



Digestibility of energy, lipids and fatty acids of vegetable oils and poultry fat by pacu *Piaractus mesopotamicus* (Holmberg 1887)

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Abstract

Evaluation of vegetable oils and poultry fat digestibility is the first step to elicit their use in aquafeeds. This work aimed at determining apparent digestibility coefficients (ADCs) of energy, lipids and fatty acids of oil sources for pacu, a widely farmed neotropical Characin. A semipurified, omnivorous fish diet (344.2 g kg⁻¹ crude protein; 18.16 MJ kg⁻¹ gross energy) was used as reference diet. Test diets were obtained by adding 2 g kg⁻¹ chromium III oxide and replacing 15 g kg⁻¹ reference diet with fish, soybean, colza, corn and flaxseed oils and poultry fat. Juvenile pacu (64 ± 10.8 g; 14.6 ± 1.1 cm) were fed to apparent satiety, four times a day, and then transferred to cylindrical-conical aquaria for collection of faeces by sedimentation ($n = 3$). Apparent digestibility coefficients of energy and lipids were high for all tested oils ($P > 0.05$); ADCs of saturated fatty acid (SFA) were lower than monounsaturated fatty acids (MUFA) and polyunsaturated fatty acid (PUFA). Essential fatty acids (18:2n-6 and 18:3n-3) had high ADC (>93%), colza oil and poultry fat yielding the lowest ADC for 18:2n-6 ($P = 0.01$) and 18:3n-3 ($P < 0.01$), respectively. Corn oil, soybean oil and flaxseed oil were interesting sources of 18:2n-6 and 18:3n-3 dietary fatty acids for pacu.

KEY WORDS: apparent digestibility, colza oil, corn oil, flaxseed oil, neotropical fish, soybean oil

Received 24 March 2013; accepted 7 July 2013

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Introduction

There is widespread concern about the stagnation of world fishery resources, and reducing use of fish meal and fish oil in aquaculture feeds is an immediate need (Sargent & Tacon 1999; Naylor *et al.* 2000; Tacon & Metian 2008). Fish oil is a source of highly unsaturated fatty acid (HUFA), such as eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), essential for the nutrition of marine fish, given that C18 polyunsaturated fatty acid (PUFA) alone cannot meet their essential fatty acids (EFAs) requirements (Sargent *et al.* 1999; National Research Council 2011). On the other hand, EFA requirements of freshwater fish can be met by dietary 18:3n-3 and 18:2n-6 (Chou & Shiao 1999; National Research Council 2011). Therefore, the use of vegetable oils as surrogate lipid sources in aquafeeds is technically possible because they have high C18 fatty acid contents (Turchini *et al.* 2009). Bureau & Meeker (2010) also consider poultry fat, a by-product of the poultry industry in which fatty acid contents vary with the composition of the poultry diet, another viable alternative to fish oil.

Because of their availability and competitive prices, inclusion of vegetable oils and poultry fat in aquafeeds has become a consolidated practice (Brown & Hart 2010). It has actually been proved that some marine and freshwater fish fed diets containing vegetable oils and animal fats as partial replacement of fish oil as lipid source, do not present impaired growth nor low disease resistance (Caballero *et al.* 2002; Izquierdo *et al.* 2003; Montero *et al.* 2003; Turchini *et al.* 2003; Shapawi *et al.* 2008; Vargas *et al.* 2008).

Studies on dietary protein and energy requirements (Bicudo *et al.* 2010), lysine requirement (Bicudo *et al.* 2009; Abimorad *et al.* 2010) and digestibility of feedstuffs (Vidotti *et al.* 2002; Abimorad & Carneiro 2004; Abimorad *et al.*

2008, 2010; Stech *et al.* 2010) by pacu (*Piaractus mesopotamicus*), a widely farmed, freshwater, neotropical Characin (Urbinati *et al.* 2010), are at hand. However, in spite of the importance of oils as dietary energy sources and of fatty acid requirements for fish growth and development, research efforts for determining their digestibility for farmed, neotropical freshwater fish are still scarce, especially regarding pacu.

Vegetable oils and poultry fat are considered the main sustainable alternative for replacement of fish oil in aquafeeds, so evaluating their digestibility is the first step to elicit their use in the formulation and processing of diets for aquatic animals. The aim of this study was thus determining apparent digestibility coefficients (ADCs) of energy, lipids and fatty acids of fish oil, vegetable oils (colza, corn, flaxseed and soybean), fish oil and poultry fat for pacu.

Material and methods

Diets

Determination of ADCs of lipids was performed by the indirect method, using chromic oxide III (Cr_2O_3) as inert market (2 g kg^{-1}). A semipurified, omnivorous fish feed formulated to meet the nutritional requirements of pacu (Bicudo *et al.* 2009, 2010) was used as reference diet (RD; Table 1), and test diets were obtained by replacing 15% RD by colza, corn, flaxseed, marine fish and soybean oils and poultry fat (Table 2). Feed ingredients were ground to a 0.9-mm particle size and mixed; test oils were added to the mixture, which was then moistened (10% water:volume), pelleted into 4.0-mm granules and oven dried (forced air; 40°C ; 24 h). Dry pellets were stored in plastic bags and frozen (-20°C). Aliquots of diet for immediate use were kept in Styrofoam boxes at room temperature.

Fish, feeding practices and sampling procedures

Juvenile pacu *Piaractus mesopotamicus* ($64.0 \pm 10.8 \text{ g}$; $14.6 \pm 1.1 \text{ cm}$ of total length) were stocked in polyethylene tanks (800 L; 35 fish per tank) and acclimated to laboratory conditions for 20 days. Feeding tanks ($n = 3$) were set in a closed loop system; temperature and dissolved oxygen of recirculating water were maintained by boilers connected to thermostat in the reservoir ($28 \pm 1^\circ\text{C}$), and porous diffusers coupled to a central air blower set in each tank.

Fish were hand-fed to apparent satiation four daily meals (8:00 am, 11:00 am, 14:00 pm, and 16:00 pm) during 7 days. On the last day and 2 h after the last meal, fish

Table 1 Formulation and chemical composition of reference diet

Ingredients	Contents
Corn starch (g kg^{-1})	329.3
Corn gluten (g kg^{-1})	239.5
Soybean meal (g kg^{-1})	149.7
Casein (g kg^{-1})	129.8
Soybean oil (g kg^{-1})	39.9
Dicalcium phosphate (g kg^{-1})	34.9
Cellulose (g kg^{-1})	34.7
Carboxymethylcellulose (g kg^{-1})	30.0
Mineral and Vitamin mix (g kg^{-1}) ¹	10.0
BHT (g kg^{-1}) ²	0.2
Chromic oxide (g kg^{-1})	2.0
Proximate composition (dry matter basis)	
Moisture (g kg^{-1})	61.7
Protein (g kg^{-1})	344.2
Lipid (g kg^{-1})	57.7
Ash (g kg^{-1})	49.8
Carbohydrates (g kg^{-1}) ³	548.3
Energy (MJ kg^{-1})	18.16

¹ Mineral mix (Premix Nutrifish Guabi[®]; Campinas, SP, Brazil) per kg of product: Fe 15 000 mg; Cu 2500 mg; Zn 12 500 mg; I 375 mg; Mn 12 500 mg; Se 87.5 mg; Co 125 mg; vitamin A 2 500 000 IU; vitamin D3 600 000 IU; vitamin E 37 500 IU; vitamin K 3750 mg; vitamin C 50 000; vitamin B1 4000 mg; vitamin B2 4000 mg; vitamin B6 4000 mg; vitamin B12 4000 µg; pantothenic acid 12 000 mg; biotin 15 mg; folic acid 1250 mg; niacin 22 500 mg; BHT 15 000 mg.

² Butyl-hydroxy-toluene.

³ Carbohydrates = 100 – (protein + lipid + ash).

were transferred to cylindrical, conical-bottomed aquaria (250 L) under aeration and continuous water exchange, equipped with refrigerated plastic bottles for faeces collection (Portz & Cyrino 2004). Faecal material was collected in the morning (7:00 am) and a total of three faecal samples per diet were collected. Faeces samples were frozen, lyophilized and stocked in ultra-freezer (-80°C) until subsequent analysis.

Chemical analyses and determination of ADC

The gross energy of feedstuffs, diets and faeces was determined in adiabatic calorimeter bomb, benzoic acid standard. Lipid extractions were performed using chloroform and methanol as solvents (Bligh & Dyer 1959). Saponification and methylation were performed according to Morrison & Smith (1964). The fatty acid methyl esters were separated using a gas chromatograph (Thermo Scientific, TR-FAME: 120 m × 0.25 mm i.d.; film thickness 0.20 µm), and the fatty acids were identified by comparing the measured fatty acids to the retention times of standard fatty acids (SUPELCO 37 comp. FAME Mix 10 mg mL⁻¹

Table 2 Selected fatty acid contents of tested oils

Fatty acid	Colza oil	Corn oil	Flaxseed oil	Fish oil	Poultry fat	Soybean oil
	% of total fatty acid					
14:0	0.07	0.03	n.d.	6.58	0.58	0.10
16:0	4.67	12.01	6.22	19.28	19.72	11.11
18:0	2.53	2.19	5.56	3.81	5.49	3.82
20:0	0.70	0.70	0.21	n.d.	0.12	0.23
16:1n-7	0.24	0.11	0.06	8.24	5.88	0.07
18:1n-9	62.24	35.49	20.64	8.56	36.57	24.85
20:1n-9	1.11	0.25	0.11	0.65	0.18	0.47
18:2n-6	19.46	47.84	14.68	17.80	25.35	52.88
18:3n-6	0.16	n.d.	0.15	0.08	0.10	0.32
18:3n-3	7.65	0.72	51.88	1.70	0.30	5.24
20:5n-3	n.d.	n.d.	n.d.	17.27	n.d.	n.d.
22:6n-3	n.d.	n.d.	n.d.	11.25	n.d.	n.d.
ΣSFA	8.50	15.35	12.29	31.31	28.32	15.74
ΣMUFA	63.74	35.85	20.84	17.75	43.30	25.43
ΣPUFA	27.31	48.69	66.75	50.59	26.26	58.53
Σn-6	19.66	47.97	14.87	19.63	25.87	53.29
Σn-3	7.65	0.72	51.88	30.96	0.39	5.24
n-3/n-6	0.39	0.02	3.49	1.58	0.02	0.10
Proximate composition (dry matter basis)						
Lipid, g kg ⁻¹	996	999	999	996	979	997
Energy (MJ kg ⁻¹)	39.10	39.36	39.00	38.96	39.10	39.26

n.d., not detected.

in CH_2Cl_2 ; internal standard C23:0). Concentrations of chromium oxide III were quantified by atomic absorption spectrophotometry after acid digestion. Apparent digestibility coefficients of diets were calculated as: $100 - [(100 \times (\% \text{ feed marker}/\% \text{ faeces marker}) \times (\% \text{ nutrient or gross energy in faeces}/\% \text{ nutrient or gross energy in feed}))]$; ADCs of lipids, energy and fatty acids of the test oils were calculated according to Forster (1999) and National Research Council (2011), as follows:

$$\text{ADC}_{\text{TI}} = \text{ADC}_{\text{TD}} + [(\text{ADC}_{\text{TD}} - \text{ADC}_{\text{RD}}) \times (0.85 \times D_R/0.15 \times D_i)] \quad (1)$$

where ADC_{TI} = apparent digestibility coefficient of test ingredient; ADC_{TD} = apparent digestibility coefficient of test diet; ADC_{RD} = apparent digestibility coefficient of reference diet; D_R = % of nutrient or MJ kg⁻¹ gross energy of the reference diet; and D_i = % nutrient or MJ kg⁻¹ gross energy of the ingredient.

Statistical analyses

Data were tested for normality and homogeneity of variances, and then submitted to ANOVA ($\alpha = 0.05$), SAS Software (SAS Institute, Cary, NC, USA). Comparisons of means were done by Tukey test ($\alpha = 0.05$).

Results and discussion

Lipids, especially fatty acids, are the preferred source of digestible energy for fish (Tocher 2003; Lim *et al.* 2011). All lipid sources were effectively digested (>96%) by pacu, irrespective of type and origin (Table 3). Apparent digestibility coefficients of energy recorded for all dietary oils exceeded 85%, and no significant differences were detected among treatments ($P > 0.05$; Tables 3 and 4). Results are comparable to those reported for marine species such as the Australian shortfin eel, *Anguilla australis* (Gunasekera *et al.* 2002) and the Atlantic halibut, *Hippoglossus hippoglossus* (Martins *et al.* 2009), fed vegetable and animal oils, but digestibility of flaxseed oil for the Australian shortfin eel (90.2%) was lower than that of cod liver oil (95.6%) and sunflower oil (94.9%) (Gunasekera *et al.* 2002). The higher concentrations of both 16:0 and 18:0 in animal lipid elicited higher ADC of diets containing vegetable lipids (93–95%; flaxseed, colza and sunflower oil), in comparison with diets containing animal lipid (90%; herring oil and poultry fat) for Atlantic halibut (Martins *et al.* 2009).

Dietary lard showed lower digestibility of lipids (79.2%), but combinations of vegetable and fish oils allowed higher ADCs (91–94%) by rainbow trout *Oncorhynchus mykiss* (Caballero *et al.* 2002). As a rule, inclusion of vegetable oils in the diet did not hamper growth of many freshwater fish, such as rainbow trout, brown trout *Salmo trutta* L,

Table 3 Apparent digestibility coefficients (ADC) of selected fatty acids in pacu fed the diets with the experimental oils

Fatty acid	ADC						Statistics	
	Colza oil	Corn oil	Flaxseed oil	Fish oil	Poultry fat	Soybean oil	P value	C.V ¹
%								
14:0	84.6±2.7 ^c	97.0±0.0 ^a	n.d.	94.5±0.5 ^a	91.7±0.1 ^{ab}	87.7±1.1 ^{bc}	<0.01	2.55
16:0	89.5±0.5 ^a	87.8±1.2 ^{ab}	83.4±1.5 ^b	89.0±1.1 ^a	89.0±0.4 ^a	89.7±0.5 ^a	<0.01	1.87
18:0	88.0±0.9 ^a	81.5±2.5 ^{ab}	83.8±0.7 ^{ab}	80.6±2.2 ^b	83.6±1.0 ^{ab}	83.5±1.1 ^{ab}	0.04	3.22
20:0	85.7±1.8	80.5±2.9	87.3±0.4	n.d.	85.4±0.9	85.1±0.7	<0.01	10.05
16:1n-7	90.9±0.3 ^a	97.0±0.0 ^a	63.0±6.5 ^b	95.7±0.4 ^a	95.9±0.0 ^a	87.8±1.2 ^a	<0.01	5.30
18:1n-9	94.0±0.7	94.6±0.3	94.5±0.2	93.9±0.7	95.3±0.1	95.4±0.1	0.16	0.83
20:1n-9	94.7±0.9 ^{ab}	97.0±0.0 ^a	93.2±0.5 ^b	95.3±0.6 ^{ab}	95.7±0.1 ^a	95.4±0.1 ^{ab}	<0.01	0.91
18:2n-6	93.5±0.7 ^b	95.2±0.2 ^{ab}	94.9±0.1 ^{ab}	95.04±0.5 ^{ab}	95.5±0.1 ^a	95.9±0.1 ^a	0.01	0.68
18:3n-6	97.0±0.0 ^a	n.d.	90.8±0.0 ^d	97.0±0.0 ^a	95.7±0.1 ^c	96.3±0.1 ^b	<0.01	0.07
18:3n-3	95.0±0.8 ^{ab}	95.9±0.0 ^a	96.3±0.0 ^a	96.3±0.2 ^a	93.5±0.2 ^b	96.5±0.0 ^a	<0.01	0.62
20:5n-3	n.d.	n.d.	n.d.	96.52±0.17	n.d.	n.d.	—	—
22:6n-3	n.d.	n.d.	n.d.	96.47±0.17	n.d.	n.d.	—	—
ΣSFA	88.0±0.9 ^{ab}	85.7±1.6 ^{ab}	83.1±1.2 ^b	88.8±1.1 ^a	87.6±0.5 ^{ab}	87.3±0.7 ^{ab}	0.02	2.08
ΣMUFA	94.0±0.7	94.6±0.3	94.3±0.2	94.3±0.7	95.4±0.1	95.4±0.1	0.19	0.79
ΣPUFA	93.8±0.7 ^b	95.1±0.2 ^{ab}	95.8±0.0 ^a	95.8±0.3 ^a	95.2±0.1 ^{ab}	96.0±0.1 ^a	<0.01	0.61
Σn-6	93.5±0.7 ^b	95.2±0.2 ^{ab}	94.8±0.1 ^{ab}	95.2±0.5 ^{ab}	95.4±0.1 ^a	95.9±0.1 ^a	<0.01	0.66
Σn-3	95.0±0.8 ^{ab}	94.5±0.3 ^b	96.3±0.0 ^a	96.5±0.2 ^a	93.3±0.2 ^b	96.3±0.1 ^a	<0.01	0.66
Lipids	96.2±0.6	96.3±0.4	96.8±0.2	96.6±0.6	96.2±0.2	97.3±0.2	0.37	0.73
Crude energy	85.5±3.6	88.3±0.9	90.8±0.2	90.1±1.0	89.6±0.5	91.2±0.4	0.20	3.09

n.d., not detected.

Data are $\mu \pm$ SEM ($n = 3$); values followed by different superscript in the same row differ ($P < 0.05$).¹ Coefficient of variation.**Table 4** Digestible fatty acids, lipids and energy contents of tested oils

Fatty Acid	Colza oil	Corn oil	Flaxseed oil	Fish oil	Poultry fat	Soybean oil
	% of total fatty acids					
14:00	0.06	0.03	0.00	6.22	0.53	0.09
16:00	4.18	10.54	5.19	17.16	17.54	9.96
18:00	2.23	1.78	4.66	3.07	4.59	3.19
20:00	0.60	0.56	0.18	0.00	0.10	0.20
16:1n-7	0.22	0.11	0.04	7.89	5.64	0.06
18:1n-9	58.52	33.58	19.50	8.04	34.85	23.71
20:1n-9	1.05	0.24	0.10	0.62	0.17	0.45
18:2n-6	18.19	45.52	13.93	16.92	24.20	50.73
18:3n-6	0.16	0.00	0.14	0.08	0.10	0.31
18:3n-3	7.27	0.69	48.58	1.64	0.28	5.06
20:5n-3	0.00	0.00	0.00	16.67	0.00	0.00
22:6n-3	0.00	0.00	0.00	10.85	0.00	0.00
ΣSFA	7.48	13.16	10.22	27.81	24.82	13.73
ΣMUFA	59.93	33.92	19.65	16.74	41.29	24.25
ΣPUFA	25.63	46.32	63.95	48.46	25.01	56.17
Σn-6	18.38	45.65	14.10	18.68	24.69	51.12
Σn-3	7.27	0.68	49.98	29.86	0.36	5.05
n-3/n-6	0.40	0.01	3.54	1.60	0.01	0.10
Proximate composition (dry matter basis)						
Lipid, g kg ⁻¹	958	961	967	962	942	971
Energy (MJ kg ⁻¹)	33.41	32.10	35.40	35.11	35.03	35.79

jundiá *Rhamdia quelen*, surubim *Pseudoplatystoma coruscans* and tilapia *Oreochromis* sp. (Greene & Selivonchick 1990; Caballero *et al.* 2002; Martino *et al.* 2002; Turchini

et al. 2003; Bahurmiz & Ng 2007; Vargas *et al.* 2008), thus confirming the high digestibility of energy of those lipid sources for freshwater fish.

Apparent digestibility coefficients of fatty acids were high, but significantly affected by the oil type and source, and related to the length of carbon chain, degree of unsaturation and melting point of each fatty acid, similarly to that reported for several marine species (Olsen *et al.* 1998; Johnsen *et al.* 2000; Menoyo *et al.* 2003). In the present study, the ADCs of all fatty acids were higher than 80% in flaxseed oil, except for 16:1n-7, as also reported for the Australian shortfin eel fed diets containing flaxseed and sunflower oils (Gunasekera *et al.* 2002). On the other hand, tilapia fed diets containing pollack liver oil and a mixture of HUFA, presented ADC for all fatty acids exceeding 90% (Ishikawa *et al.* 1997), the same trend registered for pacu, which presented high ADCs for most fatty acids from fish oil.

Apparent digestibility coefficient of total saturated fatty acid (SFA) of fish oil (88.8%) was higher than that of flaxseed oil (83.1%; $P = 0.02$), but did not differ from the other oils (85.7–88.0%). As a rule, the digestibility of SFA was lower than that of monounsaturated fatty acids (MUFA) and PUFAs (Table 3), an expected trend given that the increasing degree of unsaturation of lipids is directly proportional to the digestibility of their fatty acid contents (Olsen & Ringø 1998; Johnsen *et al.* 2000; Menoyo *et al.* 2003; Bahurmiz & Ng 2007). Within SFA, the lowest ADC was recorded for 18:0 contents of all tested oils, except colza oil. This observation is consistent with that registered by Olsen *et al.* (1998) for the Arctic char *Salvelinus alpinus*, a fresh- and cold-water species, in which the saturated 18:0 was proved resistant to digestion and absorption in pyloric caeca, midgut and hindgut.

The ADC of total PUFA was higher for flaxseed oil (95.81%), fish oil (95.8%) and soybean oil (96.0%), than for colza oil (93.84%), but did not differ from that of corn oil (95.1%) and poultry fat (95.2%; $P < 0.01$). MUFA are actually less digestible than PUFA because the lower enteric absorption of MUFA resulting from the limiting utilization of 22:1n-9 (Olsen *et al.* 1998). Similarly, 22:1n-9 and 24:1n-9 presented lower ADCs within MUFA from genetically improved, farmed tilapia (GIFT tilapia), a tropical freshwater fish (Teoh *et al.* 2011). All tested oils had very low 22:1n-9 contents (0–0.3%) and 24:1n-9 (0–0.2%), it can suggest that the MUFA digestibility was not impaired, and the MUFA contained thereon was consistently high (94.0–95.4%) and close to that of PUFA (93.5–96.0%).

Digestibility of fatty acids decrease with decreasing water temperature as a result of reduced bile secretion and lipolytic activity, and lower hydrolysis rates (Ng *et al.* 2003, 2004; Boeveik *et al.* 2010). On the other hand, warmer water temperature increases enzyme activities, thus improv-

ing lipid and fatty acid digestion. Pacu is a neotropical fish, thermal comfort zone ranging from 23 to 29 °C (Urbinatti *et al.* 2010); the water temperature in the experimental setting was kept at 28 ± 1 °C, so recording high ADC values of MUFA and PUFA for pacu shall not surprise.

Digestibility of fatty acid is directly correlated with the degree of unsaturation, that is, ADCs of 18:3 > 18:2 > 18:1 > 18:0 (Francis *et al.* 2007; Martins *et al.* 2009). This characteristic was clearly observed between 18:0 and 18:1n-9, but non-existent between 18:2n-6, 18:3n-6 and 18:3n-3, that is, digestion of PUFA by pacu is correlated with the intrinsic nature of dietary lipid, a characteristic also registered for the Australian shortfin eel (Gunasekera *et al.* 2002), a catadromous species.

The main sources of 18:2n-6 are corn oil (47.8%) and soybean oil (52.9%; Table 2), and digestibility of 18:2n-6 is around 95%, therefore yielding, respectively, 45.5% and 50.7% of digestible linoleic acid contents in those oils (Table 4). On the other hand, the flaxseed oil is the main source of 18:3n-3 (51.9%), 96.3% digestible, corresponding to 48.6% of digestible linolenic acid in that dietary lipid source.

The n-3 series PUFAs were less digested in corn oil (94.5%) and poultry fat (93.3%), in which they also show lesser contents in their fatty acid profile. The use of poultry fat as the main lipid source in diets of Atlantic halibut resulted in low overall fatty acid digestibility (Martins *et al.* 2009). The n-6 series PUFAs of all tested oils were well absorbed, except the 18:2n-6 of colza oil, which presented the lowest ADC (93.5%). Similarly, ADC of 18:2n-6 and n-6 series PUFA for Atlantic halibut fed diets formulated with colza oil was lower than that recorded for sunflower and flaxseed oils, but still higher than poultry fat and fish oil (Martins *et al.* 2009).

The highly unsaturated fatty acids (HUFAs), 20:5n-3 and 22:6n-3, were detected only in fish oil, 17.3 and 11.3%, respectively, ADCs ranging on 96%. This ADC value is similar to that registered for marine species such as Atlantic halibut (96.7–98.4%), Atlantic salmon *Salmo salar* L. (95.5–99.5%), Murray cod *Maccullochella peelii* *peelii* (97.4–97.8%), and Australian shortfin eel (98.6–99.2%), and for freshwater species such as rainbow trout (95.2–99.1%), tilapia *Oreochromis niloticus* (95.9–97.4%), and GIFT tilapia and red hybrid tilapia (99.5–100%) fed diets containing fish oil as lipid source, respectively, by Martins *et al.* (2009), Boeveik *et al.* (2010) and Karalazos *et al.* (2011), Francis *et al.* (2007), Gunasekera *et al.* (2002), Caballero *et al.* (2002) and Ng *et al.* (2010), Ishikawa *et al.* (1997), and Teoh *et al.* (2011).

There are contrasting reports on digestibility of lipids by fish. Flaxseed oil has been deemed an adequate surrogate to fish oil in commercial halibut feeds (Martins *et al.* 2009); sunflower oil was better digested than flaxseed oil by the Australian shortfin eel (Gunasekera *et al.* 2002), whereas the inclusion of a vegetable oil blend reduced overall lipid digestibility by Murray cod (Francis *et al.* 2007). Pacu is herein proved to digest rather efficiently both vegetable and animal lipid sources; colza oil and poultry fat are good dietary MUFA sources for fish, but had the lowest ADCs for 18:2n-6 and 18:3n-3, respectively. Corn, flaxseed and soybean oils do not present 20:5n-3 (EPA) and 22:6n-3 (DHA) in their composition but were shown to be adequate source of dietary essential fatty acid for pacu. Considering that freshwater fish can desaturate and elongate the 18-carbon chained fatty acids, that is, 18:2n-6 to 20:4n-6 and 18:3n-3 to 20:5n-3 and 22:6n-3 (Sargent *et al.* 2002; National Research Council 2011), a blend of vegetable oils, for instance the combination of 18:2n-6-rich corn or soybean oils, with 18:3n-3-rich flaxseed oil, may provide adequate dietary contents of both highly digestible, essential fatty acid for pacu nutrition. Specially because of the scarcity of reports on digestibility of lipids, energy and fatty acids for freshwater, neotropical species, it is safe to state that further research is required to evaluate the growth performance of pacu fed with vegetable and/or animal dietary lipid sources.

Acknowledgements

L.U.G holds a postdoctoral scholarship from Fundação de Amparo à Pesquisa do Estado de São Paulo (São Paulo State Research Foundation – FAPESP; 2010/52102-3), which also granted the research funds (2011/06146-1). J.E.P.C. is a research scholar from Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brazil's National Council for Scientific and Technological Development – CNPq).

References

Abimorad, E.G. & Carneiro, D.J. (2004) Fecal collection methods and determination of crude protein and of gross energy digestibility coefficients of feedstuffs for pacu, *Piaractus mesopotamicus* (Holmberg, 1887). *R. Bras. Zootec.*, -Braz, *J. of Animal Sci.*, **33**, 1101–1109.

Abimorad, E.G., Squassoni, G.H. & Carneiro, D.J. (2008) Apparent digestibility of protein, energy, and amino acids in some selected feed ingredients for pacu *Piaractus mesopotamicus*. *Aquacult. Nutr.*, **14**, 374–380.

Abimorad, E.G., Favero, G.C., Squassoni, G.H. & Carneiro, D. (2010) Dietary digestible lysine requirement and essential amino acid to lysine ratio for pacu *Piaractus mesopotamicus*. *Aquacult. Nutr.*, **16**, 370–377.

Bahurmiz, O.M. & Ng, W.K. (2007) Effects of dietary palm oil source on growth, tissue fatty acid composition and nutrient digestibility of red hybrid tilapia, *Oreochromis* sp., raised from stocking to marketable size. *Aquaculture*, **262**, 382–392.

Bicudo, A.J.A., Sado, R.Y. & Cyrino, J.E.P. (2009) Dietary lysine requirement of juvenile pacu *Piaractus mesopotamicus* (Holmberg, 1887). *Aquaculture*, **297**, 151–156.

Bicudo, A.J.A., Sado, R.Y. & Cyrino, J.E.P. (2010) Growth performance and body composition of pacu *Piaractus mesopotamicus* (Holmberg 1887) in response to dietary protein and energy levels. *Aquacult. Nutr.*, **16**, 213–222.

Bligh, E.G. & Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, **37**, 911–917.

Bogevik, A.S., Henderson, R.J., Mundheim, H., Waagbo, R., Tocher, D.R. & Olsen, R.E. (2010) The influence of temperature on the apparent lipid digestibility in Atlantic salmon (*Salmo salar*) fed *Calanus finmarchicus* oil at two dietary levels. *Aquaculture*, **309**, 143–151.

Brown, P.B. & Hart, S.D. (2010) Soybean oil and other n-6 polyunsaturated fatty acid-rich vegetable oils. In: *Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds*. Vol. 1 (Turchini, G.M. *et al.* eds), pp. 133–160. CRC Press, Boca Raton, FL, USA.

Bureau, D.P. & Meeker, D.L. (2010) Terrestrial animal fats in fish oil replacement and alternative lipid sources in aquaculture feeds. In: *Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds*. Vol. 1 (Turchini, G.M. *et al.* eds), pp. 245–266. CRC Press, Boca Raton, FL, USA.

Caballero, M.J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M. & Izquierdo, M.S. (2002) Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, **214**, 253–271.

Chou, B.S. & Shiau, S.Y. (1999) Both n-6 and n-3 fatty acids are required for maximal growth of juvenile hybrid tilapia. *N Am J Aquac.*, **61**, 13–20.

Forster, I. (1999) A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. *Aquacult. Nutr.*, **5**, 143–145.

Francis, D.S., Turchini, G.M., Jones, P.L. & De Silva, S.S. (2007) Effects of fish oil substitution with a mix blend vegetable oil on nutrient digestibility in Murray cod, *Maccullochella peelii peelii*. *Aquaculture*, **269**, 447–455.

Greene, D.H.S. & Selivonchick, D.P. (1990) Effects of dietary vegetable, animal and marine lipids on muscle lipid and hematology of rainbow-trout (*Oncorhynchus mykiss*). *Aquaculture*, **89**, 165–182.

Gunasekera, R.M., Leelarasamee, K. & De Silva, S.S. (2002) Lipid and fatty acid digestibility of three oil types in the Australian shortfin eel, *Anguilla australis*. *Aquaculture*, **203**, 335–347.

Ishikawa, M., Teshima, S.I., Kanazawa, A. & Koshio, S. (1997) Measurements of digestibilities of cholesterol and fatty acids using 5 α -cholestane as an inert marker in the tilapia, *Oreochromis niloticus*, and the freshwater prawn, *Macrobrachium rosenbergii*. *J. Appl. Ichthyol.*, **13**, 31–35.

Izquierdo, M.S., Obach, A., Arantamendi, L., Montero, D., Robaina, L. & Rosenlund, G. (2003) Dietary lipid sources for seabream and seabass: growth performance, tissue composition and flesh quality. *Aquacult. Nutr.*, **9**, 397–407.

Johnsen, R.I., Grahl-Nielsen, O. & Roem, A. (2000) Relative absorption of fatty acids by Atlantic salmon *Salmo salar* from

different diets, as evaluated by multivariate statistics. *Aquacult. Nutr.*, **6**, 255–261.

Karalazos, V., Bendiksen, E.A. & Bell, J.G. (2011) Interactive effects of dietary protein/lipid level and oil source on growth, feed utilization and nutrient and fatty acid digestibility of Atlantic salmon. *Aquaculture*, **311**, 193–200.

Lim, C., Yildirim-Aksoy, M. & Klesius, P. (2011) Lipid and fatty acid requirements of tilapias. *N Am J Aquac.*, **73**, 188–193.

Martino, R.C., Cyrino, J.E.P., Portz, L. & Trugo, L.C. (2002) Performance and fatty acid composition of surubim (*Pseudoplatystoma coruscans*) fed diets with animal and plant lipids. *Aquaculture*, **209**, 233–246.

Martins, D.A., Valente, L.M.P. & Lall, S.P. (2009) Apparent digestibility of lipid and fatty acids in fish oil, poultry fat and vegetable oil diets by Atlantic halibut, *Hippoglossus hippoglossus* L. *Aquaculture*, **294**, 132–137.

Menoyo, D., Lopez-Bote, C.J., Bautista, J.M. & Obach, A. (2003) Growth, digestibility and fatty acid utilization in large Atlantic salmon (*Salmo salar*) fed varying levels of n-3 and saturated fatty acids. *Aquaculture*, **225**, 295–307.

Montero, D., Kalinowski, T., Obach, A., Robaina, L., Tort, L., Caballero, M.J. & Izquierdo, M.S. (2003) Vegetable lipid sources for gilthead seabream (*Sparus aurata*): effects on fish health. *Aquaculture*, **225**, 353–370.

Morrison, W.R. & Smith, L.M. (1964) Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.*, **5**, 600–608.

National Research Council. (2011) Nutrient Requirement of Fish, pp. 376. National Academic Press, Washington, DC.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. & Troell, M. (2000) Effect of aquaculture on world fish supplies. *Nature*, **405**, 1017–1024.

Ng, W.K., Campbell, P.J., Dick, J.R. & Bell, J.G. (2003) Interactive effects of dietary palm oil concentration and water temperature on lipid digestibility in rainbow trout, *Oncorhynchus mykiss*. *Lipids*, **38**, 1031–1038.

Ng, W.K., Sigholt, T. & Bell, J.G. (2004) The influence of environmental temperature on the apparent nutrient and fatty acid digestibility in Atlantic salmon (*Salmo salar* L.) fed finishing diets containing different blends of fish oil, rapeseed oil and palm oil. *Aquacult. Res.*, **35**, 1228–1237.

Ng, W.K., Codabaccus, B.M., Carter, C.G. & Nichols, P.D. (2010) Replacing dietary fish oil with palm fatty acid distillate improves fatty acid digestibility in rainbow trout, *Oncorhynchus mykiss*, maintained at optimal or elevated water temperature. *Aquaculture*, **309**, 165–172.

Olsen, R.E. & Ringø, E. (1998) The influence of temperature on the apparent nutrient and fatty acid digestibility of Arctic charr, *Salvelinus alpinus* L. *Aquacult. Res.*, **29**, 695–701.

Olsen, R.E., Henderson, R.J. & Ringø, E. (1998) The digestion and selective absorption of dietary fatty acids in Arctic charr, *Salvelinus alpinus*. *Aquacult. Nutr.*, **4**, 13–21.

Portz, L. & Cyrino, J.E.P. (2004) Digestibility of nutrients and amino acids of different protein sources in practical diets by largemouth bass *Micropterus salmoides* (Lacepede, 1802). *Aquacult. Res.*, **35**, 312–320.

Sargent, J.R. & Tacon, A.G.J. (1999) Development of farmed fish: a nutritionally necessary alternative to meat. *Proc. Nutr. Soc.*, **58**, 377–383.

Sargent, J., Bell, G., McEvoy, L., Tocher, D. & Estevez, A. (1999) Recent developments in the essential fatty acid nutrition of fish. *Aquaculture*, **177**, 191–199.

Sargent, J.R., Tocher, D.R. & Bell, J.G. (2002) The lipids. In: *Fish Nutrition* (Halver, J.E. & Hardy, R.W. eds), pp 181–257. Elsevier, San Diego, USA.

Shapawi, R., Mustafa, S. & Ng, W.K. (2008) Effects of dietary fish oil replacement with vegetable oils on growth and tissue fatty acid composition of humpback grouper, *Cromileptes altivelis* (Valenciennes). *Aquacult. Res.*, **39**, 315–323.

Stech, M.R., Carneiro, D.J. & Carvalho, M.R.B. (2010) Fatores antinutricionais e coeficientes de digestibilidade aparente da proteína de produtos de soja para o pacu (*Piaractus mesopotamicus*). *Acta Sci. Anim. Sci.*, **32**, 255–262.

Tacon, A.G.J. & Metian, M. (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. *Aquaculture*, **285**, 146–158.

Teoh, C.Y., Turchini, G.M. & Ng, W.K. (2011) Genetically improved farmed Nile tilapia and red hybrid tilapia showed differences in fatty acid metabolism when fed diets with added fish oil or a vegetable oil blend. *Aquaculture*, **316**, 144–154.

Tocher, D.R. (2003) Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fish. Sci.*, **11**, 107–184.

Turchini, G.M., Mentasti, T., Froyland, L., Orban, E., Caprino, F., Moretti, V.M. & Valfre, F. (2003) Effects of alternative dietary lipid sources on performance, tissue chemical composition, mitochondrial fatty acid oxidation capabilities and sensory characteristics in brown trout (*Salmo trutta* L.). *Aquaculture*, **225**, 251–267.

Turchini, G.M., Torstensen, B.E. & Ng, W.K. (2009) Fish oil replacement in finfish nutrition. *Rev. Aquacult.*, **1**, 10–57.

Urbinati, E.C., Gonçalves, F.D. & Takahashi, L.S. (2010) Pacu (*Piaractus mesopotamicus*). In: *Espécies Nativas Para Piscicultura no Brasil* (Baldisserto, B. & Gomes, L.D.C. eds), pp. 205–244. Editora da UFMS, Santa Maria, RS, Brazil.

Vargas, R.J., de Souza, S.M.G., Kessler, A.M. & Baggio, S.R. (2008) Replacement of fish oil with vegetable oils in diets for jundia (*Rhamdia quelen* Quoy and Gaimard 1824): effects on performance and whole body fatty acid composition. *Aquacult. Res.*, **39**, 657–665.

Vidotti, R.M., Carneiro, D.J. & Viegas, E.M.M. (2002) Acid and fermented silage characterization and determination of apparent digestibility coefficient of crude protein for pacu *Piaractus mesopotamicus*. *J. World Aquacult. Soc.*, **33**, 57–62.