Digestibility coefficients of sun dried and fermented aquatic macrophytes for Cachama blanca, *Piaractus brachypomus* (Cuvier, 1818)

Coeficientes de digestibilidad de macrófitas acuáticas, secadas al sol y fermentadas, para Cachama blanca, *Piaractus brachypomus* (Cuvier, 1818)

Coeficientes de digestibilidade de macrófitas aquáticas, secas ao sol e fermentadas, para a Pirapitinga, *Piaractus brachypomus* (Cuvier, 1818)

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Abstract

The apparent digestibility coefficients of dry matter ($ADC_{drymatter}$), crude protein ($ADC_{protein}$) and the gross energy (ADC_{energy}) of the aquatic macrophytes *Spirodela polyrhiza, Lemna minor* and *Azolla filiculoides* treated by sun drying and lactic acid fermentation were determined for juveniles (31.0 ± 5.2 g) of Cachama blanca, *Piaractus brachypomus*. Each test ingredient was included at 300 g kg^{-1} in a semipurified reference diet containing chromium oxide as an indicator. Faeces were collected by a specific sedimentation system. The $ADC_{drymatter}$, $ADC_{protein}$ and ADC_{energy} of the reference diet were 56.8%, 97.2% and 70.1%, respectively. The 3 x 2 factorial analysis of variances indicated that the digestibility coefficients of the tested macrophytes were significantly different between plant material and treatments, but not between their combinations. $ADC_{protein}$ ranged from 74.9% to 84.5% in fermented macrophytes and from 51.1% to 60.4% in sundried macrophytes, indicating that the fermentation process had increased the protein digestibility of the aquatic plants ($P<0.05$). Among the plants, fermented *Spirodela polyrhiza* showed the highest nutrient and energy digestibility. Lactic acid fermentation is a highly recommendable treatment when aquatic macrophytes should be include into diets for Cachama blanca.

Keywords: Cachama, protein digestiblity, energy digestibility, fermentation

Resumen

Los coeficientes de digestibilidad aparente de la materia seca (ADC materia seca), la proteína cruda (ADC proteína cruda) y la energía bruta (ADC energía bruta) de las macrófitas acuáticas *Spirodela polyrhiza, Lemna minor* y *Azolla filiculoides*, tratadas mediante secado al sol y fermentación ácido láctica, fueron determinados para juveniles de Cachama blanca, *Piaractus brachypomus* (31.0 ± 5.2 g). Cada ingrediente de ensayo se incluyó en 300 g kg^{-1} en una dieta de referencia semipurificada que contenía óxido de cromo como indicador. Las heces se recogieron mediante un sistema de sedimentación específico. Los ADC materia seca, ADC proteína cruda y ADC energía bruta de la dieta de referencia fueron 56.8%, 97.2% y 70.1%, respectivamente. El análisis de varianzas factorial 3 x 2 indicó que los coeficientes de digestibilidad de las macrófitas estudiadas variaron significativamente entre el material vegetal y los tratamientos, pero no entre sus combinaciones. ADC proteína cruda varió de 74.9% a 84.5% en los macrófitas fermentadas y de 51.1% a 60.4% en los macrófitas secados al sol, lo que indica que el proceso de fermentación incrementó la digestibilidad de la proteína disponible en las plantas acuáticas ($P<0.05$). Entre las plantas estudiadas, la *Spirodela polyrhiza* fermentada mostró la mayor digestibilidad de los nutrientes y la energía. La fermentación ácido láctica es así un tratamiento altamente recomendable cuando se incluyen macrófitas acuáticas en las dietas para Cachama blanca.

Palabras clave: Cachama, digestibilidad de la proteína, digestibilidad de la energía, fermentación

Resumo

Os coeficientes de digestibilidade aparente da matéria seca (ADC matéria seca), a proteína bruta (ADC proteína bruta) e energia bruta (ADC energia bruta) de macrófitas aquáticas *Spirodela polyrhiza, Lemna minor* e *Azolla filiculoides* tratado por secagem ao sol e fermentação de ácido lático foram determinados para juvenil da Pirapitinga (31.0 ± 5.2 g). Cada ingrediente teste foi incluído a 300 g kg-1 em uma dieta de referência semipurificada contendo óxido de crómio como um indicador. As fezes foram coletadas através de um sistema de sedimentação específico. A ADC matéria seca, ADC proteína bruta e ADC energia bruta da dieta referência foram 56.8%, 97.2% e 70.1%, respectivamente. A análise fatorial de variância 3 x 2 indicaram que os coeficientes de digestibilidade das macrófitas estudadas variou significativamente entre os materiais e tratamentos de planta, mas não entre as suas combinações. A ADC Proteína bruta variou de 74.9% a 84.5% em macrófitas fermentadas e de 51.1% para 60.4% em macrófitas desidratadas, indicando que o processo de fermentação aumentou a digestibilidade de proteína disponíveis nas plantas aquáticas ($P< 0.05$). Entre as plantas estudadas, a Spirodela polyrhiza fermentada apresentou a maior digestibilidade dos nutrientes e energia. Fermentação de ácido láctico é, portanto, um tratamento altamente recomendado quando macrófitas aquáticas estão incluídas em dietas para a Pirapitinga.

Palavras-chave: Pirapitinga, digestibilidade da proteína, digestibilidade da energia, fermentação

Introduction

In feed formulation, the most important characteristics of the components are their nutrient quantity and quality as well as their digestibility, mainly digestible protein and energy (Austreng 1978; Fagbenro 1996). The apparent digestibility coefficients (ADCs) provide valuable information for the formulation of nutritional and economically feasible diets. Particularly, diet and dietary compound digestibility are essential for exact determination of nutrient demands in fish metabolism. In addition, ensuring a high diet digestibility preserves the aquatic environment by avoiding the accumulation of indigestible ingredients.

For the Amazonian fish *Piaractus brachypomus* as for the majority of characids, the scarce information on nutritional demand existent refers mainly to ingredients which are frequently used in common commercial aquafeeds, namely fish meal, soybean or soybean-derived products and wheat products (Quintero *et al.,* 1993; Hernández 1993; Torres adn Uribe 1995; Fernandes *et al.,* 2004; Gutierrez-Espinoza and Vásquez-Torres 2008). Considering increased prices of aquafeeds, the identification of less expensive and locally available feed sources has become necessary to assure the proposed aquaculture development, particularly in rural areas. In fact, the use of non-conventional feed sources is an important key to reduce feed costs in fish cultivation and therefore to ensure small-scale farmers' income in the marginal areas of the Neotropics.

Aquatic macrophytes are one of the most abundant plant materials in the Neotropical floodplain systems and a natural feed source for some native fish species. Although, they have been widely used as a nutrient source in diets for tropical freshwater fish, principally tilapia and carp (Edwards 1980; Ray and Das 1992; Bairagi *et al.,* 2002; El-Sayed 2003; Henry-Silva *et al.,* 2006), information on their nutritional value for *Piaractus brachypomus* is lacking.

In spite of the great nutritional potential of aquatic macrophytes, their utilization in native forms as fish feed remains low due to their relative high fibre and ash content as well as the presence of trypsin inhibitors, phytates, tannins and oxalates, among other antinutritional substances, which reduce their digestibility. To enhance the nutritional value of plant materials for fish, simple methods for processing suitable for marginal areas have been investigated. Thus, the fermentation process of aquatic macrophytes seems to be adequate, since it considerably reduces fibre content and antinutritional substances present in the plants (Bairagiet *al.,* 2002, El-Sayed 2003, Cruz *et al.,* 2011).

The South American fish Cachama blanca or Pirapitinga (common names in Colombia and Brazil, respectively) *Piaractus brachypomus* (Characidae), is an omnivorous fish with a predominantly herbivorous-feeding behaviour (Silva 1985). Its natural diet consists principally of plant products and in a lower amount of fish and crustacean. It is widely found in the Amazon and Orinoco river basins (Jégu 2003) and one of the most important native fish species in Colombia. Moreover, it is the primary fish species in the Colombian national program of food safety and occupies the second place in the Colombian national aquaculture production (Espinal *et al.,* 2005).

Considering that processed aquatic macrophytes may be a suitable source of plant protein for the small-scale characid production in Colombia, the present study was conducted to determine, dry matter, crude protein and gross energy digestibility of selected aquatic macrophytes (*Spirodela polyrhiza, Lemna minor* and *Azolla filiculoides*) treated by sun drying and lactic acid fermentation methods for juveniles of *Piaractus brachypomus.*

Material and methods

Sun Drying and Fermentation of Aquatic Plants

The aquatic macrophytes used for experimental diets were harvested as wild or uncultivated plant material from water bodies in northern Colombia. Plants were collected continuously during a period of 12 weeks. After taxonomical identification, the plant material was cleaned and divided in two parts. The first part was sundried until its dry weight was constant. The second part was fermented. In order to obtain the required DM content for silages, which is approximately 300-400 g kg^{-1} for grass and forages (DLG 2006), the freshly harvested aquatic plants were mixed with wilted aquatic plants of the same sample. Afterwards, these mixtures were fermented.

The lactic acid silage fermentation process was carried out using a commercial silage lactic acid bacteria (LAB) inoculant as described by Cruz *et al.,* (2011) following to the methodology proposed by Johnson *et al*., (2005). Finally, the silages were opened and dried in an oven at 45 °C for 48 h before their inclusion into the experimental diets.

Experimental Diets

The six experimental diets were composed of 700 g kg^{-1} of a reference diet based on a semipurified diet performed by Vásquez-Torres *et al.,* (2002) and 300 g kg^{-1} of each tested ingredients: *Spirodela polyrhiza* (Giant Duckweed), *Lemna minor* (Duckweed) and *Azolla filiculoides* (Fern Azolla) treated by sun drying or lactic acid fermentation (Table 1.1). Chromic oxide (Cr_2O_3) at an inclusion level of 5 g kg^{-1} was used as external inert marker. Diets were finely ground, mixed and pelleted to sizes of 4 mm.

Animals and Feeding Trial

A total of 630 juveniles of *Piaractus brachypomus* with a similar initial body weight of 31.0 ± 5.2 g (mean ± SD) were randomly stocked in 21 experimental 250L-tanks. The experiment was conducted in a closed recirculation-system with constant aeration and a photoperiod of approximately 12 h of light. The water quality was monitored daily in the morning with respect to temperature, pH, and dissolved oxygen. Prior to the experiment, the fish were fed with the reference diet twice daily and for a period of two weeks in order to accustom them to the experimental conditions. Afterwards, seven experimental diets (one reference diet and six test diets) were randomly assigned to triplicate experimental groups. Fish were manually fed the experimental diets once daily to apparent satiety.

For faeces collection nine cylindrical-conical tanks (200 L) equipped with a terminal faeces removal container system as described by Vásquez-Torres *et al*., (2011a) were used. Stocked fish of each experimental tank were transferred to the cylindrical-conical tanks after feeding. Faeces collection was done according to a specific schedule. The samples were collected at 60 min intervals over a period of 12 h per day during two wks. The faeces samples removed from the containers were subsequently rinsed in distilled water, oven dried at 60 °C for 72 h and stored until laboratory analysis.

Chemical Analysis

The nutrient composition of the sundried and fermented material, as well as the diets and the faecal samples collected from each tank were determined in triplicates and performed following the AOAC (1990) procedures. The energy content as gross energy was determined by using an adiabatic bomb calorimeter (PARR 121 EA, USA).

Table 1.1. Composition of the reference and test diets evaluated for *Piaractus brachypomus.*

Ingredients (g kg^{-1})	Reference Diet (Ref)	Test Diet
Caseine	354	248
Gelatine	43	30
Dextrine	242	169
α-Cellulose	180	126
Carboximetil Cellulose	68	48
Fishoil	33	23
Sunfloweroil	33	23
Vitamin premix[1]	2	1
Microminerals premix[2]	1	1
Macrominerals premix[3]	38	27
Ascorbicacid (Stay C-35)	1	1
Chromiumoxide (Cr_2O_3)	5	4
Test Ingredient	0	300

1 Rovimix vitamin: ®Lab. Roche S.A. 0.5 (Vit A 8.0*10^6 UI, Vit D3, 1.8*10^6 UI, Vit E 66.66 g, Vit B1 6.66 g, Vit B2 13.33 g, Vit B6 6.66 g, Calcium pantothenic 33.33 g, Biotin 533.3 mg, Folic acid 2.66 g, Ascorbic acid 400.0 g, Nicotinic acid 100.0 g, Vit B12 20.0 mg, Vit K3 6.66 g, csp vehicle 1.0 kg.

2 Micro-minerals premix: ®Lab. Roche S.A. 1.0 (Composition per 100 g the product: Mg 1.0, Zn 16.0, Fe 4.0, Cu 1.0, I 0.5, Se 0.05, Co 0.01).

3 Macro-minerals premix: 4.02 (Composition g 100 g^{-1} the product: Ca (H_2PO_4) 13.6 g, Calcium lactate 34.85 g, $2MgSO_4.7H_2O$ 13.2 g, KH_2PO_4 24 g, NaCl 4.5 g, $AlCl_3$ 0.015 g, CMC 9.835 g).

Calculations

The amount of inert marker (Cr_2O_3) contained in the diets and the faecal samples was determined by wet-acid digestion method (Furukawa & Tsukahara 1966). The ADCs for the nutrients and energy of the reference and test diets were calculated using the formula described by Nose (1960), as follows:

$$ADC_{diet} = 100 - \left\lfloor 100 \times (\%Cr_2O_{3diet} / \%Cr_2O_{3faeces}) \times (\%Nut_{faeces} / \%Nut_{diet}) \right\rfloor$$

Where:

ADC_{diet} = Apparent digestibility coefficient of the nu trients or energy in diets
$\%Cr_2O_{3diet}$ = % of chromium content in diets
$\%Cr_2O_{3faeces}$ = % of chromium content in faeces
$\%Nut_{diet}$ = % of nutrient or energy in diets
$\%Nut_{faeces}$ = % of nutrient or energy in faeces

ADCs of the test ingredients were calculated based on the digestibility of the reference diet and test diets using the formula suggested by Bureau *et al.,* (1999), which considers the relative nutrient contribution from the basal diet and the test single ingredients (Bureau andHua 2006).

$$ADC_{ing} - ADC_{comdiet} + \left\lfloor \begin{matrix} (ADC_{comdiet} - ADC_{refdiet}) \times \\ (0.7 \times \%Nut_{refdiet} / 0.3 \times \%Nut_{ing}) \end{matrix} \right\rfloor$$

Where:

ADC_{ing} = Apparent digestibility coefficient of the nutrients or energy in test ingredients
$ADC_{testdiet}$ = Apparent digestibility coefficient of the nutrients or energy in test diets
$ADC_{refdiet}$ = Apparent digestibility coefficient of the nutrients or energy in reference diet
$\%Nut_{refdiet}$ = % of nutrient or energy in reference diet
$\%Nut_{ing}$ = % of nutrient or energy in ingredients

Digestible protein (DP) and digestible energy (DE) were calculated using the data corresponding to protein and energy digestibility, crude protein (CP) and gross energy (GE) content of test ingredients.

$$DP_{ing} = (\%CP_{ing} \times ADC_{ing}) / 100$$
$$DE_{ing} = ((kJ / g)GE_{ing} \times ADC_{ing}) / 100$$

Statistical Analysis

In order to assess the separate and combined effect of the plant material (three species) and treatment (sundried and fermentation) on the dry matter, protein and energy digestibility in Cachama blanca a 3 x 2 factorial analysis of variances (ANOVA) was conducted. The means and standard error of means (SEM) for dry matter, protein and energy digestibility as a function of the two factors are presented in Table 1.3.The *F* test and Tukey's test for Post Hoc comparisons ($P < 0.05$) were applied. Homogeneity of the variances was determined by Levene's Test and by Dunnett's T3 Test ($P < 0.05$) for homogeneous and inhomogeneous variances, respectively. All statistical analysis was carried out using the SPSS (version 19) software package.

Results

The water quality parameters presented during the experimental period were within optimal requirements for *Piaractus brachypomus,* reported by Vasquez-Torres (2005): temperature 25.6 ± 0.99 °C; pH 6.42 ± 0.16; and dissolved oxygen 5.28 ± 1.45 mg L^{-1}. All diets were satisfactorily consumed, indicating that no palatability problem was encountered with any of the treatments during the experiment.

The proximate composition of the test ingredients, reference diet and test diets (Table 1.2) indicates that the reference diet had the lowest ash (38.5 g kg^{-1}) and crude fibre (58.5 g kg^{-1}) content, and the highest crude protein (342.1 g kg^{-1}) and energy (19.7 kJ g^{-1}) content. Likewise, among test diets those containing fermented plants showed the lowest crude fibre and ash content, whereas protein and lipids contents were slightly higher compared to diets containing sundried macrophytes. The dietary gross energy was not different in the test diets.

The $ADC_{drymatter}$, $ADC_{protein}$ and ADC_{energy} of the tested ingredients were significantly different between plant material types and processing methods, but not between their combinations (Table 1.3). However, values tended to be higher for the fermented aquatic plants indicating the major effect of the processing method on the digestibility. Among test ingredients, the highest $ADC_{drymatter}$, $ADC_{protein}$ and ADC_{energy} were obtained for fermented macrophytes, whereas the significantly lowest value was displayed by the sundried macrophytes. Fermented *Spirodela polyrhiza* showed the highest $ADC_{drymatter}$ (84.6 %), $ADC_{protein}$ (84.5%) and ADC_{energy} (62.6 %), whereas sundried *Azolla filiculoides* showed the lowest $ADC_{drymatter}$ (43.8 %), $ADC_{protein}$ (51.1 %) and ADC_{energy} (37.8 %).

Discussion

High ash content negatively influence the digestibility of plant materials. The lower $ADC_{drymatter}$ of sundried aquatic macrophytes could be explained by their high mineral content (ash content) when compared to the fermented aquatic plants. Edwards (1980) reported that it reduces the nutritional value of aquatic macrophytes and it is considered as the main reason why animals refuse to eat them in large quantities. The lower $ADC_{protein}$ of sundried plants may be related to their high fibre content, which ranged closely to 140 g kg^{-1}, whereas in fermented plants the fibre content ranged between 69.9 and 101.6 g kg^{-1}. Similar results were reported by Fernandes *et al.*, (2004) in diets for *Piaractus brachypomus*, who attributed the low $ADC_{protein}$ of wheat bran (61.6%) to the high crude fibre content of wheat products (about 100 g kg^{-1} according to NRC 1993) as well as to its high level of phytates (8.8 g kg^{-1}). In general, the fibre content as well as the ash content was strongly diminished in diets containing fermented plants.

The $ADC_{protein}$ of fermented aquatic macrophytes (between 74.6 and 84.5%) may be compared to those

Table 1.2. Chemical composition (g kg^{-1}) and gross energy (kJ g^{-1}) of the ingredients, reference and test diets in the digestibility trials. Values (mean ± SD) are expressed on a dry matter basis.

	Ash	Crude protein	Crude lipids	Crude Fibre	NFE[1]	Gross Energy
Ingredients						
FermentedSpirodela(FS)	241.9	229.7	38.4	76.3	413.8	14.9
FermentedLemna(FL)	246.3	227.9	37.3	69.9	418.6	14.6
FermentedAzolla(FA)	205.1	224.3	34.8	101.6	434.2	13.7
SundriedSpirodela(SS)	297.8	149.3	23.8	142.4	386.7	14.1
SundriedLemna(SL)	375.5	135.7	12.7	141.5	334.6	14.1
SundriedAzolla(SA)	343.3	145.8	13.6	142.0	355.2	13.3
Test diets						
Reference Diet (Ref)	38.5	342.1	21.8	58.5	539.1	19.7
Ref+FS	90.3	310.5	31.7	63.9	503.7	17.9
Ref+FL	91.8	312.7	31.4	61.9	502.1	17.9
Ref+FA	79.1	319.7	34.3	71.4	495.5	17.5
Ref+SS	116.7	283.4	22.5	84.5	492.9	17.6
Ref+SL	141.5	279.4	19.0	85.5	474.8	17.5
Ref+SA	130.8	283.9	18.6	85.3	481.4	17.4

[1] Nitrogen-free Extract (NFE) = 100 - (Ash + Protein + Fibre + Fat)

Table 1.3. Apparent digestibility coefficients of dry matter, crude protein (CP) and gross energy (GE) of the test ingredients (mean ± SD, n = 3).

	ADC dry matter (%)	ADC crude protein (%)	ADC gross energy (%)	Digestible CP (g kg^{-1})	Digestible GE (Kj/g)
Test Ingredients	**Mean ± SD**				
FermentedSpirodela	84.6 ± 6.7	84.5 ± 2.4	62.6 ± 8.7	194 ± 5.9	9.3 ± 1.2
FermentedLemna	68.1 ± 3.6	77.8 ± 1.7	56.6 ± 3.5	177 ± 5.6	8.2 ± 0.7
FermentedAzolla	65.8 ± 4.5	74.6 ± 2.1	47.2 ± 5.9	167 ± 3.6	6.4 ± 0.8
SundriedSpirodela	61.3 ± 3.6	60.4 ± 1.2	51.1 ± 3.2	90.1 ± 0.8	7.1 ± 0.4
SundriedLemna	56.6 ± 7.7	55.4 ± 4.3	48.4 ± 5.1	75.1 ± 6.1	6.8 ± 0.6
SundriedAzolla	43.8 ± 2.3	51.1 ± 1.4	37.8 ± 2.5	74.5 ± 4.5	5.0 ± 0.4
Plants (P)	**Mean values**				
Spirodela	69.93 ^{a}	72.42 ^{a}	56.82 ^{a}	142.08 ^{a}	8.20 ^{a}
Lemna	62.37 ^{ab}	66.58 ^{b}	52.52 ^{a}	126.20 ^{b}	7.52 ^{a}
Azolla	54.8 ^{b}	62.85 ^{c}	42.47 ^{b}	120.96 ^{b}	5.72 ^{b}
SEM	2.078	0.977	2.144	1.954	0.289
Treatment (T)					
Lacticacid ferm.	70.8 ^{a}	79.0 ^{a}	55.4 ^{a}	179.6 ^{a}	7.98 ^{a}
Sun drying	53.9 ^{b}	55.6 ^{b}	45.7 ^{b}	79.9 ^{b}	6.31 ^{b}
SEM	1.697	0.798	1.750	1.595	0.236
Statistics	**F values**				
Plants (P)	13.26 *	24.33 *	11.80 *	31.67 *	19.66 *
Treatment (T)	49.79 *	428.59 *	15.39 *	1951.71 *	24.89 *
Interaction PxT	1.631 ^{NS}	0.190 ^{NS}	0.149 ^{NS}	2.281 ^{NS}	0.488 ^{NS}

NS = Not significant,
* = Significant ($P<0.05$)
^{abc} Means in same column without common superscript are different at $P<0.05$

reported for ingredients commonly used in diets for *Piaractus brachypomus*. The $ADC_{protein}$ of toasted whole soybean (81.1%), soy cake (83.2%) (Gutiérrez-Espinosa & Vásquez-Torres 2008), wheat bran (82.9%), corn gluten (84.3%), wheat bran (82.9%) (Vásquez-Torres *et al.,* 2007), raw whole soybean (75.6%) and corn (85.1%) (Fernandes *et al.,* 2004) were nearly related to those obtained for fermented aquatic plants in the present study, particularly for fermented *Spirodela*. Lower $ADC_{protein}$ were found in wheat bran (61.6%) (Fernandes *et al.* ,2004) and Colombian fish meal (68.5%) (Vásquez-Torres *et al.,* 2007), whereas higher $ADC_{protein}$ were reported by Fernandes *et al.* (2004) for Brazilian fish meal (90.5%) and by Vásquez-Torres *et al.,* (2007) for palmiste (87.9%), yellow corn (87.2%) and sunflower cake (86.3%).

In another study with Pacu (*Piaractus mesopotamicus*), a closely-related species to *Piaractus brachypomus*, Abimorad *et al.* (2007) reported similar $ADC_{protein}$ for yeast (81.5%), Brazilian fish meal (84.6%) and corn (85.8%), whereas $ADC_{protein}$ obtained for wheat bran (87.7%), soybean meal (90.6%) and corn gluten meal (95.6%) were higher compared to those reported in the present study for fermented *Spirodela polyrhiza*.

As a consequence of their higher $ADC_{protein}$, fermented macrophytes showed a significantly higher digestible protein content (from 167 g kg^{-1} to 194 g kg^{-1}) than sundried macrophytes (from 74.5 g kg^{-1} to 90.1 g kg^{-1}). These results are also consistent to those previously reported for other warm water fish by several authors.

In earlier studies with Indian major carp, Ray and Das (1992) suggested that fermented macrophytes had a higher protein digestibility in relation to sundried macrophytes. More recently, Bairagi*et al.,* (2002) evaluated raw and fermented *Lemna polyrhiza* leaf meal in formulated diets for a fish of the carp family, Rohu (*Labeorohita*), and reported that the $ADC_{protein}$ for raw material meal was much lower at all levels of inclusion in comparison to the those obtained for the fermented meal. Also, El-Sayed (2003) demonstrated that fermentation of *Eichornia crassipes* may be necessary when it is incorporated into Nile tilapia diets at levels up to 20%.

Differences of $ADC_{protein}$ among plants could be attributed to the variances owing to the species. The population density, the ecological conditions, and the growth status of the plants at harvesting are all factors that affect their chemical composition. In fact, it must also be considered that plants were taken from natural water bodies instead of cultures.

Differences of ADC_{energy} and therefore digestible energy between treatments were found. All the energy values of the tested ingredients were relatively low and aquatic macrophytes can be described as a feed source poor in lipids and soluble carbohydrates, which do not possess desirable features to be used as an energy source. Interestingly, the ADC_{energy} obtained for both fermented (between 47.2% and 62.6%) and sundried macrophytes (between 37.8% and 51.1%) did not differ largely from those reported in the literature for common plant ingredients used in diets for *Piaractus brachypomus*. Gutiérrez-Espinosa and Vásquez-Torres (2008) reported similar ADC_{energy} for toasted whole soybean (59.1%) and soybean cake (59.9%). Closely related ADC_{energy} were reported by Vásquez-Torres *et al.,* (2007) for palmiste (52.6 %), wheat bran (49.6 %) and sunflower cake (46.9%). Likewise, in diets for *P. mesopotamicus*, Abimorad and Carneiro (2004) also reported comparable ADC_{energy} for alcohol yeast (45.8%) and cottonseed meal (59.6%).

The low ADC_{energy} can be also attributed to the relatively high content of indigestible fibre in the tested aquatic macrophytes, since fibre might reduce the nutrient and energy availability of ingredients (Robinson and Li 2006). The most obvious difference between diets containing fish meal and alternative plant ingredients is the lower available digestible energy content.

Digestibility of non-conventional plant material is highly variable depending not only on the processing methods and the dietary inclusion level but on the tested fish species. Studies involving *Piaractus* regularly relate the high digestibility of several ingredients to morphological and histological advantages of the digestive system of this genus (Abimorad *et al.,* 2007). *Piaractus brachypomus* is a typical omnivorous fish, although some aspects of its digestive tract morphology present similarities with carnivorous species as the trout, the Bacalao and the striped Bass. One of those characteristics is the presence of a clearly defined stomach and pyloric caeca (Muñoz *et al.,* 2006). Further, Pacu possesses pharyngeal teeth (Muñoz *et al.,* 2006), which participate in the crushing of diverse materials before reaching the stomach (Vásquez 2001). Also, as corresponded to omnivorous species, its intestine length is long compared to the standard length of fish (Muñoz *et al.*, 2006).

The presence of the stomach is an important feature from a perspective of increased ability of fish to digest complex proteins and therefore to adapt to variable diets (Grabner and Hofer 1989), since the gastric glands present in the stomach secret pepsin and hydrochloric acid with the purpose to contribute to the enzymatic degradation of foods in the stomach (Kaye *et al.,* 1995; Heath and Young 2000). Thus, fish species of the genus *Piaractus* are desired for using unconventional plant ingredients as nutrient sources in the rural aquaculture.

In conclusion, this study showed that $ADC_{drymatter}$, $ADC_{protein}$ and $ADC_{energy}$of fermented aquatic macrophytes were significantly higher than those of sundried aquatic macrophytes. Thus, fermented aquatic macrophytes, particularly *Spirodela polyrhiza, Lemna minor*, and *Azolla filiculoides* can be recommended as dietary ingredients into diets for the Cachama blanca (*Piaractus brachypomus*).

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