Notas Científicas

Apparent digestibility of nutrients, energy, and amino acid of nontoxic and detoxified physic nut cakes for Nile tilapia

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Abstract – The objective of this work was to evaluate the apparent digestibility coefficients of nutrients, energy, and amino acids of nontoxic and detoxified physic nut cakes treated with solvent plus posterior extrusion, for Nile tilapia. The apparent digestibility coefficients of crude protein and gross energy were higher for detoxified than for nontoxic physic nut cake. However, the apparent digestibility coefficient of ether extract of the nontoxic physic nut cake was higher than that of the detoxified one. The apparent digestibility coefficient of amino acids of both feed ingredients was superior to 80%, except for glycine, for the nontoxic psychic nut cake, and for threonine, for the detoxified one. Nontoxic and detoxified physic nut cakes show apparent digestibility coefficient values equivalent to those of the other evaluated oilseeds and potential for inclusion in Nile tilapia diets.

Index terms: *Jatropha curcas*, *Oreochromis niloticus*, alternative feedstuff, biodiesel.

Digestibilidade aparente de nutrientes, energia e aminoácidos de tortas de pinhão-manso atóxica e destoxificada para tilápia-do-nilo

Resumo – O objetivo deste trabalho foi avaliar os coeficientes de digestibilidade aparente de nutrientes, energia e aminoácidos de tortas de pinhão-manso atóxica e destoxificada tratadas com solvente e posterior extrusão, para tilápia-do-nilo. Os coeficientes de digestibilidade aparente da proteína bruta e da energia bruta foram maiores para a torta destoxificada do que para a atóxica. No entanto, o coeficiente de digestibilidade aparente do extrato etéreo da torta atóxica foi superior ao da destoxificada. O coeficiente de digestibilidade aparente dos aminoácidos dos dois alimentos foi maior que 80%, exceto para glicina, para a torta atóxica, e para treonina, para a destoxificada. Tanto a torta atóxica quanto a destoxificada de pinhão-manso apresentam valores similares aos das outras oleaginosas avaliadas e potencial para utilização em dietas para tilápia-do-nilo.

Termos para indexação: *Jatropha curcas*, *Oreochromis niloticus*, alimento alternativo, biodiesel.

The physic nut (*Jatropha curcas* L.) crop is increasing in the tropic and subtropic regions due to its resistance to drought, high oil content (60%) and quality, which are suitable for biofuel production (Kumar et al., 2011). The cake/meal obtained after oil extraction contains high nutritional value, with 25–63% crude protein, 4.0–14.21% ether extract, 36.68–45% crude fiber, and 12.82 KJ g^{-1} gross energy, showing good amino acid profile, except for lysine (Martínez-Herrera et al., 2006).

Despite the potential of physic nut cake/meal as a protein source in animal feeding, its use is limited mainly due to the presence of phorbol ester, a toxic constituent, as well as of anti-nutritional factors, such as phytates, lectins, protease inhibitors, non-starch polysaccharides, saponins, and high fiber contents (Makkar et al., 1998b; Kumar et al., 2011). Therefore, further research is needed to recommend the use of physic nut cake/meal as an alternative feedstuff.

In aquafeeds, different physic nut varieties have been evaluated: detoxified (Kumar et al., 2011); and nontoxic, after roasting, from the Quintana Roo, Veracruz, and Morelos regions of Mexico (Makkar et al., 1998a). The knowledge of the digestibility of agro-industrial by-products is essential to allow their use in balanced diets for fish (Pezzato et al., 2002). Some studies have shown the potential of physic nut cake/meal in fish diets (Makkar & Becker, 1999; Kumar et al., 2011); however, information about the digestibility of nutrients and amino acids is limited.

The objective of this work was to evaluate the apparent digestibility coefficients of nutrients, energy, and amino acids of nontoxic and detoxified physic nut cakes treated with solvent plus posterior extrusion for Nile tilapia (*Oreochromis niloticus*).

The nontoxic physic nut seeds were obtained from a private farm and registered as CNPAE 169 and CNPAE 170 in the germplasm bank of Embrapa Agroenergia. The seeds were dried for 30 min, at 100ºC, in a rotary dryer and then mechanically processed in a ERT 60 expeller press (Scott Tech, Vinhedo, SP, Brazil) for oil extraction, to obtain the nontoxic cake, at Embrapa Agroenergia. The detoxified cake was produced using a mechanically-extracted toxic cake, subjected to a sequential solvent extraction with hexane at 45–50ºC (30 min; 1.6 L hexane per kg of cake), followed by three more wash-out extraction cycles with the same solvent at 45–50ºC (15 min each) and a new extraction with ethanol at 60–65ºC (30 min; 1.6 L ethanol per kg of cake), followed by three more wash-out cycles with the same solvent at 60–65ºC (15 min each). This process was performed at the Ercitec pilot plant unit of Instituto de Tecnologia de Alimentos, in the municipality of Campinas, in the state of São Paulo, Brazil. The cake was conditioned to 21,5% of moisture and then extruded in a Clextral twin-screw extruder (Clextral, Firminy, France), with the addition of 2% sodium hydroxide, at 160ºC, at Embrapa Agroindústria de Alimentos, in the municipality of Rio de Janeiro, in the state of Rio de Janeiro, Brazil.

The trial of apparent digestibility coefficients was conducted at the fisheries laboratory of Embrapa Agropecuária Oeste, in the municipality of Dourados, in the state of Mato Grosso do Sul, Brazil. Sixty fish with average weight of 55 g were stocked in three cylindrical 70-L plastic cages, totaling 20 fish per cage, allocated in a 1,000-L cylindrical tank with recirculating water system. In addition, three 250-L cylindrical tanks with conical bottom were used for feces collection.

Prior to feces collection, Nile tilapia juveniles were adapted to the experimental diets for 5 days. During the trial period, fish were fed ad libitum, and the procedure described by Pezzato et al. (2002) was adopted for feces collection, which was performed every 2 days in order to minimize stress. The following water quality variables were measured daily in all tanks and remained within acceptable values for tilapia: temperature, 27.41±0.75ºC; dissolved oxygen, 6.12±0.11 mg L^{-1}; total ammonia, 0.06±0.01 mg L^{-1}; and pH, 7.6±0.31.

The experimental diets corresponded to: nontoxic physic nut cake; detoxified physic nut cake treated with solvent plus extrusion; and a reference diet formulated according to the National Research Council (Nutrients…, 1983), with soybean meal as a major protein source, in order to calculate the digestibility of the test ingredients (Pezzato et al., 2002). The nontoxic and detoxified physic nut cake diets consisted of the replacement of 30% of the reference diet by a test ingredient. Chromium oxide (Cr_2O_3) 0.1% was used as an inert marker.

The ingredients were ground to <0.5 mm, moistened with approximately 20% water, and processed into a 3.0–5.0-mm diameter in a laboratory pellet mill. Crude protein, ether extract, gross energy, neutral detergent fiber, acid detergent fiber, chromium, and amino acids were determined according to the AOAC methods of analysis (Horwitz & Latimer Jr., 2010). The following anti-nutritional compounds were also determined: trypsin inhibitors, phytates (Horwitz & Latimer Jr., 2010), and the toxic compound phorbol ester (Makkar et al., 1998a). A completely randomized design was used, with two treatments (nontoxic and detoxified physic nut cakes) and four replicates. The data were analyzed by the SPSS statistical software, version 13.0 (IBM, Armonk, NY, USA), by Student's test, at 5% probability. The means of the nutritional composition of nontoxic and detoxified physic nut cakes corresponded to the average of three replicates, and the means of amino acids (values and apparent digestibility coefficients) to a polled sample.

The crude protein values obtained in the present study for nontoxic and detoxified physic nut cakes (Table 1) were similar to those reported by Guedes et al. (2014), of 12.55–18.07%, but lower than those found by Aderibigbe et al. (1997), of 45.8–61.2%, and by Makkar & Becker (1999), of 35.3%. In these studies, the cake was processed from the kernel

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(dehulled seed), where most protein is located, which increased protein content.

The values of the evaluated anti-nutritional factors, for the nontoxic and detoxified physic nut cakes, were, respectively: 16.75 (1.67%) and 21.49 mg g^{-1} (2.15%) phytates; and 10,302.41 and 1,153.89 g^{-1} units of trypsin inhibitor activity. Phorbol ester, however, was not detected for the nontoxic physic nut cake, whereas 0.023 mg g^{-1} phorbol was obtained for the detoxified physic nut cake. Makkar et al. (1998a) analyzed the previously mentioned anti-nutritional factors of physic nut meals and obtained higher values than those found in the present study. According to these authors, variations in anti-nutritional contents can be explained by differences among genotypes.

Soybean (*Glycine max* L.) meal is the most widely used plant protein source in fish diets, due to its high digestibility of nutrients, energy, and amino acids. In the present study, soybean meal, as well as some cakes/meals from other oilseeds with potential for biofuel production, was used as a comparative reference to physic nut cakes. The apparent digestibility coefficient of crude protein differed between nontoxic and detoxified physic nut cakes ($p<0.05$), corresponding to 77.51 and 81.11%, respectively (Table 1). Riche et al. (2001) found similar results (84.40%) for soybean meal fed to tilapia. However, while evaluating Nile tilapia, Pezzato et al. (2002), Guo et al. (2011), and Zhou & Yue (2012) observed higher values of apparent digestibility coefficients of crude protein for soybean meal, with values ranging from 91.56 to 94.90%. Compared to cakes/meals derived from oilseeds with potential for biofuel production, the apparent digestibility of crude protein of nontoxic and detoxified physic nut cakes was similar to those reported for: radish (*Raphanus sativus* L.) forage meal (Santos et al., 2010); palm kernel (*Elaeis guineensis* Jacq.) meal (Braga et al., 2010); cottonseed (*Gossypium hirsutum* L.), rapeseed (*Brassica napus* L.), and peanut (*Arachis hypogaea* L.) meals (Zhou & Yue, 2012), with values of 82.10, 75.87, 76.70, 77.60, and 77.60%, highlighting the biological value of protein of the nontoxic and detoxified physic nut cakes for Nile tilapia. Furthermore, the nontoxic physic nut cake showed higher values of acid detergent fiber and neutral detergent fiber than the detoxified one, which can explain the reduction in crude protein digestibility of the nontoxic physic nut cake ($p<0.05$). According to Guimarães et al. (2008), high fiber increases the digesta flow rate, reducing the nutrient utilization by the short time of contact with endogenous digestive enzymes.

Table 1. Total values and apparent digestibility coefficients (ADC) of dry matter, crude protein, ether extract, and energy of nontoxic and detoxified physic nut (*Jatropha curcas*) cakes for Nile tilapia (*Oreochromis niloticus*).

Nutrient	Nontoxic		Detoxified	
	Values(1)	ADC (%)(2)	Values(1)	ADC (%)(2)
Dry matter (%)	89.08	65.10	95.52	63.99
Crude protein (%)	17.76	77.51b	27.19	81.11a
Ether extract (%)	18.93	90.48a	1.65	52.10b
Gross energy (kJ kg^{-1})	20.70	65.31b	18.10	71.77a
Acid detergent fiber (%)	49.37	-	40.92	-
Neutral detergent fiber (%)	63.21	-	61.50	-

(1)Mean values of the nutritional composition of nontoxic and detoxified physic nut cakes correspond to the average of three replicates. (2)Means of apparent digestibility coefficients followed by different letters in the same lines differ by Student's test, at 5% probability.

The apparent digestibility coefficient of ether extract was superior ($p<0.05$) for the nontoxic physic nut cake (90.48%), in comparison to the detoxified physic nut cake (52.10%) (Table 1). Pezzato et al. (2002) and Guo et al. (2011), while evaluating soybean meal, observed similar values of 82.67 and 87.23%, respectively, for apparent digestibility of ether extract of the nontoxic physic nut cake for Nile tilapia. However, the results obtained in the present study were lower than those recorded by Zhou & Yue (2012), of 92.60%, for the apparent digestibility coefficient of ether extract. The lower results for the detoxified physic nut cake can be interfered by residual phorbol ester, which is lipophilic and found principally in physic nut oil and kernel (Makkar et al., 1998b), reducing the apparent digestibility of ether extract. Moreover, in the present study, a large difference was observed between nontoxic and detoxified physic nut cakes regarding ether extract, due to the adopted solvent extraction (solvent plus extrusion).

Other co-products (cakes and meals) derived from oilseeds with potential for biofuel production showed similar results to those found for rapeseed meal, of 88.19% (Furuya et al., 2001), and cottonseed meal, of 81.80% (Guimarães et al., 2008). However, higher apparent digestibility values of 97.2 and 98.37%, respectively, were observed for cottonseed and rapeseed meals (Zhou & Yue, 2012).

The apparent digestibility of gross energy was higher for the detoxified physic nut cake (71.77%) than for the nontoxic one (65.31%). Guimarães et al. (2012) reported 62.4% of apparent digestibility for conventional soybean meal. However, Guo et al. (2011) and Zhou & Yue (2012) found higher values, of 81.31 and 87%, respectively, for the apparent digestibility of gross energy for both soybean meal and tilapia.

In a study with Nile tilapia, Santos et al. (2010) also observed similar efficiency for the apparent digestibility of gross energy (75.26%) of radish forage meal. In addition, Braga et al. (2010) reported 75.87% apparent digestibility of gross energy of palm kernel meal for Nile tilapia, whereas Zhou & Yue (2012) found 73.70, 78.9, and 81.5% for rapeseed, peanut, and cottonseed meals, respectively, for hybrid tilapia.

In general, the different responses of the apparent digestibility coefficient of detoxified and nontoxic physic nut cakes can be related to anti-nutritional factors. There is also evidence of interaction between phytates and lipids, known as lipophytins, which are complexes of Ca/Mg-phytate with lipids and peptides (Leeson, 1993), hindering the absorption and possibly contributing to the lower digestibility of ether extract, gross energy, and crude protein.

Table 2. Total values and apparent digestibility coefficients (ADC) of nontoxic and detoxified physic nut (*Jatropha curcas*) cakes for Nile tilapia (*Oreochromis niloticus*)[(1)].

Amino acids	Nontoxic		Detoxified	
	Values (%)	ADC (%)	Values (%)	ADC (%)
Histidine	0.39	86.11	0.58	87.78
Isoleucine	0.67	87.21	1.05	89.11
Leucine	1.12	86.28	1.71	90.83
Lysine	0.55	86.48	0.75	88.28
Methionine	0.22	92.97	0.15	95.08
Arginine	1.75	88.74	2.52	92.06
Phenylalanine	0.74	87.54	1.11	91.56
Tryptophan	0.13	88.15	0.29	84.32
Threonine	0.60	84.42	0.85	79.66
Valine	0.83	85.39	1.29	87.31
Nonessential				
Alanine	0.75	81.55	1.15	85.67
Aspartic acid	1.48	84.37	2.22	91.65
Glycine	0.73	79.80	1.10	84.75
Glutamic acid	2.47	88.65	3.83	92.87
Cystine	0.14	87.59	0.19	89.28
Tyrosine	0.46	87.87	0.67	87.03
Proline	0.71	85.92	1.08	85.69
Serine	0.81	80.81	1.17	88.12

[(1)]Means of amino acid values and ADC correspond to a polled sample.

The amino acids of detoxified and nontoxic physic nut cakes showed apparent digestibility coefficient up to 80%, except for threonine (79.66%), for the detoxified physic nut cake, and for glycine (79.80%), for the nontoxic physic nut cake (Table 2). Compared to other amino acids, methionine presented the highest apparent digestibility coefficients, of 95.08 and 92.97%, respectively, for detoxified and nontoxic physic nut cakes. The lowest values for the apparent digestibility coefficient of amino acids were obtained for threonine (79.66%), for the detoxified physic nut cake, and for glycine (79.80%), for the nontoxic one.

In comparison to the apparent digestibility coefficients of soybean meal obtained by Guimarães et al. (2008) and Guimarães et al. (2012), the apparent digestibility of physic nut amino acids was superior only for methionine (95.08%) and glycine (84.75%), for the detoxified physic nut cake, and for glycine (79.80%), for the nontoxic physic nut cake. The high digestibility of methionine in the physic nut cake is relevant because this amino acid limits weight gain, is incorporated into proteins, and is also used for the synthesis of other essential compounds (Nutrient…, 1983).

The digestibility of the amino acids of some oilseeds used for biofuel production was evaluated by Zhou & Yue (2012) for hybrid tilapia. The authors reported apparent digestibility coefficients of peanut, rapeseed, and cotton meals of 74.10, 63.90, and 73.30% for methionine; 89, 84.50, and 79.40% for threonine; and 82.60, 85.10, 80.50% for glycine, respectively.

Furthermore, the imbalance of the amino acid profile and the high level of physic nut anti-nutrients contributed to the lowest apparent digestibility. The detoxification process was efficient to inactivate some anti-nutrients, because, in general, the apparent digestibility of amino acids showed the highest values for the detoxified physic nut cake, except for tryptophan and threonine. The apparent digestibility coefficients of nutrients, amino acids, and energy of nontoxic and detoxified physic nut cakes is equivalent to those reported for most oilseeds and shows potential for inclusion in Nile tilapia diets.

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