JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 40, No. 1
February, 2009

Effects of Carbohydrate-Rich Alternative Feedstuffs on Growth, Survival, Body Composition, Hematology, and Nonspecific Immune Response of Black Pacu, *Colossoma macropomum*, and Red Pacu, *Piaractus brachypomus*

REBECCA LOCHMANN[1] AND RUGUANG CHEN

Department of Aquaculture and Fisheries, University of Arkansas at Pine Bluff, Pine Bluff, Arkansas 70601 USA

FRED W. CHU-KOO

Programa de Ecosistemas Acuáticos, Instituto de Investigaciones de la Amazonía Peruana – IIAP, PO Box 784, Iquitos, Perú

WILLIAM N. CAMARGO AND CHRISTOPHER C. KOHLER

Fisheries and Illinois Aquaculture Center, Southern Illinois University, Carbondale, Illinois 62901 USA

CRAIG KASPER

Department of Biology, Hillsborough Community College, Tampa, Florida 33619 USA

Abstract

To facilitate economical culture of black pacu, *Colossoma macropomum*, and red pacu, *Piaractus brachypomus*, in the Amazon region of South America, we assessed locally available alternative energy sources for practical diets. We tested the effects of control diets (containing wheat products) versus diets with different Amazonian feedstuffs (yucca, *Manihot sculenta*, plantain, *Musa paradisiaca*, or pijuayo, *Bactris gasipaes*) on the performance of the pacus in three feeding trials. Black pacu (22.5 ± 0.03 g; Trial 1) or red pacu (2.56 ± 0.01 g; Trial 2) were fed diets containing 30% wheat bran (control) or cooked or uncooked yucca, plantain, or pijuayo for 12 wk. In Trial 3, larger black pacu (86.9 ± 6.4 g) were grown to market size in 24 wk on similar diets. Weight gain, feed conversion, survival, alternative complement activity, and lysozyme were similar among diets. Hepatosomatic index, liver glycogen, and dry matter were affected by diet in Trials 1 and 2, but effects were not consistent among trials. In Trial 3, protein efficiency ratio was lower in fish fed the diet containing wheat middlings. However, relative to wheat bran or wheat middlings, all feedstuffs tested were effective energy sources for juvenile black pacu and red pacu.

Black pacu, *Colossoma macropomum*, and red pacu, *Piaractus brachypomus*, are high-value species cultured for human consumption in Bolivia, Brazil, Colombia, Ecuador, Peru, and Venezuela. The black pacu has also been introduced to Costa Rica, Cuba, Mexico, and Panama (Campos-Baca and Kohler 2005). Natural supplies of these fish cannot meet market demand, and aquaculture production is intensifying. Practical diet formulations for these species are evolving. Their natural diets are rich in plant products (Kubitzki and Ziburski 1993; Araujo-Lima and Goulding 1997), and many Amazonian plant feedstuffs may be suitable for inclusion in practical diets for characids. Some native ingredients have been used in feeds produced on farm, but there is little research on their efficacy as feedstuffs compared to more typical products such as wheat. Although wheat is one of the chief sources of energy in formulated diets for characid fish, it is not traditionally cultured in the Amazonian region and must be imported at a premium price. In contrast, yucca, *Manihot sculenta*, pijuayo, *Bactris gasipaes*, and plantain, *Musa paradisiaca*, are often grown

[1] Corresponding author.

in proximity to Amazonian culture ponds as they are an important part of the human diet in the region. Production of yucca, pijuayo, and plantain is sometimes so high that prices fall to levels more suitable for animal feedstuffs than for direct marketing as human food.

Based on growth in previous trials under different conditions, these feedstuffs have good potential to supply nutrients and energy in compound diets for characids (Alcántara and Colace 2001; Campos-Baca and Kohler 2005). However, more response variables must be measured under controlled conditions to ensure that overall performance of characids fed compound diets containing these products is equal to or better than diets with traditional ingredients.

We conducted three separate feeding trials to determine the effects of a control diet (with wheat bran or middlings) versus diets with one of the three native Amazonian plant feedstuffs on the performance of juvenile *C. macropomum* (Trial 1) and *P. brachypomus* (Trial 2). Wheat and the test ingredients were also evaluated in larger *C. macropomum* grown to market size (Trial 3). Diets were formulated to contain similar amounts of total protein and energy and differed only in the test feedstuff (yucca, pijuayo, or plantain at 30% of the diet) being evaluated as an energy source. Because cooking increases the available energy in starch (Lovell 1989), we also tested cooked and uncooked versions of each of the native feedstuffs in Trials 1 and 2. Growth performance, feed utilization, liver composition, hematology, and nonspecific immune parameters (lysozyme and alternative complement activity [ACH50]) were measured to assess diet effects.

Materials and Methods

Experimental Diets

Three separate feeding trials were conducted with characids in recirculating systems. Trials 1 and 2 were conducted at the University of Arkansas at Pine Bluff (UAPB) using juvenile black and red pacu, respectively, maintained under similar conditions. Trial 3 was conducted at the Southern Illinois University at Carbondale (SIUC), where black pacu were grown to market size. Diet formulations were identical in Trials 1 and 2, while slightly different formulations were used at SIUC.

The composition of the seven test ingredients used in Trials 1 and 2 is shown in Table 1. The diets supplemented with uncooked or cooked pijuayo, plantain, and yucca were formulated to contain around 30% crude protein on a dry basis. The control diet containing wheat bran was higher in protein (33.5%) because of the higher crude protein content of wheat bran (16.1%). Soybean meal and menhaden fish meal were the main protein sources, and all diets met the estimated essential amino acid requirements of this species (Van der Meer and Verdegem 1996). The alternative ingredients were evaluated primarily as energy sources relative to wheat bran. All diets contained soybean oil as the primary lipid source, with traces of oil from rice bran and fish meal.

The diets supplemented with wheat bran and uncooked or cooked pijuayo contained 14–17% total lipid on a dry basis, and the diets with uncooked or cooked plantain and yucca contained about 12% total lipid because of the lower lipid content of these ingredients. Uncooked or cooked plantain and yucca contained 90–92% carbohydrate — over 30% more than wheat bran and 15% higher than uncooked or cooked pijuayo. Wheat bran was made from soft wheat dry milled without heat to obtain the bran (Caldwell Milling Company, Inc., Rose Bud, AR, USA). Raw plantain and yucca were purchased locally and cut into thin slices after peeling. Uncooked plantain and yucca were dried in an oven at 60 C. Cooked plantain and yucca were prepared in a pressure cooker (equivalent to 130 C in a conventional oven for 45 min) and then dried like the uncooked ingredients. The pijuayo was purchased in Peru, and the edible pulp and rind from the fruit were processed similarly to yucca and plantain before shipment to UAPB.

All ingredients were finely ground (1–2 mm) in a Wiley mill prior to inclusion in the diets. Diet ingredients were fully mixed in a V-mixer, and 600 mL of distilled water was added per kilogram of dry diet in a Berkel® Mixer (Berkel Company, South Bend, IN, USA) to achieve

TABLE 1. *Composition of diets (dry basis) containing cooked or uncooked Amazonian plant feedstuffs in feeding trials for juvenile black pacu,* Colossoma macropomum *(Trial 1), and red pacu,* Piaractus brachypomus *(Trial 2).*

Ingredient	Control (wheat bran)	Uncooked yucca	Cooked yucca	Uncooked pijuayo	Cooked pijuayo	Uncooked plantain	Cooked plantain
Menhaden fish meal	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Soybean meal	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Rice bran	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Corn meal	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Wheat bran	30.0	0.0	0.0	0.0	0.0	0.0	0.0
Soybean oil	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin premix[1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral premix[1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Test ingredient[2]	0.0	30.0	30.0	30.0	30.0	30.0	30.0
Analyzed proximate composition[3]							
Dry matter (%)	89.0	91.4	91.5	90.1	90.0	89.7	90.4
Crude protein (%)	33.9	30.1	30.3	31.4	31.1	30.2	30.6
Total lipid (%)	15.7	12.0	11.9	14.4	14.6	12.1	12.1
Ash (%)	8.9	7.4	7.5	7.2	7.2	7.8	7.6
Fiber (%)	6.3	3.7	3.3	4.8	4.7	3.8	3.6
NFE	35.3	46.8	47.1	42.2	42.5	46.1	46.1
Energy (kJ/g)	17.5	17.4	17.4	17.7	17.8	17.3	17.4

NFE = nitrogen-free extract.

[1] Same as Moon and Gatlin (1991).

[2] The test ingredient was included at 30% of the diet on a dry basis. Wheat bran was used in the control diet, and uncooked or cooked pijuayo, plantain, and yucca were test ingredients in Trials 1 and 2.

[3] Proximate composition data are shown on a dry basis. NFE = 100 − (protein + lipid + ash + fiber). Estimated energy content of the diets was based on values of 16.7, 16.7, and 37.7 kJ/g for carbohydrate (NFE), protein, and lipid, respectively (Serrano et al. 1992).

a doughy consistency. A meat grinder fitted with a 3-mm die was used to produce stable pellets, which were fan dried and stored at −18 C until needed. Ingredients and diets for Trials 1 and 2 were analyzed at UAPB using the Kjeldahl method for crude protein, the Folch extraction method for total lipid (Folch et al. 1957), and an Ankom 2000 Fiber Analyzer (Ankom Technologies, Inc., Macedon, NY, USA) for crude fiber. The composition of diets used in Trial 3 is shown in Table 2. Dehulled solvent-extracted soybean meal (48% crude protein) and uncooked number 2 corn were produced by Mountaire Feeds, Incorporated (North Little Rock, AR, USA). Rice bran was obtained from the RiceX Company (El Dorado Hills, CA, USA). Menhaden fish meal was purchased from Omega Protein Corporation (Houston, TX, USA). The wheat middlings were purchased from Cargill, Incorporated (Minneapolis, MN, USA). Yucca roots and plantain fruits were obtained from a local grocery market, and pijuayo meal was shipped from Iquitos, Peru. Yucca, plantain, and pijuayo were dried as described for Trials 1 and 2, but the test ingredients were not cooked in Trial 3. Diets were prepared in a mixer by slowly adding micronutrients (vitamin and minerals premixes) to the macroingredients to ensure a homogenous mixture. About 400–450 mL of water was added per kilogram of diet to achieve a consistency that would produce stable pellets. A meat grinder fitted with a 3- or 7-mm die was used to produce the pellets, which were fan dried for 24 h and stored at −18 C until use. The larger pellet was used once fish reached a size where they could readily ingest it. Moisture and dry matter of the diets were obtained by drying triplicate samples weighing 250 mg at 135 C during 3 h in an Isotemp® Oven (Fisher Scientific, Ottawa, Canada). Samples were weighed in an AB54-S analytical scale (Mettler Toledo, Greifensee, Switzerland). Crude protein was determined using a 2020 digestion unit (Foss Tecator, Höganas, Sweden) and a 2200 Kjeltec Auto-Distillation Unit (Foss Tecator, Höganas, Sweden) according to

TABLE 2. *Composition of the diets (%) used to feed black pacu juveniles for 24 wk (Trial 3).*

Ingredient	Control	Yucca	Pijuayo	Plantain
Menhaden fish meal	9.0	9.0	9.0	9.0
Soybean meal	35.0	35.0	35.0	35.0
Corn grains	9.8	9.8	9.8	9.8
Wheat middlings	30.0	0.0	0.0	0.0
Rice bran	12.0	12.0	12.0	12.0
Soybean oil	3.0	3.0	3.0	3.0
Trout vitamin premix[1]	0.5	0.5	0.5	0.5
Trout mineral premix[1]	0.5	0.5	0.5	0.5
Vitamin C (Stay C 35%)	0.2	0.2	0.2	0.2
Test ingredient[2]	0.0	30.0	30.0	30.0
Analyzed proximate composition[3]				
Dry matter (%)	89.7	89.4	89.4	87.6
Crude protein (%)	31.8	27.5	27.0	28.1
Total lipid (%)	6.5	5.3	5.1	9.7
Ash (%)	7.9	6.4	6.9	6.8
Fiber (%)	6.8	5.4	5.5	4.7
Nitrogen-free extract	47.0	55.4	55.5	50.7
Energy (kJ/g)	10.7	12.3	12.3	12.3

[1] Same as Fernandes et al. (2004).

[2] The test ingredient was included at 30% of the diet on a dry basis. Wheat middlings were used in the control diet, and uncooked pijuayo, plantain, and yucca were test ingredients in Trial 3.

[3] Proximate composition data are shown on a dry basis. Nitrogen-free extract = 100 − (protein + lipid + ash + fiber). Gross energy was determined by bomb calorimetry.

manufacturer's instructions. Total lipid was determined by the lipid extraction method of Folch et al. (1957). Ash was obtained by incineration of samples at 550 C for 3 h in an Isotemp® programmable Muffle Furnace Model 650-58 (Fisher Scientific). Energy content was obtained using a Parr 1261 bomb calorimeter (Parr Instrument Co., Moline, IL, USA).

Culture System and Experimental Design

The black pacu fingerlings for Trial 1 were obtained from a producer in Iquitos, Peru, and shipped to the Aquaculture Research Center at UAPB. The fish were fed a commercial diet (Silvercup® bass feed, Murray, UT, USA) for 4 wk prior to stocking, when they had no clinical signs of diseases. Eight uniform fish were randomly selected and stocked into each of four 110-L aquaria per diet. Individual initial weight of fish was 22.5 ± 0.03 g (mean ± SEM). Aquaria were configured in a recirculating system using dechlorinated municipal water with hardness adjusted to 80–120 mg/L with calcium chloride. Water quality was monitored weekly and maintained by a biofilter, continuous aeration, and a flow rate of 1 L/min per aquaria. Water temperature was maintained at 28 ± 1 C during the feeding trial. Fish were fed to apparent satiation, which was approximately 4% of their body weight daily for the first 6 wk, followed by 3% of body weight after 6 wk. Daily rations were divided into two equal feedings (0800 and 1600 h). Fish from each tank were weighed collectively every 2 wk after stocking to monitor growth and adjust feed rations.

Final average individual weight gain and feed conversion ratio (FCR) (dry feed weight/weight gain) were calculated at 12 wk. Two fish per tank were euthanized with an overdose of tricaine methanesulfonate (MS-222), and their livers were removed, pooled, homogenized, and frozen at −70 C for glycogen analysis. Remaining fish were returned to their tanks and maintained on their respective diets for another 2 wk prior to health assays (ACH50 and hematology) to minimize the influence of handling stress on the results. In each trial, three fish per tank were anesthetized with MS-222 and bled for health assays.

Red pacu fingerlings for Trial 2 were obtained from a producer in Florida, USA, and acclimated to a recirculating system with 144-L tanks. The tanks operated as a recirculation system with a 565-L sump and large bubble wash bead filter. Each tank was supplied with an airstone to maintain supplemental aeration. Fifteen fish with individual initial weight of 2.56 ± 0.01 g (mean ± SEM) were stocked into each of three tanks per diet. An additional health assay (lysozyme activity) was successfully conducted in Trial 2 only. Other experimental conditions for Trial 2 were the same as in Trial 1 except where noted.

Hatchery-produced black pacu juveniles with individual initial weight of 86.9 ± 6.4 g (mean ± SEM) were used for Trial 3 at SIUC. The trial was conducted in a 28,000-L recirculating system with flow rate regulated to obtain a complete water exchange daily for each tank. Uniform-sized fish were selected from a large population, weighed, and randomly distributed in experimental circular tanks at a rate of 28 fish in three replicate tanks per diet. Fish were acclimated to the experimental system and their respective diets for 7 d prior to initiating the experiment. Temperature and dissolved oxygen were measured twice per week before feeding using a YSI® Model 52 Dissolved Oxygen Meter (YSI Co., Inc., Yellow Springs, OH, USA). Total ammonia nitrogen, nitrite, and alkalinity levels were measured in intervals of twice per week using a LaMotte® Water Lab Kit, while pH was measured using a pH/mV/ORP/Temperature Meter (Cole-Parmer Instrument Co., Vernon Mills, IL, USA). Fish were fed daily 3% of their wet body biomass divided in two daily rations. Feed rations for each replicate were adjusted every 2 wk from estimates of total fish biomass obtained by subsampling each tank. At each subsampling, fish weight was recorded to the nearest 0.1 g.

At the end of the 24-wk trial, all fish in each tank were counted and weighed to assess growth performance. After weighing, fish were maintained in their tanks and fed the experimental diets for another 2 wk prior to bleeding for health assays as in Trials 1 and 2.

Liver Glycogen Analysis – Trials 1 and 2

One hundred milligrams of each pooled liver sample was weighed and analyzed using the method of Good et al. (1933). Samples were digested in hot concentrated (30%) potassium hydroxide solution, followed by precipitation of the glycogen with 95% ethanol, hydrolysis of the glycogen with 0.6 N hydrochloric acid, and determination of the glucose in the hydrolysate as reducing sugar. A blue color was formed by the reaction of cuprous oxide with arsenomolybdic acid after boiling a solution of copper sulfate with glucose. The absorption of the solution was measured with a Kinetic Microplate Reader (Molecular Devices, Sunnyvale, CA, USA) at 505 nm. Glycogen concentration was calculated by multiplying the glucose concentration by a conversion factor of 0.93.

Blood Sample Collection and Analysis – Trials 1–3

After 12 wk in each trial, fish were not fed for 24 h and then anesthetized with MS-222. Blood from three to four individual fish per tank was collected from the caudal vein using a heparinized 3-mL syringe with an 18-gauge needle and then transferred into a 1.5-mL microcentrifuge tube. Duplicate 8 μL samples of fresh blood were used for hemoglobin analysis (hemoglobin cyanide method; Houston 1990). Mean corpuscular hemoglobin concentration (MCHC) was calculated on an individual basis by the formula: MCHC = hemoglobin concentration/hematocrit fraction. Duplicate blood samples collected in heparinized microhematocrit tubes were centrifuged at 3500 *g* to determine hematocrit. Twenty-five microliters of serum was used to determine ACH50, and 50 μL of serum was used to determine lysozyme activity (Trial 2 only).

Hemolytic Assay (Alternative Complement Pathway)

The hemolytic activity driven by the alternative complement pathway was measured using washed rabbit red blood cells as target cells in the presence of ethylene glycol tetraacetic acid and Mg^{2+} as described by Tort et al. (1996).

The ACH50 was determined by measuring the optical density (OD) of the hemolytic supernatant after adjustment of the OD of the diluted fish serum at 414 nm by a Kinetic Microplate Reader (Molecular Devices) as described by Chen et al. (2003).

Lysozyme Analysis

Lysozyme was analyzed using a modification of the method of Hutchinson and Manning (1996). Fifty microliters of fish plasma was added into each well in a 96-well plate. One hundred and fifty microliters of suspended *Micrococcus lysodeikticus* (0.4 mg/mL in phosphate buffer) was mixed with the fish plasma in each well, and the reduction in the absorbance reading at 450 nm was taken every 10 sec for 5 min using the basic kinetic protocol of the microplate reader with SoftMax Pro® 4.3 (Molecular Devices). One unit of lysozyme activity was defined as a reduction in absorbance of 0.001/min (1 milli-OD/min).

Statistical Analysis

Each of the seven dietary treatments in trials at UAPB was randomly assigned to four (Trial 1) or three (Trial 2) aquaria in a completely randomized design. Data were analyzed by one-way ANOVA in a StatView® program (SAS Institute, Inc., Cary, NC, USA). When significant differences among treatment means were found ($P < 0.05$), treatment means were compared using Fisher's LSD test. All percentage data were arcsine transformed prior to analysis.

Data from Trial 3 at SIUC were analyzed by JMP® statistical software (SAS Institute, Inc.) using one-way ANOVA and expressed as the mean ± standard error (SEM). Multiple comparisons of means were performed using Tukey's HSD test. The level of significance applied was $P < 0.05$.

Results

Growth, Feed Conversion, and Liver Composition

Trial 1. There were no differences in weight gain or feed conversion efficiency of black pacu fed diets with different feedstuffs (Table 3), indicating that all the test ingredients were suitable energy sources relative to wheat bran. Hepatosomatic index (HSI) of fish fed the diet with uncooked plantain was higher than that of fish fed all other diets (Table 3). Fish fed the diets

TABLE 3. *Average individual weight gain, FCR, HSI, and liver glycogen and dry matter of black pacu fed diets containing wheat bran (control) or uncooked or cooked yucca, pijuayo, or plantain for 12 wk (Trial 1).*[1]

				Liver	
Test ingredient	Weight gain[2] (g)	FCR[3]	HSI[4] (%)	Glycogen[5] (%)	Dry matter (%)
Wheat bran	88.0 ± 4.5[a]	1.86 ± 0.1[a]	1.22 ± 0.08[a]	3.09 ± 0.7[d]	20.2 ± 0.7[b]
Uncooked yucca	91.9 ± 7.1[a]	1.77 ± 0.1[a]	1.27 ± 0.08[a]	3.43 ± 0.8[d]	22.5 ± 0.1[a]
Cooked yucca	113.6 ± 9.0[a]	1.56 ± 0.1[a]	1.18 ± 0.05[a]	1.38 ± 0.6[e]	20.5 ± 0.6[b]
Uncooked pijuayo	89.7 ± 4.0[a]	1.80 ± 0.1[a]	1.38 ± 0.13[a]	4.64 ± 1.5[bc]	22.6 ± 0.9[a]
Cooked pijuayo	93.5 ± 3.2[a]	1.75 ± 0.04[a]	1.31 ± 0.06[a]	4.94 ± 1.2[b]	23.5 ± 0.7[a]
Uncooked plantain	100.6 ± 9.1[a]	1.66 ± 0.1[a]	1.83 ± 0.10[b]	6.20 ± 0.2[a]	3.4 ± 0.4[a]
Cooked plantain	94.3 ± 10.4[a]	1.76 ± 0.2[a]	1.24 ± 0.04[a]	3.67 ± 0.6[cd]	22.2 ± 0.2[a]
ANOVA (*P* value)	0.24	0.43	0.0003	0.002	0.003

FCR = feed conversion ratio; HSI = hepatosomatic index.

[1] Values are means ± SEM from four replicate aquaria initially containing eight fish each before 6 wk of feeding and then five fish each after 6 wk of feeding. Means in each column with different superscript letter are significantly different ($P < 0.05$).

[2] Initial average individual weight of fish in all treatments was 22.5 ± 0.1 g. Weight gain = final average individual weight (g) – initial average individual weight (g).

[3] FCR = feed consumed (g) dry basis/wet weight gain (g).

[4] HSI = wet liver weight (g)/individual fish weight (g) × 100.

[5] Glycogen is shown on a wet basis. Values are means ± SEM of four pooled samples, each consisting of livers from two fish. Each pooled sample was analyzed in duplicate.

with cooked or uncooked yucca or wheat bran had lower liver glycogen than those fed diets with cooked or uncooked pijuayo or uncooked plantain. Liver dry matter was lowest in fish fed diets with wheat bran or cooked yucca. Survival was 100% in all treatments. Water quality parameters were suitable for red pacu (Fernandes et al. 2001).

Trial 2. Weight gain and FCR also did not differ by diet for red pacu (Table 4). Fish fed the diet with cooked yucca had the highest HSI (Table 4), while fish fed diets with wheat bran or uncooked plantain had the lowest. Liver glycogen was higher in fish fed the diet with cooked yucca than in those fed diets with wheat bran or uncooked plantain or yucca (Table 4). Fish fed the diet with wheat bran had lower liver dry matter than those fed other diets except cooked or uncooked yucca (Table 4.) Fish fed the diets with cooked or uncooked pijuayo had higher liver dry matter than those fed cooked or uncooked yucca (Table 4). Three fish disappeared from one tank during the study, but this incident was not related to treatment, and survival was 100% in all other tanks. Water quality parameters were suitable for black pacu (Castagnolli 2000).

Trial 3. Final mean weight gain for black pacu fed the control, yucca, pijuayo, or plantain diets was 458.2, 476.2, 437.8, and 465.8 g, respectively, and did not differ among treatments (Table 5) Final weight gain (%), specific growth rate, and FCR also were similar for fish fed different diets (Table 5). The protein efficiency ratio (PER) value for fish fed the control diet was lower than for those fed all the test diets (Table 5). Survival was 100%. Mean values for water quality parameters were: DO = 5.59 ± 0.47 mg/L, temperature = 27.3 ± 1.1 C, pH = 6.8 ± 0.1, ammonia = 0.26 ± 0.1 mg/L, nitrite = 0.05 ± 0.01 mg/L, and alkalinity 50.4 ± 9.8 mg/L. All parameters were suitable for black pacu (Carneiro 1983; Torloni et al. 1984; Castagnolli 2000; Fernandes et al. 2001).

Hematology, ACH50, and Lysozyme

In Trial 1, there were no differences in hematocrit, hemoglobin, MCHC, or ACH50 among black pacu fed different diets (Table 6). Lysozyme activity was not detected in black pacu. In Trial 2, red pacu fed the diet with wheat bran had lower MCHC than those fed other diets (Table 7). The MCHC was also lower in fish fed uncooked plantain than those fed other diets except for uncooked pijuayo. There were no

TABLE 4. *Average individual weight gain, FCR, HSI, and liver glycogen and dry matter of red pacu fed diets containing wheat bran (control) or uncooked or cooked yucca, pijuayo, or plantain for 12 wk (Trial 2).*[1]

				Liver	
Test ingredient	Weight gain[2] (g)	FCR[3]	HSI[4] (%)	Glycogen[5] (%)	Dry matter (%)
Wheat bran	88.5 ± 8.0[a]	1.90 ± 0.06[a]	1.88 ± 0.08[d]	6.46 ± 0.46[d]	27.7 ± 0.56[c]
Uncooked yucca	110.7 ± 7.2[a]	1.66 ± 0.03[a]	2.11 ± 0.10[c]	8.50 ± 0.40[bc]	28.6 ± 0.40[bc]
Cooked yucca	106.4 ± 1.4[a]	1.66 ± 0.04[a]	2.70 ± 0.06[a]	9.85 ± 0.45[a]	28.7 ± 0.35[bc]
Uncooked pijuayo	92.6 ± 7.3[a]	1.80 ± 0.07[a]	2.48 ± 0.08[b]	9.35 ± 0.40[ab]	29.9 ± 0.24[a]
Cooked pijuayo	95.0 ± 7.3[a]	1.82 ± 0.15[a]	2.32 ± 0.11[b]	8.50 ± 0.41[abc]	30.0 ± 0.44[a]
Uncooked plantain	91.1 ± 8.0[a]	1.73 ± 0.09[a]	1.93 ± 0.07[cd]	8.09 ± 0.40[c]	29.3 ± 0.26[ab]
Cooked plantain	98.9 ± 8.5[a]	1.74 ± 0.08[a]	2.35 ± 0.06[b]	9.66 ± 0.36[ab]	29.3 ± 0.21[ab]
ANOVA (*P* value)	0.31	0.40	<0.0001	<0.0001	0.0004

FCR = feed conversion ratio; HSI = hepatosomatic index.

[1] Values are means ± SEM from three replicate aquaria initially containing 15 fish each. Means in each column with different superscript letters are significantly different ($P < 0.05$).

[2] Initial average individual weight of fish in all treatments was 2.55 ± 0.0 g. Weight gain = final average individual weight (g) – initial average individual weight (g).

[3] FCR = feed consumed (g) dry basis/wet weight gain (g).

[4] HSI = wet liver weight (g)/individual fish weight (g) × 100.

[5] Glycogen is shown on a wet basis. Values are means ± SEM of three pooled samples per treatment analyzed in duplicate. Each pooled sample consisted of livers from three individual fish.

TABLE 5. *Growth performance and feed utilization of black pacu juveniles fed diets containing wheat middlings (control), yucca, pijuayo, or plantain for 24 wk (Trial 3).*[1]

Test ingredient	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Weight gain (%)	SGR[2]	FCR[3]	PER[4]
Wheat middlings	80.6 ± 2.2[a]	538.8 ± 12.4[a]	458.2 ± 11.3a	669.0 ± 16.4[a]	1.02 ± 0.01[a]	1.98 ± 0.04[a]	1.42 ± 0.03[a]
Yucca	82.8 ± 4.0[a]	559.0 ± 23.1[a]	476.2 ± 22.7[a]	678.0 ± 39.4[a]	1.03 ± 0.03[a]	1.86 ± 0.08[a]	1.76 ± 0.07[b]
Pijuayo	89.6 ± 7.2[a]	527.4 ± 15.4[a]	437.8 ± 22.1[a]	599.0 ± 64.1[a]	0.95 ± 0.06[a]	1.84 ± 0.09[a]	1.78 ± 0.02[b]
Plantain	86.9 ± 4.1[a]	552.7 ± 34.8[a]	465.8 ± 36.9[a]	640.6 ± 58.2[a]	0.99 ± 0.05[a]	1.84 ± 0.01[a]	1.83 ± 0.08[b]
ANOVA (*P* value)	0.57	0.77	0.74	0.67	0.62	0.38	0.004

SGR = specific growth rate; FCR = feed conversion ratio; PER = protein efficiency ratio.

[1] Values are means ± SEM of three replicates per treatments. Means in each row followed by different superscript letters are significantly different ($P < 0.05$).

[2] SGR = (ln final body weight – ln initial body weight)/t (d) × 100.

[3] FCR = feed consumed (g) dry basis/wet weight gain (g).

[4] PER = wet weight gain (g)/protein intake (g) dry basis.

differences in hematocrit, hemoglobin, ACH50, or lysozyme activity among treatments (Table 7). In Trial 3, there were no differences in hematocrit, hemoglobin, MCHC, or ACH50 of black pacu fed diets containing wheat middlings, pijuayo, plantain, or yucca (Table 8).

Discussion

Traditionally, wheat inclusion in prepared characid diets ranges from 5 to 35% (Roubach and Saint-Paul 1994; Vásquez-Torres et al. 2002). We substituted 30% yucca, pijuayo, or plantain for wheat middlings or wheat bran in our study and obtained equal or better growth performance of red and black pacu fed the control diets. Daily growth rates obtained in Trial 3 (0.99–1.03%/d) for fish fed the control diet with wheat middlings or diets with yucca, pijuayo, or plantain were similar or slightly better than those obtained by other researchers. In a 42-d trial with *Colossoma juveniles* (~18 g) fed rubber tree, *Hevea* spp., wild rice, *Oryza* spp., or munguba, *Pseudobombax munguba*, seeds, specific daily growth rates of 0.80–1.26 were obtained (Roubach and Saint-Paul 1994). FCRs obtained for *C. macropomum* and *P. brachypomus* (1.56–1.98), and PERs (1.42–1.83) in our study were similar to those of Roubach and Saint-Paul (1994) for *C. macropomum* fed similar compound diets. Mori-Pinedo et al. (1999) also

TABLE 6. *Hematocrit (Hk), hemoglobin concentration (Hb), mean corpuscular hemoglobin concentration (MCHC), and alternative complement activity (ACH50) of black pacu fed diets containing wheat bran (control), uncooked or cooked yucca, pijuayo, or plantain for 12 wk (Trial 1).*[1]

Test ingredient	Hk[2] (%)	Hb[2] (g/dL)	MCHC[3] (g/dL)	ACH50 (units/25 μL)
Wheat bran	33.2 ± 1.3[a]	7.3 ± 0.3[b]	22.1 ± 0.6[a]	23.2 ± 2.2[a]
Uncooked yucca	34.2 ± 1.3[a]	8.1 ± 0.3[a]	23.6 ± 0.5[a]	20.8 ± 2.0[a]
Cooked yucca	35.3 ± 1.1[a]	8.0 ± 0.3[a]	22.6 ± 0.6[a]	24.1 ± 2.2[a]
Uncooked pijuayo	37.1 ± 1.1[a]	8.6 ± 0.3[a]	23.2 ± 0.3[a]	21.9 ± 1.7[a]
Cooked pijuayo	35.0 ± 0.8[a]	8.2 ± 0.3[a]	23.4 ± 0.5[a]	21.6 ± 2.3[a]
Uncooked plantain	35.5 ± 1.3[a]	8.3 ± 0.4[a]	23.3 ± 0.5[a]	21.8 ± 2.3[a]
Cooked plantain	35.9 ± 1.2[a]	8.1 ± 0.1[a]	22.9 ± 0.6[a]	19.2 ± 1.5[a]
ANOVA (*P* value)	0.36	0.064	0.45	0.70

[1] Values are means ± SEM of four replicates per treatment. Three individual fish were sampled per replicate (12 per treatment). Means in each column with different superscript letters are significantly different ($P < 0.05$).

[2] Hematocrit value and hemoglobin content from each fish were analyzed in duplicate. Hematocrit = number of red blood cells as a fraction of whole blood.

[3] MCHC = hemoglobin content (g/dL)/hematocrit.

TABLE 7. *Hematocrit (Hk), hemoglobin concentration (Hb), mean corpuscular hemoglobin concentration (MCHC), serum lysozyme, and alternative complement activity (ACH50) of red pacu fed diets containing wheat bran (control) or uncooked or cooked yucca, pijuayo, or plantain for 12 wk (Trial 2).*[1]

Test ingredient	Hk[2] (%)	Hb[2] (g/dL)	MCHC[3] (g/dL)	Lysozyme[4] (units/50 μL)	ACH50 (units/25 μL)
Wheat bran	39.3 ± 0.9[a]	8.2 ± 0.2[a]	20.8 ± 0.5[c]	31.6 ± 1.7[a]	37.4 ± 2.9[a]
Uncooked yucca	38.9 ± 0.8[a]	9.0 ± 0.2[a]	23.0 ± 0.3[a]	31.5 ± 1.6[a]	38.7 ± 2.7[a]
Cooked yucca	40.0 ± 1.1[a]	9.1 ± 0.3[a]	22.6 ± 0.3[a]	33.1 ± 1.2[a]	39.1 ± 3.3[a]
Uncooked pijuayo	40.5 ± 1.0[a]	9.1 ± 0.2[a]	22.5 ± 0.4[ab]	30.7 ± 1.1[a]	38.4 ± 3.0[a]
Cooked pijuayo	39.2 ± 1.3[a]	9.0 ± 0.3[a]	22.9 ± 0.4[a]	31.7 ± 1.2[a]	37.8 ± 2.4[a]
Uncooked plantain	41.0 ± 0.8[a]	8.9 ± 0.2[a]	21.7 ± 0.5[b]	33.8 ± 1.5[a]	36.7 ± 2.6[a]
Cooked plantain	38.2 ± 0.9[a]	8.8 ± 0.3[a]	23.1 ± 0.4[a]	33.2 ± 1.5[a]	31.9 ± 3.0[a]
ANOVA (*P* value)	0.44	0.13	0.0003	0.69	0.63

[1] Values are means ± SEM of three replicates per treatment. Four individual fish were sampled per replicate. Means in each column with different superscript letters are significantly different ($P \leq 0.05$).

[2] Hematocrit value and hemoglobin content from each fish were analyzed in duplicate.

[3] MCHC = hemoglobin content (g/dL)/hematocrit.

[4] Lysozyme was analyzed in duplicate using 50 μL of serum per fish, and alternative complement was analyzed using 25 μL of serum per fish.

substituted pijuayo for corn in diets for *C. macropomum* fingerlings with no reduction in growth, suggesting that pijuayo has the potential to replace a variety of carbohydrate feedstuffs in balanced diets for characids.

In Trials 1 and 2, we compared cooked and uncooked feedstuffs as energy sources because heating increases the available energy of starch by 10–15% (Lovell 1989). However, there was no indication that raw and cooked versions of the yucca, pijuayo, or plantain differed in available energy for *C. macropomum* because weight gain and glycogen accumulation in liver were not higher in fish fed cooked versions of the feedstuffs. In *P. brachypomus*, however, the cooked yucca and cooked plantain resulted in more glycogen accumulation in the liver, indicating that cooked versions of these ingredients did provide more available energy. It is difficult to determine how much of the differences in responses of black pacu and red pacu were because of species differences versus fish size. The black pacu in Trial 1 were approximately ten times larger than the red pacu initially. Within a species, small fish generally have a more rapid growth rate and a higher energy requirement than large fish (Webster and Lim 2002). Small fish tend to have lower glycolytic capacity than larger ones (Garenc et al. 1998), but glycogen accumulation in the liver is also affected by diet, health, and the energy status of the fish (e.g., Craig et al. 1999; Hemre et al.

TABLE 8. *Hematocrit (Hk), hemoglobin concentration (Hb), mean corpuscular hemoglobin concentration (MCHC), and alternative complement activity (ACH50) of black pacu fed diets containing wheat middlings (control) and uncooked pijuayo, plantain, or yucca for 24 wk (Trial 3).*[1]

Test ingredient	Hk[2] (%)	Hb[2] (g/dL)	MCHC[3] (g/dL)	ACH50 (units/25 μL)
Wheat middlings	33.2 ± 0.7	2.0 ± 0.04	6.1 ± 0.1	48.4 ± 4.7
Yucca	35.2 ± 0.9	2.0 ± 0.03	5.8 ± 0.1	45.0 ± 5.0
Pijuayo	36.9 ± 1.2	2.0 ± 0.06	5.6 ± 0.2	31.6 ± 7.6
Plantain	34.9 ± 1.2	2.0 ± 0.08	5.7 ± 0.1	53.6 ± 5.0
ANOVA (*P* value)	0.10	0.88	0.06	0.06

[1] Values are means ± SEM of four replicates per treatment. Three or four individual fish were sampled per replicate (8–9 per treatment). Means were not significantly different ($P > 0.05$).

[2] Hematocrit value and hemoglobin content from each fish were analyzed in duplicate. Hematocrit = number of red blood cells as a fraction of whole blood.

[3] MCHC = hemoglobin content (g/dL)/hematocrit.

2000). Red pacu in Trial 2 consumed feed voraciously, and it appears that the fish had excess dietary energy for glycogen synthesis after their metabolic needs were met. We did not sacrifice additional fish to obtain comparative body composition data because of the restricted availability of black pacu in the USA. However, excess dietary energy might also affect lipid deposition and product quality in pacus, which should be addressed in evaluation of alternative feedstuffs.

The most obvious difference between the control diets with wheat bran (Trials 1 and 2) or wheat middlings (Trial 3) and the diets with alternative feedstuffs was the lower available energy content of wheat bran and wheat middlings compared to pijuayo, yucca, or plantain. This was because of the higher percentage of indigestible fiber in wheat bran (9.9%) and wheat middlings (8%) compared to the other feedstuffs (0.2–4.5%). Fernandes et al. (2004) found that wheat bran had only about half as much apparent digestible energy as corn for red pacu. Fiber is not an energy source for monogastric animals, and most fish do not benefit from high levels (NRC 1993). Least cost feed formulations for channel catfish, *Ictalurus punctatus*, stipulate a maximum of 7% indigestible fiber because it reduces total dietary energy and can reduce the availability of energy and nutrients from other diet ingredients (Robinson and Li 2006). In addition, increased fecal output in fish fed high-fiber diets can reduce water quality (Robinson and Li 2002). However, fiber appears to stimulate growth in some characids. *Piaractus mesopotamicus* fed a diet with 16% fiber grew better than fish fed diets containing 4–12% fiber (Zanoni 1996). Indigestible fiber may stimulate growth indirectly, perhaps through a prebiotic effect on the intestinal microflora (Vazquez et al. 2006), but this has not been investigated in characids. In the present study, weight gain of red or black pacu fed higher fiber diets with wheat bran or middlings was not enhanced relative to fish fed the lower fiber diets with pijuayo, plantain, or yucca.

In the present study, MCHC was depressed in red pacu fed the diet with wheat bran compared to fish fed the other diets. However, there were no other hematological effects of diet in red pacu and no differential diet effects on hematology in black pacu. The hematocrit and hemoglobin values in this study were higher than those reported in Ranzani-Paiva et al. (1998) for characids. However, hematological parameters are affected by factors other than diet such as age (Ranzani-Paiva et al. 1998), water quality (Tucker et al. 1989), and stress (Danley et al. 2005). Therefore, the small diet effects seen in our study probably have limited health implications. Immune competence, as indicated by ACH50 in both species and lysozyme activity in red pacu, was not differentially affected by diet. Survival was also high in all trials.

The absence of detectable lysozyme activity in black pacu using identical methodology to that for red pacu indicates that this method is not suitable for black pacu or that black pacu lacks lysozyme activity. The pH optimum (6.2) for lysozyme detection in red pacu is also suitable for other species such as channel catfish and largemouth bass. The pH optimum is lower (5.8–5.9) in species with weaker lysozyme activity such as golden shiner and goldfish (D. Gatlin, Texas A&M University, personal communication). However, no lysozyme activity was detected in black pacu over the pH range of 5.8–6.2.

Conclusions

All the alternative feedstuffs tested were good sources of available energy for *C. macropomum* and *P. brachypomus* relative to wheat bran or wheat middlings. The availability of energy from feedstuffs is inversely associated with fiber content, and wheat bran and wheat middlings are both higher in fiber than all the other ingredients tested. The extra energy in the test feedstuffs, however, was used partly for hepatic glycogen synthesis rather than for whole-body growth because no significant differences in body weight occurred among fish fed different diets. Differences in the available energy of the diets might also affect lipid deposition in the fish, which should be addressed in future studies. Nevertheless, dietary effects on fish health in this study were minimal, indicating that yucca, pijuayo, and plantain are relatively safe energy sources with no obvious antinutritional effects. Plantain and yucca production are high in Latin America (FAO 2007), and pijuayo is abundant throughout most of Amazonia (Brazil, Colombia, Ecuador, and Peru). Thus, the utilization of these ingredients in formulated diets for characid fish production should be economical alternatives to wheat feedstuffs.

Acknowledgments

We thank Harold Phillips, Felicia Bearden, Baby Suja, Todd Sink, and Daryl Weldon for assistance with feeding trials and conducting health assays at UAPB. We thank Angela Merkel for assistance in the lab at SIUC and Miriam Alvan-Aguilar, Guillermo Amico, Alonso Córdova, Melyna Silva-Pezo, and Julio Barrenzuela for helping us in subsampling fishes biweekly. We extend special thanks to Lamberto Arévalo for processing and shipping the pijuayo from Peru. This research was supported by United States Agency for International Development (USAID) grant no. LAG-G-00-96-90015-00.

Literature Cited

Alcántara, F. B. and M. B. Colace. 2001. Piscicultura, seguridad alimentaria y desarrollo sostenible en la carretera Iquitos-Nauta y el río Tigre: Valorando y preservando nuestros peces amazónicos *in* A. M. Lauro, editor. Instituto de Investigaciones de la Amazonía Peruana, Iquitos, Peru.

Araujo-Lima, C. and M. Goulding. 1997. So fruitful a fish: ecology, conservation, and aquaculture of the Amazon's tambaqui. Columbia University Press, New York, New York, USA.

Campos-Baca, L. and C. Kohler. 2005. Aquaculture of *Colossoma macropomum* and related species in Latin America. American Fisheries Society. American Fisheries Society Symposium 46:451–561.

Carneiro, D. J. 1983. Níveis de proteína e energies na alimentação do pacu (*Colossoma mitrei* Berg 1895). Master's thesis. Universidade Estadual Paulista, Jaboticabal, Brazil.

Castagnolli, N. 2000. Piscicultura intensiva e sustentável. Pages 181–195 *in* W. C. Valenti, C. R. Poli, J. A. Pereira, and J. R. Borghetti, editors. Aqüicultura no Brasil: bases para um desenvolvimento sustentável Brasilia. Conselho Nacional de Desenvolvimento Cientifico a Tecnologico, Brazil.

Chen, R., R. Lochmann, A. Goodwin, K. Praveen, K. Dabrowski, and K.-J. Lee. 2003. Alternative complement activity and resistance to heat stress in golden shiners (*Notemigonus crysoleucas*) are increased by dietary vitamin C levels in excess of requirements for prevention of deficiency signs. Journal of Nutrition 33:2281–2286.

Craig, S. R., B. S. Washburn, and D. M. Gatlin III. 1999. Effects of dietary lipids on body composition and liver function in juvenile red drum, *Sciaenops ocellatus*. Fish Physiology and Biochemistry 21:249–255.

Danley, M. L., P. B. Kenney, P. M. Mazik, R. Kizer, and J. A. Hankins. 2005. Effects of carbon dioxide exposure on intensively cultured rainbow trout *Oncorhynchus mykiss*: physiological responses and fillet attributes. Journal of the World Aquaculture Society 36:249–261.

FAO (Food and Agricultural Organization). 2007. Crop production in Latin America and the Caribbean, 2006. FAOSTAT [WWW Document]. URL http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567 [accessed on September 19, 2007].

Fernandes, J. B. K., D. J. Carneiro, and N. K. Sakomura. 2001. Fontes e níveis de proteína bruta em dietas para juvenis de pacu (*Piaractus mesopotamicus*). Revista Brasileira de Zootecnia 30:617–626.

Fernandes, J. B. K., R. Lochmann, and F. A. Bocanegra. 2004. Apparent digestible energy and nutrient digestibility coefficients of diet ingredients for pacu *Piaractus brachypomus*. Journal of the World Aquaculture Society 35:237–244.

Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. Journal of Biochemistry 226:497–509.

Garenc, C., F. G. Silversides, and H. E. Guderley. 1998. Burst swimming and its enzymatic correlates in the threespine stickleback (Gasterosteus aculeatus): full-sib heritabilities. Canadian Journal of Zoology 76:680–688.

Good, C. A., H. Kramer, and M. Somogyi. 1933. The determination of glycogen. Journal of Biological Chemistry 100:485–491.

Hemre, G. I., S. Y. Shiau, D. F. Deng, T. Storrebakken, and S. S. O. Hung. 2000. Utilization of hydrolysed potato starch by juvenile Atlantic salmon Salmo salar L., when using a restricted feeding regime. Aquaculture Research 31:207–212.

Houston, A. H. 1990. Blood and circulation. Pages 273–334 *in* C. G. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland, USA.

Hutchinson, T. H. and M. J. Manning. 1996. Seasonal trends in serum lysozyme activity and total protein concentration in dab sampled from Lyme Bay, U.K. Fish and Shellfish Immunology 6:473–482.

Kubitzki, K. and A. Ziburski. 1993. Seed dispersal in floodplain forests of Amazonia. Biotropica 26:30–43.

Lovell, T. 1989. Nutrition and feeding of fish. Van Nostrand Reinhold, New York, USA.

Moon, H. Y., and D. M. Gatlin III. 1991. Total sulfur amino acid requirement of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 95:97–106.

Mori-Pinedo, L. A., M. Pereira-Filho, and M. I. Oliveira-Pereira. 1999. Substituição do fubá de milho (Zea mays, L) por farinha de pupunha (Bactris gasipaes, H.B.K) em racões para alevinos de tambaqui (Colossoma macropomum, Cuvier 1818). Acta Amazonica 29:447–453.

NRC (National Research Council). 1993. Nutrient requirements of fish. National Academy Press, Washington, DC, USA.

Ranzani-Paiva, M. J. T., F. A. Salles, J. C. Eiras, C. C. Das Eiras, C. M. Ishikawa, and A. C. Alexandrino. 1998. Blood analysis of ''curimbata'' (*Prochilodus scrofa*), ''pacu'' (*Piaractus mesopotamicus*), and ''tambaqui'' (*Colossoma macropomum*), from Instituto de Pesca Experimental Stations, Sao Paulo, Brazil. Boletim do Instituto de Pesca Sao Paulo 25:77–83.

Robinson, E. and M. Li. 2002. Channel catfish, *Ictalurus punctatus*. Pages 293–318 *in* C. Lim and C. Webster, editors. Nutrient requirements and feeding of finfish for aquaculture. CAB International Publishers, New York, New York, USA.

Robinson, E. and M. Li. 2006. Catfish nutrition: feeds. Mississippi State University Extension Service. Mississippi State University, Publication No. 2413. Starkville, Mississippi, USA.

Roubach, R. and U. Saint-Paul. 1994. Use of fruits and seeds from Amazonian inundated forests in feeding trials with *Colossoma macropomum* (Cuvier 1818) (Pisces, Characidae). Journal of Applied Ichthyology 10:134–140.

Serrano, J. A., G. R. Nematipour, and D. M. Gatlin III. 1992. Dietary protein requirement of the red drum (*Sciaenops ocellatus*) and relative use of dietary carbohydrate and lipid. Aquaculture 101:283–291.

Torloni, C. E. C., J. A. Silva-Filho, J. R. Verani, and J. A. Pereira. 1984. Estudos experimentais sobre o cultivo do pacu Colossoma mitrei, no Sudeste do Brasil. Pages 559–576 *in* Anais do Simpósio Brasileiro de Aqüicultura. Associaçáo Brasileira de Aquicultura, São Carlos, Brazil.

Tort, L., E. Gomez, D. Montero, and J. O. Sunyer. 1996. Serum hemolytic and agglutinating activity as indicators of fish immunocompetance: their suitability in stress and dietary studies. Aquaculture International 4:31–41.

Tucker, C. S., R. Francis-Floyd, and M. H. Beleau. 1989. Nitrite-induced anemia in channel catfish *Ictalurus punctatus* rafinesque. Bulletin of Environmental Contamination and Toxicology 43:295–301.

Van der Meer, M. B. and M. C. J. Verdegem. 1996. Comparison of amino acid profiles of feeds and fish as a quick method for selection of feed ingredients: a case study for *Colossoma macropomum* (Cuvier). Aquaculture Research 27:487–495.

Vásquez-Torres, W., M. Pereira-Filho, and J. A. Arias-Castellanos. 2002. Estudos para composição de uma dieta referência semipurificada para avaliação de exigencies nutricionais em juvenis de pirapitinga, *Piaractus brachypomus* (Cuvier 1818). Revista Brasileira de Zootecnia 31:283–292.

Vazquez, M. J., J. L. Alonso, H. Dominguez, and J. C. Parajo. 2006. Enhancing the potential of oligosaccharides from corncob autohydrolysis as prebiotic food ingredients. Industrial Crops and Products 24:152–159.

Webster, C. D. and C. Lim. 2002. Introduction to fish nutrition. Pages 1–27 *in* C. Lim and C. Webster, editors. Nutrient requirements and feeding of finfish for aquaculture. CAB International Publishers, New York, New York, USA.

Zanoni, M. A. 1996. Niveis de fibra bruta em dietas de crescimento do pacu (*Piaractus mesopotamicus*) Holmberg, 1887. Master's thesis. Universidade Federal de Santa Catarina, Florianopolis, Brazil.