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Responses of digestive enzymes of tambaqui (*Colossoma macropomum*) to dietary cornstarch changes and metabolic inferences

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Abstract

Digestive enzyme responses plus metabolic implications were studied in tambaqui (*Colossoma macropomum*) fed isoproteic diets containing 28% crude protein, 3300 kcal of gross energy/kg and different amounts of cornstarch (30, 40 and 50%). Amylase, maltase, acid protease, trypsin and chymotrypsin from the alimentary tract were assayed. Plasma, liver and white muscle metabolites were gauged to profile metabolism of the fish. The alimentary tract of tambaqui is compartmentalized morphologically and enzymatically. Amylase was present through the gut; acid protease was detected in stomach; maltase, trypsin and chymotrypsin were found in pyloric caeca and proximal and distal intestine sections. Increase of cornstarch levels from 40 to 50% in the diet resulted in an increase in amylase and maltase. Trypsin and chymotrypsin were unresponsive to starch levels. Acid protease follows the protein/carbohydrate ratio decrease. The increase of dietary cornstarch resulted in liver glycogenesis and the increase in plasma triglycerides is suggestive of lipogenesis. Digestive biochemical responses of tambaqui correlated with changes of feeding plus the analyses of metabolic profile are assumed as a tool for optimizing diet formulation and are a clue to other feeding optimizations for freshwater tropical species.

Keywords: *Colossoma macropomum*; Cornstarch; Digestive enzymes; Fish metabolism; Fish nutrition; Freshwater fish; Tambaqui

1. Introduction

Several aspects of digestive process in fish remain still unclear. In spite of digestive process in fish is not as much known of that in mammals, the enzyme profile seems well related (Hidalgo et al., 1999). It is tough to compare the current data concerning digestive juice of fish since many different technical approaches have been reported (Das et al., 1987; Fagbenro, 1990; Munilla-Móran and Saborido-Rey, 1996; Hidalgo et al., 1999; Deguara et al., 2003). The ability to transform and use the dietary nutrients depends on the enzyme distribution over the digestive tract. The enzyme content in the digestive juice is crucial to chemical digestion. Recent issues have shown that in fish this is related to factors as: the age (Kuz'mina, 1996); the feeding habit and gut morphology (Smith, 1988; Kuz'mina and Smirnova, 1992; Sabapathy and Teo, 1993); the frequency of feeding (Ugolev and Kuz'mina, 1994); the year season and/or the acclimatization temperature (Kuz'mina et al., 1996). However, only recently some data concerning diet composition versus digestive enzyme adaptation have been put forward. Ugolev and Kuz'mina (1994) reported different patterns of digestive enzymes in fish associated to the type of feeding. This ability was recently reported in the carnivorous freshwater fish pintado *Pseudoplatystoma corruscans* (Lundstedt et al., 2004), in the omnivorous matrinxã *Brycon cephalus* (Vieira et al., 2005), and pacu *Piaractus mesopotamicus* (Bidinotto et al., 1997). There are several freshwater teleost species in the continental waters of South America with increasing interest for farm culture. Among those is tambaqui *Colossoma macropomum* a species from the Amazon basin. It is reported as omnivorous with a shift to frugivory (Honda, 1974; Goulding and Carvalho, 1982; Val and Honczaryk, 1995). This species is usually exposed to environmental water quality oscillations and nutrient availability (Val and Honczaryk, 1995). Inasmuch as 1) the feeding is the source of nutrients and energy; 2) tambaqui is naturally susceptible to dietary changes and; 3) this species is very promising to farm cultures, it is relevant to study the influence of dietary changes on the digestive enzymes and the adaptations of the metabolical profile. The present study reports

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the effect of the dietary levels of cornstarch on the intermediary metabolism and the digestive enzyme profile of tambaqui. This approach has been proposed as a tool to optimize the nutrient composition of the feeding as well as to access the feeding strategy of the species.

2. Materials and methods

2.1. Experimental design

Juvenile tambaquis ($n=18$) from CEPTA–IBAMA — Pirassununga, Sao Paulo, average mass 167.7±8.09 g (means± S.E.M.) and size 21.9±0.72 cm, were placed into 2000 L fiberglass containers and kept under aerated water at 7.5 mg/L dissolved oxygen (DO), 25±2 °C and pH 6.5. The fish were fed ad libitum with commercial fish pellets over 30 days for acclimatization. After that, they were allocated equally to three 250 L tanks and kept starved for 48 h before starting the experimental feeding. Fish were fed a semi-purified experimental diet, containing 30%, 40%, or 50% cornstarch (Table 1). Nourishment was offered twice a day at 8:00 and 17:00 h with 3% of stocked biomass for 15 days. The tanks were assigned A, B and C for 30%, 40% and 50% of cornstarch respectively. The protein content was kept constant at 28% (Table 1). Attained at the end of the trial span the fish were starved for 24 h, sampled, anesthetized with 0.27 g/L 3-aminobenzoic acid ethyl ester (MS222), and the blood was collect from caudal vein with heparinized syringe. Hematocrit was done to appraise fish health condition. The blood was centrifuged at 11,400 ×*g* for 3 min and the plasma was set aside for glucose, pyruvate, triglycerides and free amino acid determination. Afterwards, the fish were killed by cervical dislocation, digestive tracts excised, eventual gut stuffs were quickly removed, and the gut was rinsed with cold saline and kept at −20 °C for subsequent enzyme assays. Liver and white muscle samples were quickly collected and plunged into liquid nitrogen. These samples were kept at −20 °C for quantification of metabolites.

Table 1
Ingredients and estimated composition of the semi-purified experimental diets (dry matter bases)

	Experimental diets [a]		
	$P_{28}C_{30}$	$P_{28}C_{40}$	$P_{28}C_{50}$
Ingredients (%)			
Albumin	25.93	23.00	25.00
Corn starch	29.97	39.96	49.97
Fish meal [b]	10.50	14.70	11.85
Cellulose	21.13	14.77	8.36
Vegetable oil [c]	8.93	4.33	1.33
Bi-calcium phosphate	0.80	0.50	0.75
dl-methionine	0.54	0.54	0.54
l-lysine HCl	1.65	1.65	1.65
Vitamin–mineral mixture [d]	0.50	0.50	0.50
Vitamin C	0.05	0.05	0.05
Total	100	100	100
Composition (%)			
Crude protein	28.00	28.00	28.01
Total carbohydrate	30.00	40.00	50.00
Crude fiber	16.53	11.63	6.62
Fat	10.46	6.30	2.91
Calcium	0.91	1.13	0.99
Phosphorus	0.52	0.58	0.53
Methionine+cystine	0.86	0.95	0.89
Lysine	1.75	1.93	1.81
Vitamin C	0.04	0.04	0.04
Gross energy (kcal/kg)	3302.3	3300.1	3300.9

[a] P = crude protein percent; C = cornstarch percent.
[b] Fish meal composition: crude protein 50.99%; dry matter 91.83%; ash 24.77%; fat 10.48% and crude fiber 0.60%.
[c] Soy bean oil.
[d] Vitamin–mineral premix: vitamin A 650.000 UI; vitamin D 175.000 UI; vitamin E 15.000 mg; vitamin K 2.500 mg; vitamin B_1 500 mg; vitamin B_2 2.500 mg; vitamin B_6 875 mg; vitamin B_{12} 375 mg; vitamin C 28.750 mg; folic acid 500 mg; niacin 5.000 mg; pantothenic acid 5.000 mg; choline 112.500 mg and antioxidant BHT (3,5-di-ter-butyl-4-hydroxytoluene) 25.000 mg.

2.2. Tissue homogenates and extracts

The digestive tract was placed on a cold Petri dish and sectioned into oesophagus, stomach, pyloric caeca, and proximal and distal intestine sections. The fractions were homogenized on ice in 0.02 M Tris/phosphate 0.01 M buffer pH 7.0 dissolved into v/v glycerol with a Potter-Elvehjem homogenizer. The homogenates were centrifuged at 11,400 ×*g* for 3 min and the supernatants (crude extracts) were used as enzyme source.

Glucose and pyruvate from plasma and tissues were each quantified in acid extracts. Plasma was diluted at 1:10 into 0.6 N perchloric acid (PCA); liver and white muscle were disrupted in 20% trichloroacetic acid (TCA) with a Potter-Elvehjem homogenizer. Homogenates were centrifuged at 11,400 ×*g* for 3 min and the metabolites glucose and pyruvate were quantified in the supernatant.

Free amino acids were quantified in neutral extract. Plasma extracts were prepared by adding 0.5 mL $Ba(OH)_2$ 0.3 N and 0.5 mL $ZnSO_4$ 5% into 0.6 mL plasma. The samples were immediately centrifuged at 11,000 ×*g* for 3 min and amino acids were determined in the supernatant. Liver and white muscle 0.1 g samples were disrupted into 1.0 mL cold distilled water and immediately centrifuged at 11,000 ×*g* for 3 min. Suitable aliquots were treated with 1.0 mL of Ba^{2+}–Zn^{2+} solution and re-centrifuged at 11,000 ×*g* for 3 min, as reported above. Amino acids were determined in the supernatant.

Glycogen was quantified after total tissue alkaline digestion adapted from Bidinotto et al. (1997). Liver and white muscle were disrupted in 0.6 N KOH under boiling water bath for 3–5 min. Glycogen was precipitated by 96% ethanol plus 1% K_2SO_4. The cloudy white precipitate was centrifuged at 1000 ×*g* for 3 min. The supernatant was discharged and the glycogen pellet was fully dissolved into distilled water and quantified by the sulfuric hydrolytic method of Dubois et al. (1956). Triglycerides were estimated directly in the plasma at 510 nm with LABTEST enzymatic kit, and expressed in mg/dL.

2.3. Enzyme assay

All the samples were assayed in duplicate and two blanks were used: the non-enzymatically hydrolyzed substrate reaction