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Short communication

Coefficients of total tract apparent digestibility of some feedstuffs for Tambaqui (*Colossoma macropomum*)

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ABSTRACT

This research aimed at evaluating the coefficients of total tract apparent digestibility (CTTAD) of dry matter (DM), gross energy (GE) and crude protein (CP) for conventional products (whole ground corn, wheat middlings, soybean oil, broken rice and whole ground sorghum) and alternative by-products (cassava root meal, mesquite pod meal and copra meal) by tambaqui (*Colossoma macropomum*). A semi-purified reference diet containing 345 g kg^{-1} CP and 13.44 MJ DE kg diet^{-1} was used and test diets were produced by incorporation of test ingredients in a 7:3 ratio (700 g kg^{-1} reference diet and 300 g kg^{-1} test ingredient). Chromic oxide was used as an indigestible inert marker at 1 g kg^{-1} and feces were collected following a modified Guelph procedure. CTTAD of DM ranged from 0.426 to 0.838 among the feedstuffs. CTTADs of GE showed trend similar to DM, ranging from 0.449 to 0.927. Soybean oil, cassava root meal and broken rice had the highest CTTAD of GE while whole ground sorghum and mesquite pod meal showed the lowest values. The CTTAD of crude protein of the feedstuffs evaluated ranged from 0.714 to 0.875. These results indicate that copra meal and cassava root meal have great potential to replace commonly used ingredients in diets for tambaqui and further research to determine their level of inclusion is needed. Additionally, further research is necessary to determine if the fiber digestibility is influenced by dietary fat content in tambaqui diets. The CTTAD of protein above 0.700 observed in this study indicated that tambaqui is able to efficiently digest protein in several types of ingredients.

1. Introduction

Brazil is known worldwide for its great freshwater fish diversity accounting for approximately 41% of total bony fish species (Crescêncio, 2005). However, only around 30 species have been used in aquaculture production. Tambaqui is the third most produced species in Brazilian aquaculture and its farming has been increasingly growing in the past few years (MPA, 2010). The growing interest in tambaqui farming is due to its flesh quality, high growth and feed efficiency rates. Additionally, this fish is an omnivorous species and is well adapted to high stocking densities and artificial diets (Doria and Leonhardt, 1993; Abimorad and Carneiro, 2004; Araújo-Lima and Gomes, 2005).

Despite the importance of tambaqui to aquaculture production, to our knowledge, there is paucity in information regarding the nutrient requirements and apparent digestibility coefficients of ingredients commonly used in formulated

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Table 1
Composition and proximate analysis of reference diet.

Ingredient	$g\,kg^{-1}$
Albumin	320.0
Gelatin	77.0
Corn starch	441.3
Soybean oil	60.0
Cellulose	60.0
Dicalcium phosphate	30.0
Vit/min supplement[a]	5.0
Ascorbic acid[b]	0.5
NaCl[c]	5.0
Chromic oxide III	1.0
BHT[d]	0.2
Proximate analysis (dry matter basis)[e]	$g\,kg^{-1}$
Moisture	85.9
Crude protein	344.8
DE[f] ($MJ\,kg^{-1}$)	12.77
Lipid	62.3
Ash	81.2
Crude fiber	50.6
aNDFom[g]	70.9

[a] Vitamin and mineral mix (IU or mg kg^{-1} premix): folic acid 600 mg, biotin 24 mg, choline chloride 54 g, niacin 12,000 mg, calcium panthothenate 6000 mg, vit. A 600000 IU, vit. B1 2400 mg, vit. B12 2400 mg, vit. B2 2400 mg, vit. B6 2400 mg, vit. C 24 g, vit. D3 100000 IU, vit. E 6000 mg, vit. K3 1200 mg. Cobalt 1 mg copper 300 mg, iron 5000 mg, iodine 10 mg, magnesium 2000 mg, selenium 10 mg and zinc 3000 mg.
[b] Ascorbyl polyphosphate (DSM Stay C 35.0% activity).
[c] Sodium chloride.
[d] Antioxidant: butylated hydroxytoluene.
[e] Analyzed.
[f] Calculated value according to the CTTAD of nutrients reported by NRC (1993).
[g] Neutral detergent fiber with a heat stable amylase and expressed exclusive of residual ash.

diets for this species. Thus, this study aimed to determine the CTTAD of nutrients of whole ground corn, wheat middlings, broken rice, whole ground sorghum, cassava root meal, soybean oil, mesquite meal, and coconut meal.

2. Materials and methods

2.1. Feed ingredients and diet preparation

Test ingredients were mixed with a semi-purified reference diet (Table 1) in a 7:3 ratio while soybean oil was mixed in an 8.5:1.5 ratio. Thus, nine compound diets were evaluated, eight containing the test ingredients and a reference diet. The reference diet was formulated to meet or exceed the protein and energy requirements for tambaqui (Oishi et al., 2010). Chromic oxide (Cr_2O_3) was used as external inert marker at $1\,g\,kg^{-1}$. Test ingredients, vitamin and mineral premixes were purchased from a feed mill industry (Nutreco Fri-Ribe Nutrição Animal S.A.). Test ingredients were ground until sieve in a mesh diameter of 400 μm to present the same particle size of the reference diet mixture.

Chemical composition of test ingredients is shown in Table 2. Diets were mechanically mixed with distilled water at 45 °C (25% of dry-weight); the moist mixture was extruded in a 4.0 mm die of a meat grinder. Diets were oven-dried at 55 °C for 48 h, crumbled to obtain 3 cm long pellets, and stored at −18 °C until used.

Table 2
Chemical composition of test ingredients (mean of two samples).[a]

Ingredient	IFN[b]	Dry matter ($g\,kg^{-1}$)	Crude protein ($g\,kg^{-1}$)	Gross energy ($MJ\,kg^{-1}$)	Crude fiber ($g\,kg^{-1}$)	aNDFom[c] ($g\,kg^{-1}$)	Ash ($g\,kg^{-1}$)
Mesquite meal	–	870.7	83.4	14.62	228.1	242.4	34.5
Cassava meal	4-11-937	873.5	30.9	16.33	54.2	69.3	28.3
Sorghum	4-04-444	903.5	78.4	16.63	19.6	40.3	21.8
Corn	4-02-935	893.5	72.1	17.00	20.0	41.9	12.0
Broken rice	4-03-932	907.2	82.1	15.66	7.5	47.0	7.8
Copra meal	5-01-572	895.0	230.1	21.10	140.7	193.5	55.0
Wheat middlings	4-05-205	915.8	144.7	16.75	88.9	121.0	47.3
Soybean oil	4-07-983	–	–	37.87	–	–	–

[a] As is basis.
[b] International feed number.
[c] Neutral detergent fiber with a heat stable amylase and expressed exclusive of residual ash.

Table 3
Coefficients of total tract apparent digestibility of gross energy, crude protein and dry matter of some conventional and alternative ingredients for tambaqui (%, $n = 3$ aquaria).[a]

Ingredient	Gross energy	Crude protein	Dry matter
Corn	.764 ± 0.007c	.875 ± 0.009a	.778 ± 0.020b
Cassava meal	.823 ± 0.013b	.817 ± 0.004c	.836 ± 0.011a
Wheat middlings	.682 ± 0.009d	.844 ± 0.004b	.626 ± 0.008d
Sorghum	.572 ± 0.017e	.714 ± 0.011d	.616 ± 0.008d
Broken rice	.807 ± 0.016bc	.728 ± 0.003d	.838 ± 0.008a
Soy oil	.927 ± 0.017a	–	–
Mesquite meal	.449 ± 0.015f	.821 ± 0.005c	.424 ± 0.011e
Copra meal	.769 ± 0.016c	.864 ± 0.006a	.717 ± 0.008c

[a] Means ± SD with different superscript letter in the same column are statistically different by Tukey test (P<0.05).

2.2. Fish and experimental condition

180 tambaqui juveniles, initial weight 86.52 ± 6.71 g, were randomly stocked into nine circular net cages inside three 500 L-aquaria. This set of aquaria was used for the feeding procedure and was connected to a recirculated water system. Three conic 300 L-aquaria were used to collect feces by sedimentation following a modified procedure of the Guelph system described previously (Guimarães et al., 2008). Both systems were connected to a biological filter and water temperature was thermostatically controlled.

Feces collection was performed according to Guimarães et al. (2008) with slightly modifications. Briefly, diets were randomly assigned to net cages and the fish were fed seven days prior to the beginning of fecal collection (acclimatization period), then the first feces collection was carried out. During the feces collection period, fish were fed twice daily until apparent satiation. The first three groups of fish were then transferred to collecting feces aquaria, remaining for eight hours. They were then returned to the feeding aquaria. On the consecutive two days the remaining six groups were transferred and feces were collected. Feces were oven-dried at 55 °C, ground and stored at −20.0 °C until chemical analysis. This procedure was repeated three times to obtain a replication (each group of fish), and test diets were reassigned to the circular net cages for each following round. The acclimatization and fecal collection process (round) were repeated three times to obtain triplicate measurements per test diet.

During the experimental period, a 12 h light:12 h dark photoperiod was maintained, dissolved oxygen content was approximately 6.1 mg L^{-1}; pH 6.7–7.8, and total ammonia–nitrogen 0.038 and 0.057 mg L^{-1}. Temperature ranged from 28.5 °C to 30.9 °C.

2.3. Chemical analysis, experimental design and data processing

The chemical composition was determined according to standard methods (AOAC, 1990) including dry matter (method 930.15), ash (method 942.05), CP (Nx6.25; method 988.05) and crude fiber (CF; method 978.10). Ether extract was determined after extraction with petroleum ether by the Soxhlet method (method 920.39). The procedure of Van Soest et al. (1991) with addition of sodium sulphite and alpha amylase was used to determine neutral detergent fiber (aNDFom). Chromic oxide was determined according to Bremer-Neto et al. (2005) and gross energy content was determined in an adiabatic calorimetric bomb (Parr Instrument Company, Moline IL, USA). Coefficients of total tract apparent digestibility coefficients (CTTAD) of nutrients were calculated according to Bureau and Hua (2006).

The trial consisted of eight treatments (test diets) and three replications (group of fish) arranged in a randomized block design. The block was considered as a round of fecal collection. The differences between ingredients were analyzed according to the following model:

$$Y_{ijk} = \mu + T_i + B_j + e_{ijk}$$

where Y is the observed response; μ the overall mean; T the effect of ingredients; B the effect of the fecal collection period; and e is the residual error.

All percentage data were arcsine transformed before analysis. The CTTADs for dry matter, protein, and energy were subjected to ANOVA using the PROC GLM procedure of SAS (Statistical Analysis System, version 8.12). Differences in CTTADs among the tested ingredients were determined using Tukey's multiple range test at P<0.05. No effect of the fecal collection period was observed.

3. Results

CTTADs of dry matter, protein and energy of test ingredients are shown in Table 3. The CTTAD of DM was highly variable and ranged from 0.426 to 0.838. Cassava root meal and broken rice had the highest CTTAD of DM, while wheat middlings, whole ground sorghum and mesquite pod meal (MPM) showed the lowest values. CTTADs of GE showed similar trend to

DM, ranging from 0.449 to 0.927. Soybean oil, cassava root meal (CRM) and broken rice had the highest CTTAD of GE while whole ground sorghum and mesquite pod meal (MPM) had the lowest values.

The CTTAD of crude protein ranged from 0.714 to 0.875 among the ingredients. Whole ground corn and copra meal had the highest CTTAD of CP while the lowest values were observed for sorghum and broken rice ($P<0.05$).

4. Discussion

CRM and broken rice had the highest CTTAD of DM for tambaqui. Generally, the CTTAD of DM for tambaqui was higher than those reported for tilapia and carp (Fagbenro, 1999; Pezzato et al., 2002, 2004) and closer to those reported for pacu, *Piaractus mesopotamicus* (Abimorad et al., 2008). However, higher CTTADs were reported in sorghum, wheat middlings and corn for pacu (Abimorad and Carneiro, 2004; Fernandes et al., 2004). Although pacu and tambaqui are species from the same phylogenetic group and have similar digestive tract anatomy, they seem to digest nutrients differently. Previous studies have demonstrated that tambaqui may probably be able to efficiently digest fiber up to 210 g kg^{-1} (Silva et al., 1999, 2003), while levels above 90 g kg^{-1} reduced the digestibility and growth in pacu. Although some reports have indicated that tambaqui may have the ability to digest fiber in some feedstuffs, studies are needed to determine the maximum dietary fiber level based on growth performance. Additionally, the mechanism which this species uses to digest complex carbohydrates is worth of studying.

Previous studies on Rohu and Nile tilapia have indicated that copra meal levels ranging from 200 to 300 g kg^{-1} do not affect growth of these fish (Mukhopadhyay and Ray, 1999; Pezzato et al., 2000). However, our previous study demonstrated the suitability of totally replacing the soybean meal by copra meal for tambaqui without any adverse effect on growth performance (Lemos et al., 2011). Similar CTTAD of gross energy and/or dry matter in copra meal have been reported for *Mystus nemurus* (Khan, 1994) and pacu (Oliveira et al., 1997); on the other hand, lower CTTADs were reported in grass carp (Law, 1986), *Megalobrama amblycephala* (Zhou et al., 2008) and Nile tilapia (Pezzato et al., 2000). Tambaqui seems to efficiently digest copra meal since the CTTADs of energy and dry matter were higher than for wheat middlings and whole ground sorghum, and comparable to those values of whole ground corn. These results support the hypothesis that tambaqui is able to efficiently digest the fiber in copra meal, and the presence of lipid could probably influence the fiber digestibility, since the CTTAD of dry matter was higher in this feedstuff while CTTADs in the other feedstuffs with higher and/or similar crude fiber and NDF content was lower.

Mesquite, *Prosopis juliflora*, is a xerophytic evergreen legume tree well adapted to dry and hot weathers and world widely distributed in the most tropical regions. Mesquite pods are rich in saccharose (20–25% of DM) and reduced sugar (10–20% of DM) (Silva, 1986), while the seeds contain around 34–39% crude protein (CP) on a DM basis (Mendes, 1986), 21.6–29.1% mucilage (with >85% Nitrogen Free Extract NFE). The cotyledon fraction represents 30–40% of the seeds and contains 27.4–70.3% CP and 62.9–71.2% NFE (Escober et al., 1987). Their pods have been incorporated into feeds for cattle, sheep, camel, buffalo, rabbits, poultry and rats, especially in South America, Africa and India (Mendes, 1986; Sawa et al., 2004). Despite the potential of mesquite pod as a supplementary feed for some fish species, few studies have evaluated the nutritive value of this ingredient. Tambaqui seems to have limited capacity for digesting mesquite pod meal since the lowest CTTAD of dry matter and energy values were observed for this product. Similar results were reported for Nile tilapia showing CTTADs of DM and GE around 0.487 and 0.305, respectively (Braga et al., 2010). However, Pezzato et al. (2004) reported higher CTTAD of GE for the same species (0.745). These differences on mesquite pod meal CTTAD values may be related to the wide variation in proximate composition of the pods (Sawa et al., 2004), the presence of antinutritional factors, and the processing method used to produce the meal. Carp (*Labeo rohita*) fed diets with soaked *Prosopis juliflora* seed meal up to 20% had better growth performance than carp fed raw and autoclaved pods (Bhatt et al., 2011). The authors also attributed the lower growth performance of fish fed high levels of mesquite seeds to the imbalance of amino acids and the presence of non-starch polysaccharides.

The high CTTAD of protein in this study (above 0.700) shows that tambaqui efficiently digests the protein in several types of ingredients. The crude fiber and/or starch content may have minor effects on protein digestibility in tambaqui since cereal grain products are rich in these compounds. Although we did not measure the tannin content in sorghum, the lowest protein digestibility observed in this ingredient may be attributed to the presence of tannins, which can link to certain amino acids and therefore reduce the overall protein digestibility. Similar results were reported for Nile tilapia (Pezzato et al., 2002; Freire et al., 2005; Guimarães et al., 2008); however, those values were lower than in this study. Higher CTTADs of protein in sorghum were reported for Pacu (Abimorad and Carneiro, 2004). In general, the sorghum available in Brazil for the feed industry has less than 0.7% of total tannins and approximately 0.2% of condensed tannins. These amounts are not detrimental to the growth performance and does not affect nutrient digestibility of farm animals, fish included (Magalhães et al., 1997; Pinto et al., 2001, 2008). Although tambaqui is a freshwater frugivorous species in adult stages, no studies have been conducted on the composition of antinutrients in these fruits/nuts and thus, the effect of the tannins in the physiology of this fish remains unknown. According to Guimarães et al. (2008), differences among CTTAD of protein observed in different studies may be either a result of differences in feces collection method, feed processing or statistical approach. The most used feces collecting methods in nutrient digestibility studies with fish are the stripping, dissection, and sedimentation in the water column. The choice of the method can affect the CTTAD of nutrients: in general, stripping and dissection methods tend to underestimate the CTTAD values since feces contamination with blood, urine and intestinal cells may occur in the first method, while in the dissection, incomplete digestion and absorption of nutrients and contamination with blood during excision of the gut may occur, increasing the nutrient content in feces, therefore, reducing the CTTAD. On the other hand, these methods do not have the problem of nutrient leaching, in contrast to when the feces are collected by sedimentation in the water column. The leaching problem becomes more severe if the fish species do not have a mucous protection of the feces, such as tilapia. Thus, the feces sedimentation method usually overestimates the CTTAD of nutrients. Despite these specific concerns, all the methods can be used if care is taken on the feces collecting procedure. It is well established that feed processing can affect nutrient digestibility in farm animals, fish included. Although the extent of the effect of extrusion on nutrient digestibility may vary according to the feeding habit of the fish, increased nutrient digestibility is expected in extruded diets and/or ingredients. However, amino acid and/or protein digestibility may be reduced due to severe extrusion conditions. Therefore, care must be taken when comparing results from different studies.

In sum, our study demonstrated that, except for MPM, tambaqui is able to efficiently digest several types of feedstuffs, showing higher CTTAD values than other omnivorous species. Since this is the first report on nutrient digestibility values of feed ingredients for tambaqui, further studies on nutritional physiology are necessary to explain differences among ingredients and fish species. Our results indicated that copra meal and cassava root meal have great potential to replace commonly used ingredients in diets for tambaqui and further research to determine their level of inclusion is needed. Additionally, further research is necessary to determine if the fat content influences fiber digestibility in tambaqui. The CTTAD of CP above 0.700 observed in this study indicated that tambaqui is able to efficiently digest protein in several types of ingredients. Further studies on nutrient digestibility of other feedstuffs are necessary to build up a platform of knowledge based on nutrient availability for this species.

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