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Use of phytase to improve the digestibility of alternative feed ingredients by Amazon tambaqui, *Colossoma macropomum*

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ABSTRACT: The study assessed the effect of phytase on the digestibility of alternative plant feed ingredients Brazil nut (*Bertholletia excelsa*) and leucaena (*Leucaena leucocephala*) leaf meal fed to tambaqui (*Colossoma macropomum*) and on fish growth. Five treatments were used: UPI denoted the negative control diet with unfermented plant ingredients; UPIP was positive control diet with unfermented plant ingredient supplemented with 3 g/kg phosphorus (NaH_2PO_4); FPI indicated diet with fermented plant ingredients; FPIPT1 denoted diet with fermented plant ingredients supplemented with phytase 4000 U/kg; and FPIPT2 was diet with plant ingredients fermented together with phytase 4000 U/kg. Chromic oxide was added to the diets as inert marker for digestibility study. The diets were fed to juveniles of tambaqui (70.0 ± 6.3 g) to satiation twice daily in recirculation systems for 56 days. Results indicated high and better digestibility of protein and lipid by fish fed diet with phytase, which resulted in a corresponding better fish growth. Growth rate of the fish was high and increased from initial weight of 70 g to 132 g almost doubling the initial weight. No significant differences were found in weight gain, specific growth rate, and feed conversion ration, but performance was better in fish fed diets with phytase. The fish grew well in low dissolved oxygen levels of 4.23–4.34 mg/l, pH of 5.80–5.91 and high ammonia concentration of 1.76–2.17 mg/l without compromising growth and physiological functions.

KEYWORDS: alternative plant feed ingredients, fish growth

INTRODUCTION

In Manaus Brazil, the soil conditions are unsuitable for mass production of arable crops resulting in shortage of grains, corns, and soybean meal conventionally used in fish and animal feeds[1]. Consequently, these essential ingredients are purchased from other regions with high transportation costs, leading to high cost of fish feeds and fish production. At the same time, there are considerable quantities of by-products from tropical fruits processing plants which can be converted into fish feeds[2]. One such by-product is the residue meal from Brazil nut (*Bertholletia excelsa*) processing plants. The Brazil nut is found throughout the Amazon rainforest in Brazil, Peru, Colombia, Venezuela, Ecuador, and Bolivia[3]. It is high in essential fatty acids[3], amino acids[4,5], zinc, vitamin A and E, and selenium[6], an important antioxidant. The Brazil nut is used to produce oil, soaps and shampoos, and for the cosmetic industries[7]. It therefore carries a high value in international markets and is usually exported to the US and Europe[3]. The nut is processed by breaking the pod and shells of individual nuts before exportation. The discards make up the Brazil nut residue meal.

Another potential alternative feed ingredient in Manaus is leucaena leaf meal. Leucaena (*Leucaena leucocephala*) is a tropical forest legume rich in protein and is used as a protein supplement for ruminants fed on poor quality roughages such as maize stover[8,9]. The foliage is highly digestible (60–70%)[10] and its leaves (dry weight) contain 21% crude protein, 18% crude fibre, 8% ash, 6% fat, and 46% total digestible nutrients[11].

The tambaqui (*Colossoma macropomum* Cuvier, 1818) is a popular food fish of the Amazon basin, and is well known in Brazil, Colombia, Peru, and Venezuela[12]. This widespread popularity and the biological characteristics (fast growth, big size, low water quality tolerance, and disease resistance) have made it one of the indigenous species with the most potential for aquaculture development within Latin American tropical fresh waters[13,14]. This fish has been cultured in mono- and polyculture systems in earthen ponds since the 1970s with food varying from agricultural by-products (maize, palm nut cake, peach palm meal) to commercial poultry feeds[15–17]. Including leucaena leaf meal in the diet of tambaqui increases growth[18]. Similarly, including Brazil nut residue meal in the fish diet increases digestibility of the diet and growth[19].

Phytase is known to improve nutrients digestibility and fish growth[20–22]. However, there is little information on the effect of phytase on Brazil nut residue and leucaena leaf meal digestibility and growth of tambaqui. Most of the plant feed ingredients contain bound phosphorus in form of phytate which is not available to fish because they lack the enzyme phytase to digest it. Phytic acid chelates divalent and trivalent cations such as iron, zinc, magnesium, copper, and calcium and this results in a decreased bioavailability of the minerals and ultimately leads to poor animal growth[23]. Phytases are acid phosphatase enzymes of the histidine acid phosphatase family that can liberate inorganic phosphorus from phytate[24] and other bound minerals. Previous studies[25, 26] have shown that phytase works best when the plant feed ingredients are pre-treated. Therefore the present study assessed the effect of phytase on the digestibility of Brazil nut residue and leucaena leaf meal based diets, and on the growth of tambaqui.

MATERIALS AND METHODS

Preparation of plant feed ingredients

The two alternative plant ingredients used in the diets were leucaena (*Leucaena leucocephala*) leaf meal and Brazil nut (*Bertholletia excelsa*) meal. Leaves of leucaena were harvested from trees around the Instituto Nacional de Pesquisas da Amazônia (INPA), dried at 60 °C in an oven for 48 h, and blended into powdery form as the meal. The discards of Brazil nuts were collected from a processing plant. These were the small parts of the nuts chopped off during processing. The discards were blended into granules as the Brazil nut residue meal. The defatted soybean meal was purchased from food vendors in Manaus. The meals were analysed for their proximate composition using described methods[27]. The nutrient compositions of the leucaena leaf meal and Brazil nut residue meal are presented in Table 1.

Table 1 Nutrient compositions of Brazil nut and leucaena leaf meals.

	Brazil nut meal	Leucaena leaf meal
Crude protein (%)	22.7	21.0
Crude fat (%)	48.8	3.5
Ash (%)	5.3	6.1
Crude fibre (%)	4.9	12.0
N-free extract (%)	16.0	48.6
Phosphorus (mg/g)	23.8	3.5
Ca (mg/g)	6.06	17.6
Mg (mg/g)	13.4	4.9
K (mg/g)	19.7	18.0
Na (mg/g)	20	0.2
Mn (µg/g)	50	9.3
Fe (µg/g)	80	255.0
Zn (µg/g)	115	22.6
Cu (µg/g)		49.3
Arginine (g/16 g N)	14.9	2.20
Histidine (g/16 g N)	2.2	0.72
Isoleucine (g/16 g N)	2.8	2.44
Leucine (g/16 g N)	6.75	3.02
Lysine (g/16 g N)	2.94	2.37
Methionine (g/16 g N)	6.52	0.58
Phenylalanine (g/16 g N)	3.74	1.89
Threonine (g/16 g N)	2.86	1.94
Tryptophan (g/16 g N)	2.36	0.31
Valine (g/16 g N)	4.02	2.31

Proximate compositions from the present study. Mineral composition of Brazil nut meal after Ref. 3 and its amino acid composition after Ref. 28. Mineral and amino acid compositions of leucaena leaf meal after Ref. 29.

Diets preparation

Five diets designated as UPI, UPIP, FPI, FPIPT1, FPIPT2 were formulated so as to contain 36% protein, with plant feeds (defatted soybean meal, Brazil nut meal, leucaena leaf meal, and yellow maize) making up 59% of the total protein and about 75% of the total feed. Fish meal made up 40.6% of the total protein and about 24.9% of the total feed ingredients. UPI stands for the negative control diet with unfermented plant ingredients. UPIP denotes the positive control diet with unfermented plant ingredient supplemented with 3 g/kg of phosphorus (NaH_2PO_4). FPI is the diet with fermented plant ingredients. FPIPT1 denotes a diet with fermented plant ingredients supplemented with phytase 4000 U/kg. FPIPT2 is the diet with plant ingredients fermented together with phytase 4000 U/kg. The phytase with activity of 5000 U/g was provided by Animal Health, SSP 300 Cravinhos-São Paulo, Brazil. The process of fermentation of all plant ingredients was as follows: 1.5 l of warm water (50 °C) was mixed with 1 kg of the blended plant ingredients in plastic containers with covers and allowed to stand for 15 h[30, 31] before mixing with the other ingredients (Table 2) prior to pelleting. Gross energy of the diets was calculated using values of 23.0, 38.1, and 17.2 kJ/g for protein, lipid, and carbohydrate[32]. Note that plant feed as used in this text means all the

Table 2 Gross composition of experimental diets.

Ingredients:	Diets				
(g/kg DM)	UPI	UPIP	FPI	FPIPT1	FPIPT2
Fish meal (65% CP)	225	225	225	225	225
Soybean meal (56% CP)	400	400	400	400	400
Brazil nut meal (22.7% CP)	146	146	146	146	146
Leucaena leaf meal (21% CP)	100	100	100	100	100
Yellow maize	37.2	22.2	37.2	36.4	36.4
Soy bean oil	60.0	60.0	60.0	60.0	60.0
Vitamin-mineral premix[a]	25.0	25.0	25.0	25.0	25.0
DL- Methionine	0.40	0.40	0.40	0.40	0.40
Lysin-HCl	1.45	1.45	1.45	1.45	1.45
Chromic oxide	5.00	5.00	5.00	5.00	5.00
Phytase (Ouro Fino)	0.00	0.00	0.00	0.80	0.80
NaH_2PO_4	0.00	15.0	0.00	0.00	0.00
Chemical composition:					
Protein (%)	43.3	44.3	44.1	44.2	43.4
Lipid (%)	17.7	18.9	17.1	17.9	19.7
Crude ash (%)	9.2	10.7	9.7	9.7	9.3
Crude fibre (%)	4.4	4.1	4.7	4.9	3.8
Nitrogen free extract (%)	25.4	22.3	24.4	23.3	23.8
Gross energy (kJ/g)	21.07	21.23	20.9	20.99	21.58
pH	6.02	6.57	6.04	5.95	5.99

[a] 1 kg of mix contained: Vit. A 1 000 000 IU; Vit. D_3 600 000 IU; Vit. E 12 000 IU, Vit. K_3 15 mg; Vit. C 12 500 mg; Vit. B_1 250 mg; Vit. B_2 1750 mg; Vit. B_6 875 mg; Vit. B_{12} 2500 mg; Ca-D-pantothenate 5000 mg, Nicotinic acid 3750 mg; Folic acid 250 mg; Co 24 999 mg; Cu 1999 mg; Fe 11 249 mg; Se ($Na_2SeO_3 \cdot 5\,H_2O$) 75 mg; I (KI) 106 mg; antioxidant 250 mg.

plant materials added to the diets, and these included defatted soybean meal, Brazil nut meal, leucaena leaf meal, and yellow maize, whereas plant ingredients mean the two alternative plant ingredients, Brazil nut meal and leucaena leaf meal.

Feeding experiment

The experiment was conducted at the INPA, Manaus. For the feeding trials, we used 15 cylindrical 200 l fibre glass tanks. There were five treatments and each was in triplicate. Water flow into the tanks was adjusted to 1.5 l/min. Air stones were used to aerate the tanks throughout the feeding period. About 300 juvenile tambaqui were harvested from INPA fish farm and acclimatized for one month before the experiment. Then, 180 fish of a uniform size (70.0 ± 6.3 g) were weighed individually and grouped into 12 fish per tank according to the five treatments. The fish were fed to satiation twice daily between 09:00–11:00 and 16:00–18:00 six days a week for 56 days. The weight of the fish was measured bi-weekly and used to calculate the weight gain, specific growth, and feed conversion ratio. The specific growth rate (SGR) was calculated from

$$\mathrm{SGR} = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{culture period}}.$$

The feed conversion ratio (FCR) was calculated as weight of feed fed divided by the fish weight gain. The condition factor (K) was calculated using $K = \left[(\text{fish weight})/(\text{fish length})^3\right]$.

Digestibility measurement

Each of the diets contained 0.5% chromic oxide (Cr_2O_3) as indigestible marker. Faeces were collected from the settling tube attached to the bottom of the 200 l cylindrical fibre tanks between the 5th and 7th weeks for 9 days. Faeces were collected from each of the 15 tanks representing the five treatments (3 replicates per treatment). The faeces were collected in plastic containers and stored at −20 °C. When enough were collected, they were freeze-dried and kept at −20 °C prior to analysis. Chromic oxide digestion was carried out according to described methods[33]. The apparent digestibility coefficient (ADC) of nutrients was calculated using the described methods[34].

Water quality analysis and measurement

The ammonia and nitrite concentrations in the experimental tank waters were determined weekly, while dissolved oxygen, temperature, and pH were measured daily. The ammonia concentration was determined colourimetrically at the wavelength of 530nm by forming indophenol blue with hypochlorite and salicylate in the presence of sodium nitroferricyanide as catalyst[35]. Nitrite concentration was determined by colourimetric methods employing diazotizing reagents[36]. The absorption was read at 543 nm. Dissolved oxygen was measured using a combined digital oxygen, conductivity, salinity, and temperature YSI 85 meter (YSI Inc), while pH and temperature were measured using a combined digital pH and Temperature YSI 60 meter.

Statistical analysis

Data were subjected to one way analysis of variance using SAS/SAT Institute Software (1998). The Duncan multiple range test was used to separate means among treatments at a 5% significance level[37].

RESULTS

Table 1 presents the nutrient composition of Brazil nut and leucaena leaf meals. The table indicated that Brazil nut meal had higher protein and less crude fibre and nitrogen free extract than the leucaena leaf meal. The Brazil nut meal also had about 14 times more oil than the leucaena leaf meal which explains its use as edible oil and in the soap and cosmetics industries. Phosphorus (growth factor) of the Brazil nut meal is about 7 times more abundant than in leucaena leaf meal. The Brazil nut meal is also richer in essential amino acids than the leucaena leaf meal.

The nutrient composition of the experimental diets (Table 2) showed that the protein, lipid, crude ash, crude fibre, nitrogen free extract, gross energy, and pH of all the diets were similar. Therefore, differences in the digestibility and growth parameters may not be attributed to differences in dietary composition.

The fish has high tolerance to low water quality (Table 3). The dissolved oxygen content of the culture water was almost at critical level, yet fish growth and other physiological functions were not compromised. The pH content of the water was acidic in nature, different from the pH of warm fresh water fish which usually tends towards alkaline. The fish also thrived very well in culture water with high ammonia concentrations.

During the feeding trials, tambaqui only responded to feeding hours after each routine weight measurements. It usually took the fish time to readjust to feeding. Also during heavy rains the fish would not eat. Instead they clustered at a corner very close to one another. But on bright warm days the fish usually fed to satisfaction. And if the heavy down pour was in the morning hours, and eventually turned into a bright warm afternoon, the fish usually had a compensatory feeding rate.

Digestibility of the nutrients (Table 4) indicated that the addition of 3 g/kg phosphate diet significantly improved the apparent digestibility coefficient (ADC) of protein and dry matter in comparison to other treatments. Similarly, addition of phytase into diets marginally improved the ADC of protein, lipid, and carbohydrate in comparison with the treatments without phytase supplements. This increment resulted in a corresponding increase in fish growth which was larger than those fed diets without phytase. Also there were no significant differences in the ADC of carbohydrate from all the treatments.

The growth and nutrient utilization data of tambaqui fed phytase diets are presented in Table 5. The data showed that the fish had a high growth rate and that they nearly doubled the initial weights in 56 days. This growth probably results from the good acceptance and digestibility of the diets containing the alternative feed ingredients. Although there were no significant differences in the mean weight gain, spe-

Table 3 Water quality parameters.

	UPI	UPIP	FPI	FPIPT1	FPIPT2
Dissolved oxygen (mg/l)	4.34 ± 0.56	4.41 ± 0.61	4.23 ± 0.70	4.40 ± 0.60	4.39 ± 0.67
Temperature (°C)	26.8 ± 0.58	26.8 ± 0.56	26.9 ± 0.55	26.9 ± 0.53	26.9 ± 0.53
Conductivity (µS/cm)	28.3 ± 6.76	32.2 ± 14.3	28.3 ± 6.15	27.6 ± 6.44	28.7 ± 9.00
pH	5.87 ± 0.30	5.91 ± 0.32	5.82 ± 0.39	5.82 ± 0.35	5.80 ± 0.37
Ammonia (mg/l)	2.17 ± 0.79	2.14 ± 0.75	1.81 ± 0.65	1.76 ± 0.72	1.82 ± 0.75
Nitrite (mg/l)	0.05 ± 0.08	0.06 ± 0.04	0.04 ± 0.04	0.03 ± 0.02	0.04 ± 0.04

Means of triplicate values were not significantly different.

Table 4 Apparent digestibility coefficient (ADC) of nutrients.

	UPI	UPIP	FPI	FPIPT1	FPIPT2
ADC Dry matter	$65.3^{b} \pm 4.96$	$76.3^{a} \pm 1.04$	$65.9^{b} \pm 4.19$	$67.3^{b} \pm 3.30$	$68.8^{b} \pm 3.30$
ADC Lipid	$80.2^{b} \pm 3.11$	$88.2^{a} \pm 1.27$	$82.2^{b} \pm 3.00$	$85.9^{ab} \pm 0.72$	$85.6^{ab} \pm 0.72$
ADC Protein	$73.2^{b} \pm 4.69$	$86.4^{a} \pm 2.23$	$73.7^{b} \pm 5.50$	$76.8^{b} \pm 1.81$	$79.4^{b} \pm 2.80$
ADC Carbohydrate	$31.0^{a} \pm 9.63$	$40.8^{a} \pm 5.41$	$38.5^{a} \pm 3.89$	$39.8^{a} \pm 14.0$	$40.5^{a} \pm 8.41$

^{a,b} Means of triplicate values with the same superscripts were not significantly different.

Table 5 Growth and nutrient utilization of tambaqui fed phytase diets.

	UPI	UPIP	FPI	FPIPT1	FPIPT2
Final weight (g)	125.0 ± 13.9	136.0 ± 0.78	130.3 ± 14.1	131.2 ± 18.9	139.6 ± 15.5
Initial weight (g)	69.8 ± 0.63	70.9 ± 0.83	70.6 ± 0.71	70.1 ± 1.34	70.6 ± 0.57
Weight gain (g)	55.2 ± 13.4	65.1 ± 0.25	59.7 ± 13.5	60.1 ± 17.6	69.2 ± 15.1
100 SGR (d^{-1})	1.05 ± 0.19	1.19 ± 0.01	1.11 ± 0.18	1.12 ± 0.23	1.24 ± 0.19
FCR	1.94 ± 0.27	1.78 ± 0.27	1.90 ± 0.26	1.88 ± 0.46	1.70 ± 0.05
100 K (g/cm^3)	2.20 ± 0.20	2.17 ± 0.15	2.21 ± 0.11	2.12 ± 0.15	2.15 ± 0.07

Means of triplicate values were not significantly different.

Table 6 Proximate composition of the fish (whole body) carcass after experiment.

	UPI	UPIP	FPI	FPIPT1	FPIPT2
Protein (%)	$56.0^a \pm 0.55$	$56.0^a \pm 1.82$	$58.3^a \pm 1.19$	$58.6^a \pm 2.06$	$58.8^a \pm 2.06$
Lipid (%)	$28.2^a \pm 0.96$	$28.1^a \pm 1.89$	$25.7^a \pm 2.34$	$24.5^a \pm 2.71$	$26.7^a \pm 2.71$
Ash (%)	$10.3^b \pm 0.76$	$11.5^{ab} \pm 1.42$	$11.4^{ab} \pm 0.65$	$12.0^a \pm 0.76$	$12.7^a \pm 0.76$

[a,b] Means of triplicate values with the same superscripts were not significantly different.

cific growth rate (SGR), feed conversion ratio (FCR), or condition factor (K) of the fish during various treatments, the SGR and FCR of fish fed diets with phytase were better than the values from fish fed diet without phytase (Table 5). In addition, weight gain, SGR and FCR of fish fed a diet with plant ingredients fermented together with phytase (4000 U/kg) were better than the values from fish fed a diet supplemented with phosphorus (3 g/kg). The growth performance of fish fed a diet with fermented plant ingredients was higher than that of the fish fed the negative control diet with unfermented plant ingredients. This showed a better conversion of the diet with fermented plant ingredients into flesh (Table 5). Fish fed the negative control diet also had the highest FCR indicating that other diets were better converted into flesh. This may explain the poorest performance of the fish in that treatment.

There were no significant differences in protein concentrations of the fish whole body (Table 6). However, fish fed diet containing phytase had slightly more body protein than others. The lipid content in all the fish showed no significant differences, but fish fed diets without phytase had the highest lipid concentration. Similarly, fish ash contents were statistically the same in the UPI, UPIP, and FPI treatments. However, ash content in fish in treatments supplemented with phytase was significantly higher than that in fish fed a diet without phytase, or unfermented plant ingredients. There was a tendency for larger ash concentration in all fish fed diets with phytase than in fish fed diets without phytase.

DISCUSSION

The use of phytase in the diets enhanced the digestibility of Brazil nut and leucaena leaf meal. The nutrient composition of Brazil nut meal and leucaena leaf meal revealed that they can be used as protein sources in fish feeds as recommended by the American National Research Council[38]. The high phosphorus content of the Brazil nut meal allows it to be classified as a growth promoter and enhancer of mineralization. Similarly, the high essential amino acid profile of Brazil nut meal, which compares well with that of soybean meal, makes it a good plant protein source in animal feed. The protein contents of the leucaena leaf meal and Brazil nut meal obtained from our study support the values previously reported[18,19].

The high ADC of protein and lipid recorded from the present study is evidence of the acceptability of the alternative feed ingredients. This confirms previous observations that in the wild, tambaqui usually feeds on plant seeds and fruits in rainy seasons and wild rice during the dry season[39,40]. The high digestibility also agrees with reports of high digestibility by tambaqui fed diets containing leucaena leaf meal and Brazil nut meal[18,19]. The digestibility was further enhanced by the addition of phytase into the diets. The inclusion of phytase in diets also enhanced the digestibility of nutrients by common carp (*Cyprinus carpio*)[20,22,41] and rohu (*Labeo rohita*)[42]. Supplementary exogenous enzymes in tambaqui diets increased the apparent digestibility of nutrients and crude energy[43].

Unlike cyprinids and tilapias which have a quick response to feed after routine weight measurement operation, tambaqui does not quickly respond to feed after weight measurements. The situation is the same during heavy rain. The fish does not feed satisfactorily. During equipment failures in the recirculation systems, feeding may also be delayed. Therefore, the question arises of how does this delayed feeding affect feed intake and fish growth in feeding experiments. Observations from the present study clearly indicate compensatory feeding regime and growth. This observation is in line with the report that tambaqui juveniles display compensatory growth when fed again after a period of deprivation[44]. Similarly, compensatory growth occurred in African catfish with feed restriction for 28 days and another 28 days feeding to satiation[45]. In the latter study there were no significant differences in body composition, organ indices, eviscerated carcass composition, viscera lipid and liver lipid of the fish subjected to feed restrictions. In a related study, nutritional restriction resulted in compensatory responses, including hyperphagia, rapid weight increase, and repletion of the energy reserves[46]. Compensatory growth has also been established for salmonids, cyprinids, and pleuronectids[47–50].

The fast growth rate of the fish recorded from the present study is evidence of acceptance and high digestibility of the alternative feed ingredients, and influence of phytase which further enhanced the growth of the fish. Others have also described the fish as a fast grower[13, 14, 18, 19]. The fish can attain a weight of 30 kg in the wild[51]. Common carp fed diets fortified with phytase also showed an increment in growth[20, 22, 41]. The phytase acted to release bound phosphorus and minerals, thereby making extra nutrients bio-available for improvement in fish growth. Supplementation of phytase into diets also improved the growth performance of rohu[42]. The effect of the combination of two plant protein sources in enhancing fish growth seems to be better than the use of a single plant protein source, as the nutrients of the combined protein sources complement each other[52]. This assertion agrees with the observation made here. A mean weight gain of about 33 g was obtained from a trial involving feeding tambaqui with leucaena leaf meal as the only alternative ingredient[18]. In addition, similar mean weight gain was also obtained from another trial involving feeding the fish with Brazil nut meal as the only alternative ingredient[19]. But from the present study, when we combined the leucaena leaf meal and Brazil nut meal, the mean weight gain of the fish reached 55–59 g, and with inclusion of phytase in the diets, it increased further to 60–69.2 g.

Observations on the high tolerance of the fish to low water quality is in line with previous reports[13, 14, 18]. The dissolved oxygen could be at critical levels[53, 54] without affecting the performance of the fish, perhaps because the fish has a special adaptation to low oxygen concentrations[55]. Oxygen concentrations in tambaqui aquaria and its natural habitat are 4.1–8.0 and 2.4–6.0 mg/l, respectively[56]. Tambaqui has shown a high degree of adaptation to adverse factor such as oxygen depletion[57]. Ammonia concentration was higher than the recommended value for warm fresh water fish culture[58]. Also the pH levels indicated high preference of the fish for acidic media, in agreement with results obtained from tambaqui culture tanks[44]. The low water quality requirement of the fish could explain its wide distribution in Latin and South America[13] and in the Amazon basin and forest Amazon countries[12].

CONCLUSIONS

Brazil nut and leucaena leaf meal proved to be good sources of protein for tambaqui. The study showed that the nutrients in the two alternative feed ingredients complemented each other, resulting in better fish growth than when either of the two plants was used individually. The only problem with the use of leucaena leaf meal was fouling of the experimental water with leaf residues. Therefore, further studies are necessary to determine the most effective processing method for leucaena leaf meal to reduce the incidence of the meal fouling the culture water.

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