferentiated cells at the base of the villi, which perform numerous mitotic divisions to form new cells (JOBLING, 1995).

Below the epithelial tissue it was possible to observe the lamina propria with the connective tissue featuring a loose and richly vascular appearance, and a well developed smooth muscle layer.

However, the density of the villi was not influenced ($p > 0.05$) by the source or level of inclusion of the byproduct, and an average of 18.7 villi 1,250,000 μm^{-2} was obtained (Figure 2). The lower count of villi $area^{-1}$ does not necessarily mean lower absorption capacity, because there might be compensation in terms of height. Thus, absorption capacity may be considered as villi density and height and microvilli dependent, and not only of a single variable (KINDLEIN et al., 2007).

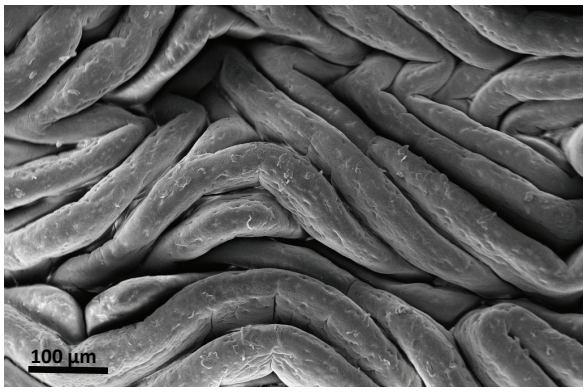

Figure 2. Electron micrographs of the proximal intestine scanning of juvenile Nile tilapia fed diets containing different levels of inclusion of mesquite bean and cassava leaves bran.

In the morphometric analysis of the small intestine of tilapia, we observed a significant effect ($p < 0.05$) of the level of inclusion of bran (mesquite or cassava) in the diets of tilapia on the intestinal villi height (Table 4) with a linear trend, indicating that the higher the level of inclusion of bran in the diet, the lower the height of the intestinal villi (Figure 3).

Table 4. Height and density of intestinal villi of juvenile Nile tilapia fed different levels of inclusion of mesquite bean bran and cassava leaf bran.

Treatment	Variable	
	Height (μm)	Density (villi 1,250,000 μm^{-2})
Mesquite bean bran	380.66	18.96
Cassava leaf bran	373.78	18.38
0%	404.73	18.50
5%	385.85	18.13
10%	371.04	19.42
20%	347.25	18.63
CV[1](%)	5.18	24.67
Level	0.0001	0.9699
Source	0.3292	0.7424
Level x Source	0.9390	0.6102

[1]Coefficient of variation.

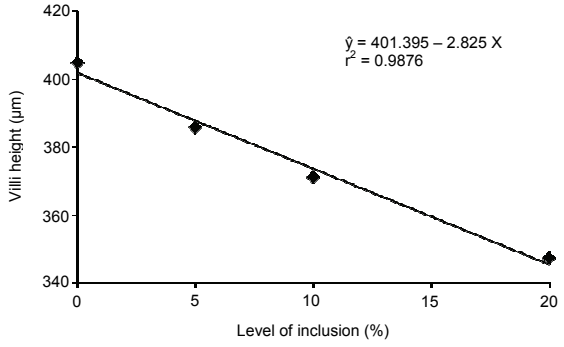

Figure 3. Villi height of juvenile Nile tilapia fed diets containing different levels of inclusion of mesquite bean bran and cassava leaves bran.

The larger size of the villi presented by the fish of the control treatment suggests greater integrity of the intestinal mucosa allowing their further development and therefore greater efficiency in the absorptive process by tilapia (CABALLERO et al., 2003).

Although the reduction in size of intestinal villi of tilapia from the control treatment was observed, the zootechnical performance of animals was not adversely affected.

However, it is noteworthy that the shortest lengths of villi presented by tilapia in the treatments containing mesquite bean bran and cassava leaf bran suggest lower absorption of nutrients, by minimizing the absorption area presented by the villi. Thus it is possible that this characteristic may adversely affect the performance of fish that are subjected to these feeds for a long time.

Conclusion

Mesquite bean bran and cassava leaf bran may be used in Nile tilapia's feed up to 20% inclusion level without compromising the zootechnical performance and changing the chemical composition of carcass. However, the presence of the studied co-products reduces the size of intestinal villi of fish.

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