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AQUACULTURE

Energy and nutrient digestibility from mulberry (*Morus alba*) leaf meal for Nile tilapia

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ABSTRACT. The aim of this study was to determine the apparent digestibility coefficients and digestible values of crude protein, ethereal extract, gross energy and dry matter of mulberry leaf meal (MLM) (*Morus alba* L.) as Nile tilapia (*Oreochromis niloticus*) feed. A total of 135 Nile tilapia juveniles were used, and the indirect methodology (Cr_2O_3) was applied for digestibility determinations. Mulberry leave meal presented good apparent digestible coefficients of protein, ethereal extract and energy with respective values of 0.94, 0.58 and 0.39. The mulberry leave meal thus comprises adequate digestible protein and digestible energy values, similar or better than other leafy foods, presenting potential for inclusion in Nile tilapia diets.

Keywords: fish farming; alternative food; fish meal free; fish nutrition.

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Introduction

The silkworm feeds on mulberry leaves (Vu, Verstegen, Hendriks, & Pham, 2011), one of the oldest crops in the world, originally from Asia and subsequently distributed to other continents. The mulberry is more efficient than grass for light harvesting and biomass production, and its roots act underground to recover water and nutrients (Martín et al., 2017a).

Mulberries combine good chemical composition and nutritional quality, presenting crude protein (CP) levels above 20% (Martín, Montejo, Milera& Garcia, 2017b). However, their application in fish rearing can be limited by essential amino acid deficiencies and the presence of antinutritional factors (Dorothy et al., 2018).

Few studies on the use of mulberry leaves as fish food are available (Bag, Ghorai, Mahapatra, Rao, & Pal, 2012) and no studies regarding Nile tilapia digestibility exist (Astuti, Becker & Richter, 2012). Mulberry leaf meal (MLM) can substitute part of fish meal in sting fish *Heteropneustes fossilis* feed (Mondal, Kaviraj & Mukhopadhyay, 2011), to provide better performance and survival for fingerlings of the same species (Bag et al., 2012) and is able replace up to 30% of fish meal in Nile tilapia diets (Astuti et al., 2012).

The aim of this study was to determine the apparent digestibility coefficients (ADC) and digestible values of CP, ethereal extract (EE), gross energy (GE) and dry matter (DM) of mulberry leaf meal (MLM) (*Morus alba* L.) as Nile tilapia (*Oreochromis niloticus*) feed.

Material and methods

The experiments were carried out at the Aquaculture Technology Laboratory (LATAq) belonging to the Federal University of Paraná, Advanced Jandaia do Sul Campus, Brazil. A total of 135 Nile tilapia juveniles (50 g) were used, and the indirect methodology (Cr_2O_3) was applied for digestibility determinations (National Research Council [NRC], 1993; 2011).

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 02/2018, approved by Ethics Committee on the Use of Animals of the Palotina Sector of UFPR (CEUA/Palotina).

Three cages (100 L) and a circular glass fiber tank (2000 L), termed the feed tank, were used. The cages were conditioned during the adaptation period and also during the day throughout the collection period. Feces were collected using three cylindrical fiberglass vats with conical bottoms (200 L).

Water temperature was measured daily at 8:00 am and 4:00 p.m., and other feed tank and feces collection vat physico-chemical variables were monitored weekly. The mean temperature, pH, dissolved oxygen and ammoniacal nitrogen values during the experimental period were of 24.6 ± 1.3°C in the morning and in the afternoon 25.4 ± 2.4°C; 7.75 ± 0.25 and 7.67 ± 0.31; 5.5 ± 1.18 mg L^{-1} and 4.9 ± 1.05 mg L^{-1}; 0.017 ± 0.002 mg L^{-1}, respectively, remaining within the range recommended for aquaculture activities (Sipaúba-Tavares, Ligeiro & Durigan, 1995).

The MLM was prepared by manually harvesting mulberry leaves, which were then dried in a forced ventilation oven (60ºC for 72 hours) and passed through a knife and hammer mill with a 0.5 mm sieve and subsequently stored in plastic bags under refrigeration.

The reference and test feeds were free of fishmeal (Table 1) and were analyzed regarding CP, GE, EE, DM and ash (AH) (Table 2) according to Association of Official Analytical Chemists [AOAC] (1995). The test feeds were composed of 70% of the reference ration and 30% of the test feed, with corrections required only for the amount of mineral and vitamin supplements present in the mixture. The feed manufacture methodology followed Hayashi, Boscolo, Soares, Boscolo, and Galdioli, (1999) and Meurer, Hayashi and Boscolo (2003).

Table 1. Diets used for determining the apparent digestibility of mulberry leaf meal to Nile tilapia.

Ingredients (%)	Reference diet	Test diet
Soybean meal	60.43	42.30
Corn	31.36	21.95
Butylated hydroxytoluene	0.01	0.01
Dicalcium phosphate	3.07	2.15
Limestone	0.09	0.06
Soybean Meal	3.32	2.32
Mineral and vitamin Supplement[1]	1.00	1.00
NaCl	0.50	0.50
Cr_2O_3	0.10	0.10
Mulberry leaf meal	0.00	29.52
Total	100.00	100.00

[1] Supremais.

Table 2. Chemical composition of feeds used to determine the apparent digestibility of mulberry leaf meal to Nile tilapia.

Chemical composition	Soybean meal	Corn	Mulberry leaf meal
Crude protein (%)	48.30	8.95	24.45
Gross energy (Kcal kg^{-1})	-	-	3.985
Dry matter (%)	93.42	92.67	75.73
Ash (%)	6.41	1.16	11.31
Ethereal extract (%)	2.32	4.12	2.15

For the determination of MLM energy and nutrient ADC (Mukhopadhyay & Ray 1997), feces were analyzed regarding CP, EE, AH and DM (AOAC 1995). The determination of the GE of both the feces and feeds was performed using an adiabatic calorimetric pump (IKA C5003 control). Chromic oxide determinations were performed according to Kimura and Miller (1957).

Results and discussion

The MLM composition is displayed in Table 3. MLM CP was similar to the values reported by Miranda, Bonacin and Takahashi (2002), Okamoto and Rodella (2006), Vu et al. (2011) above the values reported by Magalhães et al. (2003) and Wang et al. (2017), and below the values determined by Al-Kirshi et al. (2013).

For GE, the obtained value was close to that determined by Al-Kirshi et al. (2013) and below that reported by Wang et al. (2017). The EE determined in the present study was below literature values (Vu et al., 2011, Al-Kirshi et al., 2013, Wang et al., 2017). The MLM AH determined herein is in agreement with the data reported by Magalhães et al. (2003), Vu et al. (2011) and Al-Kirshi et al. (2013), but higher than that reported by Okamoto and Rodella (2006).

The MLM ADC GE, CP, EE, and DM for Nile tilapia are displayed in Table 4. Similar DM ADC are observed compared to the values presented by Al-Kirshi et al. (2013) for broilers and laying hens.

Table 3. Mulberry leaf (*Morus Alba* L.) composition values found in literature.

Reference	CP	GE	EE	DM	AH
Miranda et al. (2002)	25.13	--	--	--	--
Magalhães et al. (2003)	14.8	--	--	43.6	12.8
Okamoto & Rodella (2006)	21.62	--	--	26.43	8.06
Vu et al. (2011)	22.3	--	3.5	19.8	13.6
Al-Kirshi et al. (2013)	29.80	4,203	5.57	89.25	11.81
Wang et al. (2017)	18.81	4,940	11,65	--	--
Author data	24.45	3,985	2.15	94.81	11.31

CP: Crude protein (%); GE: gross energy (Kcal kg^{-1}); EE: ethereal extract (%); DM: dry matter (%); AH: Ash (%)

Table 4. Apparent digestible coefficients and digestible values of dry matter, protein, ether extract and energy of mulberry leaf meal.

Nutrient and Energy digestibility coefficient			
Dry matter	Protein	Ethereal extract	Energy
0.40	0.94	0.58	0.39
Digestible values			
Digestible protein		Digestible ether extract	Digestible energy
22.94 %		1.25%	1,569 Kcal kg^{-1}

The MLM DM ADC values were higher than those determined for maniçoba (*Manihot pseudoglaziovii*) (Araújo, Santos, Silva, Santos, & Meurer, 2012, Santos, Sousa, Silva, Bombardelli, & Meurer, 2014) leucena (*Leucaena leucocephala*), and manioc (*Manihot esculenta*) for Nile tilapia (Araújo et al., 2012) and for the orelha de onça, miúda, gigante and common of the *Opuntia fícus* cultivars (Daniel et al., 2016). However, the MLM presented a lower DM ADC value when compared to brown seaweed flour (*Ascophyllum nodosum*) (Alves Filho, Santos, Silva, Bombardelli, & Meurer, 2011), to manioçoba hay processed in the extruded form (Santos et al., 2014) and to yeast (Meurer et al., 2003).

The MLM CP ADC was higher than several foods of plant origin, such as leucena, maniçoba, manioc leaves, prickly pear, brown seaweed flour and yeast (Meurer et al., 2003, Alves Filho et al., 2011, Araújo et al., 2012, Santos et al., 2014, Daniel et al., 2016). The MLM EE ADC values were higher than values reported for leucena, maniçoba and manioc leaves (Araújo et al., 2012).

MLM digestible protein values were higher than leucena (Araújo et al., 2012) and maniçoba (Araújo et al., 2012, Santos et al., 2014) hay. When compared to conventional protein sources, the MLM comprises about half the digestible protein present in soybean meal and poultry offal meal (Boscolo, Hayashi, & Meurer, 2002, Pezzato et al., 2002, Meurer et al., 2003) compared to fishmeal, depending on the source (Pezzato et al., 2002, Meurer et al., 2003), which can present values ranging from half to a little less than half the value it provides to Nile tilapia.

The fraction of digestible EE provided by the MLM is similar to that presented by maniçoba and leucena hay (Araújo et al., 2012). The DE supplied by MLM for Nile tilapia was higher than maniçoba, leucena and manioc hays (Araújo et al., 2012, Santos et al., 2014). When compared to other energy or protein-energy sources, MLM provides about half of the energy present in corn, triticale, wheat bran and soybean meals (Boscolo et al., 2002; Pezzato et al., 2002).

MLM composition values can vary due to the environmental factors (Miranda et al., 2002), such as soil type, fertilization and plant development stage (Okamoto, Cunha & Furlaneto, 2011). This explains changes in protein content, which tend to decrease with mulberry leaf harvesting delays, related to leaf aging processes (Okamoto, Cunha, Bueno, Porto, & Santos, 2007).

The assessed MLM presented an excellent ADC for CP. Thus, MLM protein is easily digested by Nile tilapia. Although the amino acid composition of this food item has not been evaluated, the quality of this protein in terms of essential amino acids has been reported as good even when compared to soybean meal (Table 5). Although it is a food of vegetal origin, fibers do not seem to significantly influence protein fraction digestibility.

Over 40% of the EE was not digested and absorbed. Fibrous foods have a significant effect on the digestion of the lipid fraction, as fibers are not digested by the fish and end up imprisoning part of the chylomicrons, precluding them from completing their way to the brush border of the intestine.

Table 5. Amino acid composition (%) of mulberry leaf and soybean meal.

	Mulberry leaf			Soybean meal
	Al-Kirshi, Alimon, Zulkifli, Zahari, & Sazili (2009)	Astuti et al. (2012)	Wang et al. (2017)	Rostagno et al. (2017)
Dry matter	89.30	90.3	100.0	88.1
Crude protein	29.80	29.58	18.81	44.4
Lysine	1.88	1.84	1.11	2.74
Methionine	0.52	--	0.36	0.59
Tryptophan	0.27	0.54	0.26	0.63
Arginine	1.80	1.86	0.89	3.23
Histidine	0.69	0.92	0.35	1.17
Isoleucine	1.43	1.22	0.74	2.08
Leucine	2.58	2.46	1.45	3.43
Phenylalanine	1.94	--	0.92	2.29
Threonine	1.31	1.39	0.79	1.74
Valine	1.76	1.47	0.62	2.17
Alanine	1.54	--	1.09	1.99
Aspartate	3.06	--	1.77	3.14
Cysteine	0.30	--	0.30	0.66
Glycine	1.57	--	0.99	1.93
Glutamate	3.33	--	1.92	4.34
Proline	1.30	--	0.88	2.26
Serina	1.22	--	0.80	2.41
Tyrosine	0.82	--	0.62	1.62

The MLM energy ADC value, as well as the DE value, mainly reflects protein and lipid fraction digestibility. Although some digestible carbohydrate may be present in the MLM, the rest of the food, with the exception of protein and fat, is related to minerals and fibers, which do not contribute to the digestible energy fraction.

Conclusion

The mulberry leaf meal thus comprises adequate digestible protein and digestible energy values, similar or better than other leafy foods, presenting potential for inclusion in Nile tilapia diets.

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