COEFFICIENTS OF ENERGY AND NUTRIENT APPARENT DIGESTIBILITY OF THREE RAW AND COOKED ALTERNATIVE PLANT INGREDIENTS FOR *Colossoma macropomum* AND *Piaractus brachypomus* (SERRASALMIDAE)

Fred William CHU-KOO[1], William N. CAMARGO[2], Miriam Adriana ALVAN-AGUILAR[1], Jesse TRUSHENSKI[3], Christopher C. KOHLER[2]

1 Instituto de Investigaciones de la Amazonía Peruana – IIAP. Programa AQUAREC. Centro de Investigaciones Fernando Alcántara – CIFAB. E-mail: fchu@iiap.org.pe

2 Southern Illinois University Carbondale. Fisheries and Illinois Aquaculture Center. Carbondale, IL 62904.

3 Eagle Fish Health Laboratory. Idaho Department of Fish and Game.

ABSTRACT

The coefficients of the apparent digestibility for dry matter, crude protein, crude lipid and gross energy in cooked and raw green plantain meal (GPM), cassava root meal (CRM), and peach palm meal (PPM) were determined for juveniles of black pacu (60.8 ± 6.4 g) and red pacu (76.3 ± 5.0 g). The ADC of each alternative ingredient was determined by comparison of the digestibility (based on recovery of 1% chromic oxide as an inert marker) of a reference diet (consisting of 70% of test diet and 30% of the feedstuff) against with a test diet (24.5% of crude protein). Fish were bred in a recirculation system including 42, 110-L glass aquaria (21 tanks per species) at a density of five fish per tank, and fed their respective diets to apparent satiety, twice a day. Feces were collected after 10 days in plexiglass fecal collectors. Apparent digestibility coefficients of crude protein, crude lipid and gross energy from raw and cooked GPM and CRM were low compared to values to other common vegetal ingredients like corn and wheat. Conversely, ADC values for raw and cooked PPM were, in most cases, higher than those reported in raw or cooked GPM and CRM. In comparison with other traditional feedstuffs, apparent utilization of PPM was similar to corn and higher than reported for soybean meal and wheat bran in these fish species. Peach palm meal appears to have good potential as a feed ingredient in low-cost diets for pacu species.

KEYWORDS: aquaculture, digestibility, feeding, red pacu, black pacu.

COEFICIENTES DE DIGESTIBILIDAD APARENTE DE LOS NUTRIENTES Y LA ENERGÍA DE TRES INGREDIENTES VEGETALES ALTERNATIVOS CRUDOS Y COCIDOS EN *Colossoma macropomum* Y *Piaractus brachypomus* (SERRASALMIDAE)

RESUMEN

Los coeficientes de digestibilidad aparente (ADC) para la materia seca, proteína cruda, lípidos y energía bruta en harinas obtenidas de porciones crudas y cocidas de plátano verde (GPM), yuca (CRM) y pijuayo (PPM) fueron determinados en juveniles de gamitana (60.8 ± 6.4 g) y paco (76.3 ± 5.0 g). El ADC de los ingredientes alternativos fue determinado por comparación de la digestibilidad (basada en la recuperación de óxido crómico como marcador inerte) de una dieta referencial (70% de dieta testigo y 30% del ingrediente en estudio) versus la dieta testigo (24.5% de proteína bruta). Los peces fueron criados en un sistema de recirculación compuesto de 42 acuarios de vidrio de 110 L (21 tanques para cada especie), a una densidad de cinco peces por tanque y alimentados con sus respectivas dietas hasta la saciedad aparente, dos veces por día. Las heces fueron colectadas a los 10 días en colectores fecales. Los niveles de ADC para proteína bruta, lípidos y energía bruta registrados para las harinas crudas y cocidas de plátano y yuca fueron bajas comparadas con las existentes para otros ingredientes como el maíz y el trigo. Sin embargo, los niveles de ADC obtenidos para las harinas crudas y cocidas del pijuayo fueron en la mayoría de los casos superiores a los reportados para el plátano y la yuca. En comparación con otros ingredientes vegetales, las harinas a base de pijuayo fueron utilizadas en niveles similares a las reportadas para la soya y salvado de trigo en paco y gamitana. El pijuayo presenta mayor potencial como ingrediente de bajo costo para ser usado en dietas de alimentación del paco y gamitana.

PALABRAS CLAVE: acuicultura, digestibilidad, alimentación, paco, gamitana.

INTRODUCTION

The black pacu *Colossoma macropomum* and red pacu *Piaractus brachypomus* are native fish species being farmed in South America (Núñez *et al*., 2009). Cultured in earthen ponds at stocking rates between 0.5 – 1 fish/m^2, both fish species may reach 0.8 to 1 kg in only 6 to 8 months (Dañino *et al*., 2009a,b). They are generally fed extruded or pelleted diets formulated with traditional feedstuffs (fish meal, wheat derivatives, corn and soybean meal). However, prices for commercial aquafeeds are too high for most small-scale fish farmers, who comprise nearly 90% of those involved in pacu culture in the Peruvian Amazon (Chu-Koo *et al*., 2012). Therefore, there is an increasing need for more low-cost ingredients to be used in Peruvian aquafeeds for pacus, particularly because of the high cost of ingredients like imported soybean meal and wheat derivatives.

Traditionally, corn grains and wheat middlings are the main sources of gross energy in commercial formulated aquafeeds for pacu and other Amazonian omnivorous cultured fishes (Mori *et al*., 1999; Lochmann *et al*., 2009). Corn prices have progressively increased in the past years and wheat is not traditionally farmed in the Amazonian region and consequently must be imported from Argentina or the USA, and at a high price. In contrast, plantain (*Musa paradisiaca*), cassava root (*Manihot sculenta*), and peach palm (*Bactris gasipaes*) are substantial components of the local population's diet and they are grown in proximity to fish ponds. In fact, production of these plants is often so high that local prices fall to levels more suitable for livestock feed ingredients than for marketing as human staple foods (Lochmann *et al*., 2009).

Cassava (locally known as yuca) and its by-products (roots, skin, leaves, etc.) are used as alternative ingredients to corn and rice in formulated diets for monogastric animals in developing countries (Nhu Phuc *et al*., 1995; Rosales & Paúcar, 1996; Akinfala *et al*., 2002; Vieira *et al*., 2002), including farmed fish (Araújo-Lima & Goulding, 1997; Alcántara & Colace, 2001; Campos Baca & Kohler, 2005). Plantain is used primarily for human food but also as supplemental feed for small-scale fish farming in Brazil and Peru (Alcántara & Colace, 2001; Alcántara *et al*., 2003; Guimarães & Storti-Filho, 2004; Chuquipiondo & Galdós, 2005). Approximately 15 million metric tons of bananas and 9 million metric tons of plantains are annually available for use as feedstuffs and processed plantain meal is being used in aquafeeds in Southeast Asia (Aquafeed International, 2008; Quynh Tram, 2010). In addition, peach palm (or pijuayo) is a staple food crop whose fruit provides an edible pulp for direct human consumption, flour for infant formula and baked goods, as well as serving as an ingredient in feeds for farmed animals and fish (Blanco-Metzler *et al*., 1992; Mora-Urpí, 1993; Clement, 1995; Mori *et al*., 1999; Alcántara *et al*., 2003).

Because *Colossoma* and *Piaractus* readily accept plant materials as food, small-scale fish farmers utilize a wide variety of agricultural by-products, leaves, fruits and seeds to feed their fish (Alcántara & Colace, 2002). However, little information exists with respect to these types of feedstuffs, such as their nutritional value and digestibility, the growth performance they yield, etc.

The Food and Agriculture Organization (FAO, 2007) recommended the utilization of native crops to supply the dietary needs currently obtained from imports or feeds that might be better used by humans in Latin America; and suggested the use of cassava, plantain and peach palm as potential ingredients to be used in animal diets, in order to decrease current dependence on cereals and grains (Machin, 1992). Recently, Núñez (2009) emphasized the need for conducting sustainable aquaculture in South America by promoting the culture of species whose dependence of animal protein derivatives can be far lower than current species being cultured (e.g. salmonids, shrimps). Therefore, red and black pacu are two excellent candidates for sustainable aquaculture since they can use dietary plant proteins and carbohydrates more efficiently than carnivores due to their natural frugivorous diet (Araújo-Lima & Goulding, 1997; Lochmann *et al*., 2009).

Culture techniques for these and other native Amazon fishes could be advanced considerably with new information on nutrient utilization in fishes fed diets with different compositions. Although some of the basic nutrient requirements are known for serrasalmids (Fernandes *et al*., 2004), there is not much information on the availability of nutrients from feedstuffs of local origin. Even when cost and convenience of local feedstuffs are attractive, there is no advantage to using them in fish diets if the nutrients they contain are largely unavailable. Digestibility coefficients for many of the traditional feedstuffs used in current pacu diets have been determined (Fernandes *et al*., 2004). Comparative data from promising native feedstuffs would provide a nutritional basis for selecting low-cost accessible feedstuffs for use in pacu diets in the Amazon region. Knowledge about the nutrient digestibility of the food sources is important for the formulation of both experimental and practical fish diets as well as in studies to determine quantitative nutrient requirements. Furthermore, the formulation of low-cost fish diets requires extensive knowledge of both the chemical composition of the feedstuffs and the availability of their nutrients to the fish.

Accordingly, this study was designed to assess the apparent nutrient and energy digestibility coefficients of cooked and raw green plantain, cassava root, and peach palm, three agricultural products currently harvested and used as food sources for fish in the Peruvian Amazon.

METHODS

EXPERIMENTAL SYSTEM AND FISH

Hatchery-produced fingerlings of black pacu and red pacu were shipped from the Instituto de Investigaciones de la Amazonía Peruana–IIAP (Iquitos, Peru) to Carbondale (Illinois, USA). After a period of acclimation, they were initially trained to a commercial catfish diet (32% crude protein) and maintained in three raceways with recirculated water for four months prior their use in the trials.

Fish were individually measured and weighed and then randomly placed in groups of five into each of 42, 110-L aquaria (21 tanks per pacu species) in a recirculation aquaculture system and allowed to acclimate to ambient conditions. During acclimation fish were fed to satiation with a standard reference diet formulated according to Fernandes *et al*. (2004). Then, the seven experimental diets (Table 2) were randomly assigned to triplicate aquaria in each trial. Water temperature and dissolved oxygen were measured twice per week before feeding using a Dissolved Oxygen Meter, YSI Model 52 (YSI Instrument Co. Inc., Yellow Spring, Ohio, USA). Total ammonia nitrogen, nitrite, and alkalinity levels were measured twice per week using a AQ-2 Freshwater Water Lab Kit (LaMotte Co., Chestertown, Maryland, USA). The pH was measured using a pH/temperature/mV/ORP Meter (Col-Parmer Instrument Co., Vernon Mills, Illinois, USA).

EXPERIMENTAL DIETS

Each test diet was prepared with 70% of the standard reference diet and 30% of the test ingredient. The individual test ingredients evaluated were: green plantain meal (GPM), cassava root meal (CRM), and peach palm meal (PPM), each assessed as raw and cooked products (Table 2).

Raw cassava roots, peach palm and green plantain were washed, peeled and chopped in thin slices (4-5 mm) and then dried at 65 °C for 24 h in an oven. They were then finely ground (1-2 mm) in a Wiley mill. For cooked ingredients, thin raw slices were boiled in water for 5 min before being dried and finely ground in the same manner as raw ingredients. The proximate composition of these three ingredients is shown in Table 1.

Diets were prepared in a mixer by slowly adding micronutrients (vitamin and minerals premixes) to the macro-ingredients to ensure a homogenous mixture (Table 2). 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 mm die was used to produce the pellets, which were fan dried for 24 h and stored at –18°C until use. The composition of the test diets is shown in Table 2.

FECAL COLLECTION

In each trial, fish were fed their respective diets for a 5-d acclimation period followed by a 10-d fecal collection period. During the fecal collection period, fish were fed to apparent satiation twice daily. Two hours after feeding, uneaten food was removed to prevent ingestion of feed that may have leached nutrients. All aquaria bottoms were siphoned in order to standardize the stress on the fish. The feces were collected the following morning at approximately 12-14 hours after the previous feeding event in specially designed fecal collector devices similar to these described by Ayala & Kohler (1993). Daily fecal samples for each aquarium were dried in a drying oven for 6 h at 65 ºC to reduce moisture, and then stored frozen in aluminum pans.

ANALYTICAL METHODS

Moisture and dry matter were obtained by drying triplicate samples (approximately 250 mg) of diets at 135 ºC for 3 hours. Crude protein was determined on dried samples using a Kjeltec 1026 Distillation Unit (Tecator-Perstorp, Höganas Sweden). About 1-g samples were weighed in nitrogen-free paper in duplicate, and transferred to a digestion tube along with two Kjeldahl catalyst tablets. Concentrated sulfuric acid was added using a dispenser and the tubes were placed in a preheated digestion block at 420 ºC for 1 hour. After cooling, the tubes were distilled automatically in the Kjeltec 1026 Distillation Unit. The solution in the receiver flask was then titrated against HCl of a known concentration. Percent nitrogen of the sample was calculated using the titration volume and converted to protein using a factor of 6.25. Crude fat was determined gravimetrically following the lipid extraction according to Folch *et al*., (1957). Crude fiber content was determined in the Chemical Analysis Lab of the SIUC College of Animal Sciences, following standardized methods of acid-alkali digestion (AOAC, 1990).

Ash was determined gravimetrically by burning 2.5 g of triplicated samples at 550 ºC for 3 hours, while energy content were obtained using a Parr 1261 Bomb Calorimeter (Parr Instrument Co., Moline, Illinois, USA).

Chromic oxide recovery levels were determined by atomic absorption using standard methods used at the SIUC Wildlife Cooperative Lab (Carbondale, IL).

CALCULATIONS AND STATISTICAL ANALYSIS

The digestibility coefficient for a nutrient in either the reference or test diet and for a nutrient in each test ingredient was determined according to the formula:

$$ADC\ (\%) = 100-[100(\%C_{diet} / \%C_{feces}) \times (\%N_{feces} / \%N_{diet})]$$

where C is the inert marker and N the nutrient. The apparent digestibility coefficients (ADC) for dry matter (ADC_{DM}), crude protein (ADC_{CP}), crude lipid (ADC_{CL}) and gross energy (ADC_{GE}) for each ingredient was calculated as:

$$ADCi\ (\%) = ADC_{test} + ((0{\cdot}7 \times N_{ref}) / (0{\cdot}3 \times N_i)) \times (ADC_{test} - ADC_{ref})$$

where ADCi is the apparent digestibility coefficient for each ingredient expressed in terms of percentage; ADC test is the apparent digestibility coefficient of the test diet; N_{ref} the nutrient content of the reference diet; N_i the nutrient content of each test ingredient; ADC_{ref} the apparent digestibility of the reference diet.

The data were subjected to two-way analysis of variance (ANOVA) to test the effects of ingredient, the type of presentation and the interaction of these two variables.

If significant ($P<0.05$) differences were found in the ANOVA test, Tukey HSD test was used for pairwise comparison using SPSS version 11.5 for Windows (SPSS, Chicago, IL, USA). Results are expressed as the mean ± standard deviation (SD) of three replicates for each treatment (diets) in each trial. The level of significance applied was $P<0.05$.

RESULTS

WATER QUALITY

Water quality parameters maintained during the experiments were the following: dissolved oxygen 5.6 ± 0.4 mg/L, water temperature 27.1± 0.5 ºC, pH 6.7 ± 0.1, ammonia 0.23 ± 0.1 ppm, nitrite 0.05 ± 0.01 ppm, and alkalinity 48.9 ± 6.4 ppm. These values are considered suitable for black and red pacu culture (Soberón *et al.*, 2007).

Table 1. Proximate composition (% mean ± standard deviation) of raw and cooked green plantain meal, cassava root meal, and peach palm meal.

Chemical composition	Raw			Cooked		
	Green Plantain Meal	Cassava Root Meal	Peach Palm Meal	Green Plantain Meal	Cassava Root Meal	Peach Palm Meal
Dry matter (% as-fed)	94.6 ± 3.2	91.2 ± 4.1	92.5 ± 3.8	90.9 ± 5.1	92.5 ± 3.9	91.4 ± 4.4
Crude Protein (% DM)	4.3 ± 0.3	1.9 ± 0.1	6.2 ± 0.5	3.3 ± 0.4	2.1 ± 0.2	6.0 ± 0.6
Crude Lipid (% DM)	0.8 ± 0.1	2.8 ± 0.2	15.7 ± 1.6	0.6 ± 0.1	2.6 ± 0.3	11.5 ± 1.1
Ash (% DM)	2.1 ± 0.3	2.2 ± 0.4	2.8 ± 0.4	1.6 ± 0.2	0.8 ± 0.1	2.1 ± 0.3
Crude Fiber (% DM)	0.9 ± 0.1	4.2 ± 0.3	5.8 ± 0.4	0.8 ± 0.1	4.3 ± 0.3	5.5 ± 0.4
Carbohydrates (% DM)	91.9 ± 4.0	88.9 ± 5.1	69.5 ± 6.7	93.7 ± 4.8	92.1 ± 4.7	74.9 ± 6.7
Gross Energy (Kcal/100 g)	396.3 ± 23.1	390.3 ± 20.5	450.3 ± 26.2	396.7 ± 25.4	402.3 ± 30.2	433.1 ± 26.1

Table 2. Reference and test diet formulations (%) for determination of apparent digestibility coefficients for black pacu (*Colossoma macropomum*) and red pacu (*Piaractus brachypomus*).

Ingredient	Reference Diet	Cooked Diets			Raw Diets		
		Plantain Diet	Cassava Diet	Peach Palm Diet	Plantain Diet	Cassava Diet	Peach Palm Diet
Menhaden fish meal	10.0	7.0	7.0	7.0	7.0	7.0	7.0
Soybean Meal	26.0	18.2	18.2	18.2	18.2	18.2	18.2
Corn	30.0	21.0	21.0	21.0	21.0	21.0	21.0
Wheat Bran	27.0	18.9	18.9	18.9	18.9	18.9	18.9
Soybean Oil	4.0	2.8	2.8	2.8	2.8	2.8	2.8
Vitamin Mixture[a]	1.0	0.7	0.7	0.7	0.7	0.7	0.7
Mineral Mixture[b]	1.0	0.7	0.7	0.7	0.7	0.7	0.7
Chromic Oxide	1.0	0.7	0.7	0.7	0.7	0.7	0.7
Test Ingredient	0.0	30.0	30.0	30.0	30.0	30.0	30.0
Proximate composition (%)							
Moisture (as-fed)	10.3 ± 0.6	9.6 ± 0.5	11.5 ± 0.8	10.7 ± 0.6	11.6 ± 0.4	10.4 ± 0.7	14.2 ± 0.8
Dry Matter (as-fed)	89.7 ± 0.8	90.4 ± 1.1	88.5 ± 1.0	89.3 ± 0.8	88.4 ± 1.2	89.6 ± 1.1	85.8 ± 1.3
Protein (% DM)	27.7 ± 0.7	20.4 ± 0.8	20.7 ± 1.1	20.8 ± 0.6	19.6 ± 0.8	20.8 ± 1.2	20.2 ± 0.9
Lipid (% DM)	7.6 ± 0.4	5.3 ± 0.3	7.4 ± 0.4	8.9 ± 0.5	5.4 ± 0.4	5.4 ± 0.5	6.7 ± 0.3
Ash (% DM)	7.9 ± 0.6	6.8 ± 0.4	6.4 ± 0.3	6.2 ± 0.4	6.6 ± 0.3	6.5 ± 0.3	6.3 ± 0.4
Fiber (% DM)	6.2 ± 0.5	3.8 ± 0.4	3.7 ± 0.3	4.8 ± 0.3	3.6 ± 0.3	3.3 ± 0.2	4.7 ± 0.4
Carbohydrates (% DM)	50.6 ± 3.8	63.7 ± 3.2	62.4 ± 2.8	59.3 ± 3.8	64.8 ± 3.7	64.0 ± 3.1	62.1 ± 2.9
Gross Energy (kcal/100 g)	409.3 ± 25.8	404.6 ± 28.1	419.7 ± 22.6	421.3 ± 19.8	405.8 ± 29.8	408.7 ± 20.4	409.8 ± 23.8

[a]Vitamin mix (composition/kg): ascorbic acid, 50 g; D-calcium panthenate, 5 g; choline chloride, 100 g; inositol, 5 g; menadione, 2 g; niacin, 5 g; pyridoxine. HCl, 1 g; riboflavin, 3 g; thiamine. HCl, 0.5 g; DL-alpha-tocopherol acetate (250 IU/g), 8 g; vitamin A acetate (2000 IU/g), 5 g; biotin, 0.5 g; cholecalciferol, 0.002 g; folic acid, 0.18 g; vitamin B12, 0.002 g; cellulose, 806.48 g.

[b]Mineral mix (composition/kg): calcium phosphate monobasic, 136 g; calcium lactate, 349.9 g; ferrous sulfate, 5 g; magnesium sulfate, 132 g; potassium phosphate dibasic, 240 g; sodium phosphate monobasic, 88 g; sodium chloride, 45 g; aluminum chloride, 0.15 g; potassium iodide, 0.15 g; cupric sulfate, 0.5 g; manganese sulfate, 0.7 g; cobalt chloride, 1 g; zinc sulfate, 3 g;and sodium selenite, 0.01 g.

Dehulled solvent-extracted soybean meal (48% protein) was produced by Mountaire Feeds, Inc., North Little Rock, Arkansas, USA. Menhaden fishmeal was purchased from Omega Protein, Inc., Hammond Louisiana, USA. Uncooked # 2 corn was obtained from Mountaire Feeds, Inc., North Little Rock, Arkansas, USA. The wheat bran was purchased from Siemer Milling Company, Hopkinsville, Kentucky, USA. Green plantains and cassava roots were purchased from a local market in Carbondale, IL, and peach palm meal (cooked and raw) was shipped from Iquitos, Peru.

APPARENT DIGESTIBILITY COEFFICIENTS

In black pacu (Table 3), ADC_{DM} values for the cooked ingredients ranged from 78.6% (CRM) to 89.9% (PPM); whereas raw feedstuffs ranged from as low as 74.2% (CRM) to as high as 87% (PPM), with significant differences recorded for both factors tested (P<0.001 for test ingredients, and P=0.009 for type of presentation, respectively). ADC_{CP} values varied from 60.1% (CRM) to 83.3% (PPM) in cooked ingredients, and from 49.8% (CRM) to 80.1% (PPM), for raw feedstuffs. As reported for dry matter, the analysis of variance indicated that protein digestibility coefficients are significantly influenced by the dietary ingredients being tested (P<0.001) and also by the type of presentation (P<0.001).

ADC_{CL} values were higher when compared to the other parameters evaluated. ADC values estimated for crude lipids contained in cooked ingredients ranged from as low as 84.6% (GPM) to as high as 90.7% (PPM), while ADC_{CL} for raw feedstuffs varied from 50.5% (CRM) to 90.6% (PPM). The analysis of variance indicated that lipid digestibility is significantly influenced by the dietary ingredients (P<0.001), the type of presentation (P<0.001), and the interaction between these two variables (P=0.005). Gross energy digestibility for cooked ingredients varied from 47.8% (CRM) to 84.2% (PPM), whereas ADC for raw feedstuffs ranged from as low as 38.1% (CRM) to as high as 78.2% (PPM). As reported for dry matter and crude protein, the ANOVA indicated that ADC for gross energy may be significantly influenced by both factors, the dietary ingredients tested (P<0.001) and the type of presentation (P<0.001), but no significant effects (P=0.742) were recorded due to the interaction between these two variables according to the two-way ANOVA test.

In red pacu (Table 4), ADC_{DM} values for cooked ingredients ranged from 82.7% (CRM) to 87.1% (PPM), whereas raw feedstuffs varied from as low as 82.1% (CRM) to as high as 86.2% (PPM), with significant differences recorded only for tested ingredients (P<0.001). No significant differences were recorded due to the type presentation (P=0.366) or the interaction between these two variables (P=0.424). Crude protein digestibility values varied from 55.7% (CRM) to 86.8% (PPM) in cooked ingredients, and from 53.1% (CRM) to 85.6% (PPM, for raw feedstuffs. The analysis of variance indicated that ADC_{CP} percentages are significantly influenced by the dietary ingredients being tested (P<0.001), and the type of presentation (P<0.044). However, the two-way ANOVA test yielded no significant effects of the interaction between these two independent variables (P=0.569).

In red pacu, the crude lipid digestibility coefficients were significantly higher for cooked and raw PPM, when compared to the other ingredients (P<0.01).

ADC_{CL} values estimated for crude lipids contained in cooked ingredients ranged from as low as 56.6% (GPM) to as high as 92.2% (PPM), whereas the ADC_{CL} of raw feedstuffs varied from 54.9% (GPM) to 90.4% (PPM). No significant differences were recorded due to the type of presentation or because of interaction between these variables (P=0.005).

Gross energy digestibility percentages for cooked ingredients varied from 40.9% (CRM) to 81.4% (PPM), while ADC_{GE} for raw feedstuffs ranged from as low as 32.7% (CRM) to as high as 74.2% (PPM). The ANOVA indicated that ADC for gross energy may be significantly influenced by both factors, the dietary ingredients tested (P<0.001) and the type of presentation (P=0.003). No significant differences were recorded due to the interaction between these two variables (P=0.345).

DISCUSSION

The replacement of certain traditional feedstuffs used as ingredients in aquafeeds, for substitutes, has emerged as an alternative economic practice mainly in developing countries. However, the digestibility of most of these new items has yet to be studied to increase the knowledge of animal nutrition.

According to Hepher (1988), several factors may influence the digestibility of fish dietary feedstuffs. The author emphasizes that the main ones are: a) fish species, b) age/size, c) physiological conditions, d) water temperature, e) salinity, f) food composition, g) feed intake and f) particle size.

Other key factors reported to overestimate or underestimate fish digestibility are the methods of collection of feces. While passive methods tend to overestimate it, active methods such as abdominal stripping (sometimes even killing the fishes for intestine stripping) tend to underestimate ADCs (Ramsay *et al*., 2000).

It was found that ADC levels for crude protein, lipid and gross energy for cooked GPM and CRM in black pacu were always higher than ADC recorded for raw ingredients, as it was originally expected, since thermal treatments, such as boiling, typically have a positive effect on the digestibility of most nutrients in aquafeeds (Bergot, 1991; Wilson, 1994) by inhibiting antinutritional factors (ANF) that may be occurring in plant-based feedstuffs and/or facilitating the breakdown of dietary starch (Hardy, 1989).

Table 3. Nutrient and energy apparent digestibility coefficients (ADC) of three local Amazonian ingredients under two presentations (cooked or raw) tested for black pacu (*Colossoma macropomum*) juveniles. Values expressed as mean ± SD (standard deviation, n=3 replicates).

Test Ingredient	Feedstuff Presentation	ADC (%)			
		Dry Matter	Protein	Lipid	Gross Energy
Peach Palm	Cooked	89.9 ± 0.4^{a}	83.3 ± 1.8^{a}	90.7 ± 4.8^{a}	84.2 ± 1.5^{a}
	Raw	87.0 ± 3.9^{a}	80.1 ± 0.7^{a}	90.6 ± 1.8^{a}	78.2 ± 2.8^{b}
Plantain	Cooked	85.0 ± 5.3^{ab}	66.7 ± 3.9^{b}	84.6 ± 1.7^{ba}	54.4 ± 0.5^{c}
	Raw	76.6 ± 3.9^{bc}	55.5 ± 3.1^{cd}	53.1 ± 1.3^{c}	45.7 ± 3.5^{d}
Cassava	Cooked	78.6 ± 1.8^{bc}	60.1 ± 3.8^{b}	85.5 ± 11.2^{ba}	47.8 ± 2.0^{d}
	Raw	74.2 ± 2.1^{c}	49.8 ± 1.9^{d}	50.5 ± 5.1^{c}	38.1 ± 2.0^{e}
Two-Way ANOVA					
Ingredient		P<0.001	P<0.001	P<0.001	P<0.001
Presentation		P=0.009	P<0.001	P<0.001	P<0.001
Ingredient * Presentation		P=0.424	P=0.112	P=0.005	P=0.742

Table 4. Nutrient and energy apparent digestibility coefficients (ADC) of three local Amazonian ingredients under two presentations (cooked or raw) tested for red pacu (*Piaractus brachypomus*) juveniles. Values are expressed as mean ± SD (standard deviation, n=3 replicates); values with common letter labels are not significantly different ($P < 0.05$).

Test Ingredient	Feedstuff Presentation	ADC (%)			
		Dry Matter	Protein	Lipids	Gross Energy
Peach Palm	Cooked	87.1 ± 0.9^{a}	86.8 ± 1.8^{a}	92.2 ± 2.7^{a}	81.4 ± 0.6^{a}
	Raw	86.3 ± 0.5^{ab}	85.6 ± 2.4^{a}	90.4 ± 2.5^{a}	74.2 ± 5.8^{a}
Plantain	Cooked	84.3 ± 1.3^{ab}	62.9 ± 1.0^{b}	56.6 ± 1.9^{b}	42.4 ± 2.1^{b}
	Raw	83.5 ± 0.5^{ab}	57.5 ± 2.2^{bc}	54.9 ± 1.0^{b}	39.7 ± 2.8^{bc}
Cassava	Cooked	82.7 ± 2.9^{ab}	55.7 ± 4.6^{bc}	65.2 ± 2.9^{b}	40.9 ± 3.3^{bc}
	Raw	82.1 ± 2.7^{b}	53.1 ± 3.4^{c}	64.8 ± 3.6^{b}	32.7 ± 3.4^{c}
Two-Way ANOVA					
Ingredient		P=0.004	P<0.001	P<0.001	P<0.001
Presentation		P=0.366	P=0.044	P=0.395	P=0.003
Ingredient * Presentation		P=0.990	P=0.569	P=0.862	P=0.345

Table 5. Apparent digestibilty coefficients (ADC) determined for some ingredients in black pacu (*Colossoma macropomum*), red pacu (*Piaractus brachypomus*), and pacu (*Piaractus mesopotamicus*) as reported by other authors.

Test Ingredient	ADC (%)		
	Dry Matter	Protein	Lipid
Gutierrez et al. (2009) Corn (black pacu)	82.4 ± 1.0	75.5 ± 1.5	76.2 ± 2.4
Vidal et al. (2004) Corn (black pacu)	83.3 ± 8.7	81.5 ± 7.9	----
Soybean meal (black pacu)	82.8 ± 9.3	90.4 ± 6.4	----
Fernandes et al. (2004) Soybean meal (red pacu)	83.7 ± 6.9	75.9 ± 8.0	63.0 ± 6.3
Corn (red pacu)	89.1 ± 8.2	85.1 ± 2.4	83.0 ± 1.6
Wheat bran (red pacu)	82.1 ± 4.7	61.6 ± 4.4	82.4 ± 6.6
Gutierrez-Espinosa & Vásquez-Torres (2008) Soybean meal (red pacu)	----	83.2 ± 1.6	59.9 ± 2.9
De Oliveira et al. (1997) Dende meal (pacu)	54.0	80.7	76.9
Coco meal (pacu)	72.6	83.4	97.6

In contrast, ADC levels for dry matter, protein, lipid and gross energy for cooked and raw GPM and CRM in red pacu were surprisingly similar. However for practical terms, nutrients as well as the gross energy contained in CRM and GPM were just available for both fish species even when CRM and GPM were previously boiled, representing a limiting key factor for their future utilization in formulated low-cost compound diets for serrasalmids.

Low digestibility coefficients of CRM and GPM were unexpected outcomes since pacus are believed to efficiently utlize plant-derived nutrients such as protein, lipids and especially carbohydrates (Da Silva *et al*., 2002) as previous studies had reported the utilization of these ingredients in pacu diets with encouraging results (Guimarães & Storti-Filho, 2004; Chuquipiondo & Galdós, 2005; Delgado-Vidal *et al*., 2009). We believe that the feces collection method used in this study (sedimentation) may provide some explanation of this unexpected result, as passive methods of fish collection tend to underestimate the nutrient and gross energy digestibility in fish (Ramsay *et al*., 2000). Low ADC values for crude protein recorded for CRM and GPM in black and red pacu may also be partially explained due to the high carbohydrate content of both feedstuffs. Protein digestibility tends to be depressed as the concentration of dietary carbohydrates increases (NRC 1993). Fish age/size may be another important factor involved in the low ADC recorded in this study as it was proved that digestibility in herbivore/omnivore fishes may increase with fish size/age due to associated increases in intestinal length, microflora and enzyme production and thus digestion and assimilation time (Ferraris *et al*., 1986; Quynh Tram, 2010). For instance, Quynh Tram (2010) proved that enzymatic production and action of proteases and amylases are typically lower during the first development stages of herbivore/omnivore fish than in the later stages, and this might also hold true for red and black pacu.

Since crude fiber of experimental diets ranged from as low as 3.3% to as high as 6.4%, it cannot be assumed of having a negative effect in digestibility as reported for other fish species (Ferraris *et al*., 1986; Wang *et al*., 1985). For instance, Dongmesa *et al*. (2009) revealed that green plantain contains high levels of active tannins and cassava roots contain phytic acid and hydrogen cyanides. These compounds are chemical anti-nutritional factors (ANF) that limit a wide range of enzymatic reactions, particularly of the proteases (Francis *et al*., 2011). The presence of ANF may be partially responsible for low nutrient availability in raw GPM and CRM. In the case of tannins, which are only 50-70% deactivated by boiling (Vijayakumari *et al*., 1995), this could even explain the reasons for low uptake of nutrients in the cooked GPM in both fish species. As for CRM, Montagnac *et al*. (2009) reported that phytate and polyphenols contained in cassava roots interfere with digestion and uptake of nutrients.

Sowetan & Oyewole (2009) mentioned the fact that a new plant-based feedstuff is eaten by a fish is nothing less than just a sign of acceptability. Usually, fish nutritionists pay more attention on the analyses of protein and fiber as indicators of feedstuff quality when more importance should be paid to the presence of compounds such as ANF which may affect levels of dietary protein and fiber uptake in fish (Sowetan & Oyewole, 2009; Francis *et al*., 2001).

Additionally, low enzymatic activity may have occurred into the digestive tracts of both fish species, as a result of our feeding schedule during the experiment (fish were fed once daily), as it is known that digestive enzymes response may be affected by the feeding period, since changes in enzyme synthesis and activity in fishes can be observed after a long feeding period (Kaushik *et al*., 1995).

Conversely, black and red pacu individuals seemed to utilize the nutritional content of PPM more efficiently, regardless of presentation (raw or cooked). Only in black pacu, gross energy was more efficiently utilized when it was under the form of cooked PPM, which suggests no thermal pre-treatment is absolutely necessary to improve PPM nutrient availability for formulated pacu aquafeeds.

Without a question, digestibility of a particular feedstuff depends on the species of fish tested. A clear example of that is the study performed by Abu *et al*. (2010) in which, they found that CRM can effectively replace corn as a dietary source of energy in low-cost aquafeeds for hybrid African catfish *Clarius gariepinus*. Complementarily, Udo & Umoren (2011) found that boiled CRM is well digested by the same catfish as ADC values for dry matter, crude protein and energy were 88.5, 94.4, and 73.2% in that species. Madalla (2008) also pointed out that CRM could replace up to 75% of wheat meal in a Nile Tilapia *Oreochromis niloticus* diets without significantly affecting fish growth performance.

Higher ability of effectively utilizing CRM, showed for African catfish and Nile tilapia may be a result of drastic physiological differences respect to pacus, specifically in digestive amylases and proteases composition and/or function. For instance, De Almeida *et al*. (2006) reported that the proteolytic activity in black pacu is mainly detected in the stomach, where acidic proteases prevail, as nonspecific protease activity in black pacu's intestine is very low since it like other herbivores/omnivores fish) does not express a considerable number of alkaline proteases, likely because its diet doesn't contain high levels of protein. However, most fish have better proteolytic activities into alkaline pH levels (De Almeida *et al*., 2006), placing the stomach as the main organ to digest protein in black pacu.

As has been said, the search of new ingredients for sustainable small-scale aquaculture feeds is an increasing need in most developing countries as feeding cost is the most expensive limiting factor for fish production (Adelizi *et al*., 1998). For instance in Pakistan, Asad *et al*. (2005) evaluated the digestibility of canola and guar meal in rohu (*Labeo rohita*), and determined that ADC for dry matter and protein were higher for canola when compared to guar meal (dry matter 70.3 ± 0.1 vs. 59.9 ± 0.7; crude protein 60.7 ± 2.4 vs. 50.3 ± 0.9, respectively). In Brazil, Henry-Silva *et al*. (2006) studied the digestibility of two aquatic macrophytes (*Eichornia crassipes* and *Pistia stratiotes*) in Nile tiapia (*Oreochromis niloticus*) and found that average ADC of crude protein were 59.2% for *E. crassipes*; and 52.2% for *P. stratiotes*, respectively, with no significant differences observed between the ADC of the plants ingredients. Finally in Nigeria, Osuigwe & Okoro (2008) determined the ADC crude protein and gross energy of processed (boiled) and unprocessed (raw) mucuna seed meal (*Mucuna cochinchinensis*) in hybrid catfish (*Heterobranchus longifilis* x *Clarius gariepinus*). It was found that average ADC of crude protein and gross energy were 46.3 and 63.1% for boiled; and 40.2 and 41.2% for raw mucune seed meal, respectively. The results reported in the three studies cited only noted the low nutrient availability of these alternative feedstuffs tested for rohu, Nile tilapia and hybrid catfish. Although the nutrient availability may be limiting in these feedstuffs, now that this information is known, they may be better utilized (or avoided, as necessary) in locally produced aquafeeds.

Digestibility coefficients of the traditional feedstuffs used in pacus compound diets have been previously determined by Fernandes *et al*. (2004) and Gutiérrez *et al*. (2009). When compared to other common feedstuffs, both protein and lipid ADC of PPM are quite superior of those reported for soybean meal and wheat bran in red pacu and similar to ADC values estimated for corn. As for black pacu, dry matter, protein and lipids ADC values reported for cooked PPM revealed to be even better than ADC showed by Gutiérrez *et al*. (2009) for corn in the same species (Table 4). Without a doubt, peach palm's natural abundance in the Amazon region makes it economically viable for aquaculture purposes contributing to help small-scale farmers to reduce feeding cost, which remarkedly is the most critical limitation for the development of aquaculture in the Peruvian Amazon nowadays. As a general rule, ADC values obtained for GPM and CRM were always lower than ADC values reported for corn and wheat bran in both fishes (Fernandes *et al*., 2004; Gutiérrez *et al*., 2009).

Comparisons between ADCs for PPM, GPM and CRM obtained in this study and the ADCs obtained by other authors in black and red pacu are showed in Table 3.4. Again, only ADC levels of PPM are clearly superior to those obtained for alternative and common feedstuffs such as dende meal, wheat and similar to corn. In fact, using peach palm as a feedstuff is nothing new. Mori-Pinedo *et al*. (1999) used PPM in three different levels (replacing corn while raising black pacu fingerlings in Brazil), having demonstrated that PPM meal can completely substitute corn without negatively affecting fish growth and body composition.

An additional advantage of PPM is its low cost. Compared with corn grains and wheat derivatives, PPM can be inexpensive in the Brazilian, Colombian, and Peruvian Amazon, and its nutrient and energy apparent digestibility values suggest that it can be used at high levels of replacement for corn (as suggested by Mori-Pinedo *et al*., 1999) or wheat derivatives (as suggested by Lochmann *et al*., 2009), in diets for either black or red pacu.

In conclusion, evaluation of the ADC for dry matter, crude protein, crude lipid and gross energy indicated that PPM (cooked or raw) is the most digestible indigenous feedstuff for black and red pacu when compared to raw or cooked CRM and GPM. Energy and nutrient ADCs obtained in this investigation support PPM as the primary choice for low-cost pacu aquafeeds in the Peruvian Amazon.

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