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Productive performance and digestibility in the initial growth phase of tambaqui (*Colossoma macropomum*) fed diets with different carbohydrate and lipid levels

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ABSTRACT

The use of dietary protein can be optimized by increasing diet energy, which can be achieved by adding non-protein nutrients such as carbohydrates and lipids. If incorporated in suitable amounts, these items can promote the protein sparing effect, reducing nitrogen excretion and improving the quality of fish farming effluents. The study assessed productive performance, body composition, nutrient and energy retention efficiency and digestibility of the omnivorous fish tambaqui (*Colossoma macropomum*) fed diets with three carbohydrate (410, 460 and 510 g kg^{-1}) and two lipid levels (40 and 80 g kg^{-1}) in the initial growth phase (juvenile weighing between 10 and 250 g). The experiment was completely randomized, with six treatments and four replicas arranged in a 3 × 2 factorial design. The 1080 tambaqui tested (10.88 ± 0.13 g body weight) were randomly distributed into 24 tanks (500 L; 45 fish/tank) and fed the test diets for 120 days. The highest carbohydrate inclusion (510 g kg^{-1}) reduced food intake and fish growth. A protein sparing effect was observed in the growth of tambaqui fed 460 g kg^{-1} carbohydrates since they showed higher weight gain, protein efficiency ratio, protein productive value and crude protein participation in weight gain. The increase in lipid levels from 40 g kg^{-1} to 80 g kg^{-1} increased body fat deposition and decreased the digestibility coefficients of diet nutrients and diet energy. The results demonstrate that the ideal balanced diet to grow juvenile tambaqui is 460 g kg^{-1} carbohydrates and 40 g kg^{-1} lipids.

1. Introduction

The formulation of low cost feed limits greater fish farming expansion (Silva et al., 2007). Feeding can account for up to 80% of farming costs, and protein sources are the most expensive ingredients of diet (Cheng et al., 2003). According to Thoman et al. (1999), optimizing protein utilization can increase nutrient retention by fish, decreasing nitrogen loss and farming costs. To that end, non-protein ingredients such as carbohydrates (CHO) and lipids (LIP) can be used to increase diet energy (Watanabe et al., 2001) to achieve an ideal energy-protein ratio. When the rate of protein conversion into energy decreases, diet protein can be used for growth and tissue formation, in a process known as the protein sparing effect (Hemre et al., 2002).

Although carbohydrate requirements are not well established, its absence in feed can compromise animal growth (Wilson, 1994). CHO is a low-cost energy source for domestic animal diets (Wilson, 1994). It is widely available (Abimorad and Carneiro, 2007) and if provided at adequate levels can improve the utilization efficiency of other nutrients and contribute to diet processing (Krogdahl et al., 2005). However, excess CHO can decrease food intake, preventing the required intake of other nutrients (Winfree and Stickney, 1981). In addition, excess loads of dietary energy can increase body fat deposition in fish (Page and Andrews, 1973).

Adequate levels of LIP input in diet can minimize protein utilization. Lipids are the greatest source of metabolic energy for growth, from egg to adulthood (Mohanta et al., 2008). In addition, LIPs are a source of essential fatty acids, which are necessary for adequate development, to enhance feed palatability and as a vehicle for fat-soluble vitamins and sterols, in addition to playing an important role in the structure of biological membranes in the form of phospholipids and sterols (Johnson et al., 2002). Excess dietary LIP, however, can decrease pellet stability, compromising food quality

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and storage, as well as reducing consumption due to high energy and increasing fat deposition in tissues, devaluing fish fillet and/or carcass.

With a production of 139,21 thousand tons in 2014, tambaqui is the main native fish species farmed in Brazil (IBGE, 2014). It is also produced in other South American and Central American countries. The tambaqui is omnivorous (Honda, 1974), feeding on a variety of natural food items. In flooding periods, its diet consists mostly of fruits and seeds, which are rich in CHO and LIP and contain high energy levels. In the dry season, the main food source, zooplankton, provides a protein-rich diet (Silva et al., 2000). Studies to improve the technology of tambaqui production have been promoted given its significant commercial and socioeconomic value, meat quality and high performance for farming purposes.

In farming systems, knowledge of diet digestibility is crucial to ensure that the nutritional requirements of fish are met.If data on digestibility is not available, however, the diet can contain excess nutrient levels (especially crude protein) or can be nutritionally deficient. In both cases, the inadequate nutrient supply can compromise fish growth and performance (Gonçalves and Carneiro, 2003). Thus, to establish an adequate proportion between CHO and LIP levels in a diet, it is important to optimize the use of these nutrients, especially considering that an imbalance of non-protein energy sources can directly affect body composition (Li et al., 2013). Accordingly, the present study tested different levels of CHO and LIP in the diet of juvenile tambaqui to determine the protein sparing effect and attempt to optimize productive performance, digestibility and nutrient and energy retention efficiency.

2. Material and methods

2.1. Biological material and experimental conditions

The experiments were carried out in the Laboratory of Aquatic Organism Nutrition of the Aquaculture Center of São Paulo State University (Jaboticabal, São Paulo, Brazil). The experimental protocols were performed according to the ethical principles for animal research of the Brazilian College of Animal Experimentation (COBEA) and approved by the Animal Use Ethics Committee (CEUA), under protocol no. 018492/12. We studied 1080 juvenile tambaqui (weight = 10.88 ± 0.13 g and body length = 9.49 ± 0.24 cm) obtained from farmed in excavated tanks from a fish farming, located in the city of Manaus – Amazonas. They were acclimatized in a 500-L tank (at 29 °C) for two weeks prior to the experiment and fed a commercial diet containing 320 g of crude protein per kg. After this period, the fish were randomly distributed into twenty-four 500-L tanks (45 fish/tank) with constant air and water supply (renewal rate of 4.0 L min^{-1}). The physicochemical water variables were monitored once a week. Temperature was 29.68 ± 0.15 °C, dissolved oxygen 5.85 ± 0.32 mg L^{-1}, pH 7.81 ± 0.7, total ammonia 196.90 ± 40.76 μg L^{-1} and total phosphorous 42.85 ± 15.65 μg L^{-1} (the last two determined according to Koroleff, 1976, and Mackereth and Talling, 1978, respectively).

2.2. Diets

Six isoprotein diets (230 g kg^{-1} digestible protein) were prepared by combining three different CHO levels (410, 460 and 510 g kg^{-1}) with two LIP levels (40 and 80 g kg^{-1}). Diet formulation was based on the apparent digestibility coefficients (ADC) of the ingredients (unpublished data). The calorie content of the diets containing40 g kg^{-1} LIP and 80 g kg^{-1} LIP was 17 MJ kg^{-1} and 18 MJ kg^{-1} crude energy, respectively (Table 1).

The diets were processed in an extruder at the feed manufacturing facility of the Agricultural and Veterinary School of UNESP, Jaboticabal. Pellet size ranged from 4 to 6 mm. The fish were fed the diets until apparent satiety 3 times a day. The amount of feed consumed in each treatment was calculated weekly by the difference in the weight of the food containers before and after feeding.

2.3. Productive performance parameters

All of the fish were weighed and measured individually at the beginning and end of the experimental period, and the parameters were measured as follows:

1 Weight gain = final body weight (g) – initial body weight (g)
2 Feed conversion rate = feed intake (g)/weight gain (g)
3 Daily feed intake = feed intake per fish (g)/days
4 Specific growth rate = (*ln* final weigh – *ln* initial weight) × 100/days
5 Protein efficiency ratio = mean weight gain (g)/crude protein intake (g)

2.4. Body composition and nutrient retention parameters

Body composition was evaluated in 30 fish collected before the experiment and in 3 fish collected from each tank at the end (12 fish per treatment). The fish used to assess body composition were subjected to 24 h fasting to empty the digestive tract, euthanized by deep anesthesia with 0.2 g L^{-1} benzocaine and frozen. Fish carcasses were then ground and freeze dried to constant weight to determine moisture, ether extract, crude protein, ash and crude energy according to AOAC (2000) protocols.

Data on body composition and productive performance were used to calculate the nutrient retention parameters by estimating protein productive value (PPV), energy productive value (EPV), proportion of crude protein in weight gain (CP_{WG}) and proportion of ether extract in weight gain (EE_{WG}), as follows:

$$PPV(\%) = [(CP_F \times W_F) - (CPi \times Wi)] \times 100/I_{CP} \quad (1)$$

$$EPV(\%) = [(CE_F \times W_F) - (CEi \times Wi)] \times 100/I_{CE} \quad (2)$$

$$CP_{WG}(\%) = [(CP_F \times W_F) - (CPi \times Wi)] \times 100/(W_F - Wi) \quad (3)$$

$$EE_{WG}(\%) = [(EE_F \times W_F) - (EEi \times Wi)] \times 100/(W_F - Wi) \quad (4)$$

where:

CP_F, CE_F, EE_F: final crude protein, crude energy and ether extract in the carcass.

CP_I, CE_I, EE_I: initial crude protein, crude energy and ether extract in the carcass.

I_{CP}, I_{CE}: total protein and energy intake.

W_i, W_F: initial and final body weight.

2.5. Diet digestibility

Digestibility assays were carried out after productive performance evaluations to determine the apparent digestibility coefficients (ADC) for crude protein, ether extract, total starch and crude energy. To that end, 192 tambaqui (224.52 ± 42. 47 g) were distributed into twenty four 430-L tanks supplied with continuous water flow. Water was kept at 29.68 ± 0.15 °C temperature and 5.85 ± 0.32 mg L^{-1} dissolved oxygen. For 7 days, fish under the different treatments were fed to satiation the respective experimental diets containing 5.0 g kg^{-1} chromium oxide (Cr_2O_3) as inert marker.

The fish were then transferred to twelve 100-L tanks with continuous water circulation and built according to a modified Guelph system, as described by Gonçalves and Carneiro (2003). Feces were deposited in Falcon type collecting tubes placed on the bottom of the tank and protected by ice-filled coolers to prevent sample degradation. Feces were collected at 4 h intervals over one night.

Table 1
Diet composition (on the natural matter bases).

Ingredients (g kg^{-1})[1]	Diets (Carbohydrate/Lipid)					
	410/40	460/40	510/40	410/80	460/80	510/80
Salmon meal (65% CP)	191	191	191	191	191	191
Corn gluten	170	170	170	170	170	170
Yeast	20	20	20	20	20	20
Corn	145	145	145	145	145	145
Sorghum	50	50	50	50	50	50
Corn starch	190	270	340	210	280	350
Cellulose	199	119	49	140	70	–
Corn Oil	15	15	15	54	54	54
Vitamin-mineral supplement[2]	5	5	5	5	5	5
Dicalcium phosphate	11	11	11	11	11	11
Antioxidant (BHT)	1	1	1	1	1	1
Antifungal (Filax)	3	3	3	3	3	3
Nutritional composition[3] (g kg^{-1})						
Dry matter	928	928	928	928	928	928
Crude protein	260	260	260	260	260	260
Digestible protein[4]	236	236	236	236	236	236
Lysine	13	13	13	13	13	13
Methionine	8	8	8	8	8	8
Lipid	40	40	40	80	80	80
Mineral matter	41	41	41	41	41	41
Crude fiber	171	111	58	127	74	22
Starch	316	386	447	333	395	456
Carbohydrate[5]	413	466	513	418	464	510
Crude energy (MJ kg^{-1})	17	17	17	18	18	18
Digestible energy[4] (MJ kg^{-1})	15	15	15	16	15	15
CE to CP ratio[6] (MJ g^{-1})	65	65	65	69	69	69

(1) All ingredients were purchased from two brazilian industries Guabi® and Nutreco®, with the exception of salmon meal that was imported from Chile.
(2) Mineral mix (Premix Nutrifish Guabi®, Campinas, SP, Brazil) per kg of product: folic acid (1250 mg); calcium pantothenate (12 000 mg); cupper (125 mg); iron (15 000 mg); iodine (375 mg;) manganese (12 500 mg); selenium (87.5 mg); zinc (12 500 mg); cobalt (125 mg); vitamin A (2 500 000 IU); vitamin B12 (4000 mg); vitamin B1 (4000 mg); vitamin B2 (4000 mg); vitamin B6 (4000 mg); vitamin C (50 000 mg); vitamin D3 (600 000 IU); vitamin E (37 500); vitamin K3 (3750 mg); niacin (22 500 mg); biotin (15 mg).
(3) Diet composition was calculated according to ingredient values.
(4) Obtained from another study on digestibility for juvenile tambaquis (unpublished data).
(5) Carbohydrate = dry matter – (crude protein + lipid + mineral matter + crude fiber).
(6) CE = crude energy, CP = crude protein.

They were then freeze dried, ground in a mill and sieved to remove scales.

The chromium oxide concentration in feces and in the diet was determined by nitroperchloric digestion, as described by Furukawa and Tsukahara (1966). Ether extract was determined by the acid hydrolysis method (AOAC, 2000), crude protein by LECO FP 528 Nitrogen Analyzer (LECO Instruments, St Joseph, Michigan, USA), crude energy in a calorimetric pump and starch content according to Hendrix (1993).

As described by Nose (1966), diet ADC was determined using the equation:

$$ADC = 100 - 100 \times ((\%Cr_2O_3 in diet) / (\%Cr_2O_3 in feces) \times (\%nutrients in feces)/(\%nutrients in diet)) \quad (1)$$

2.6. Statistical analysis

The experiment was randomized and consisted of six treatments arranged in a 3 × 2 factorial scheme (diets combining 3 CHO and 2 LIP levels) with four replicates. After being tested for normality (Cramer Von-Mises test) and homoscedasticity (Brown-Forsythe test), data were analyzed by two-way factorial ANOVA. Statistically significant means were contrasted by the Tukey test ($P < 0.05$). Statistical tests were performed using Statistical Analysis System (SAS®) v.9.0 software (SAS Institute Inc., Cary, North Carolina, USA).

3. Results

3.1. Productive performance

Diet CHO levels affected fish growth, but no interaction effect was detected between CHO and LIP (Table 2).

Fish receiving the highest CHO levels (510 g kg^{-1}) had lower daily diet intake and, as a consequence, lower weight gain and specific growth rate ($P < 0.05$). The highest mean weight gain was exhibited by tambaqui fed diets with 410 and 460 g kg^{-1} CHO. Feed conversion and protein efficiency rate were higher in fish fed diets with 460 g kg^{-1} CHO. Productive performance was not affected by the LIP levels tested (Table 2).

3.2. Body composition

The interaction effect between CHO and LIP was detected in carcass moisture, ether extract and crude energy ($P < 0.05$). The crude protein and ash levels in carcass were not affected by the treatments (Table 3).

Juveniles fed diets with a CHO/LIP ratio of 410/40 and 460/40 g kg^{-1} showed higher mean moisture content than those fed diets containing 80 g kg^{-1} lipids. Groups fed 80 g kg^{-1} LIP showed similar ether extract and crude energy levels in the carcass, irrespective of CHO levels (Table 5), but in the treatments with 40 g kg^{-1} LIP, these variables increased with a rise in CHO levels.

Fish fed diets with 410 and 460 g kg^{-1} CHO showed higher fat deposition and crude energy in the carcass when the diet contained the highest lipid level (80 g kg^{-1}). For fish fed diets with 510 g kg^{-1}

Table 2
Growth performance parameters (mean ± sd; N = 4) and corresponding F-values for juvenile tambaqui fed the experimental diets.

Factors	Final weight (g)	Weight gain (g)	Feed conversion rate	Daily feed intake (g)	Specific growth rate (% day^{-1})	Protein efficiency ratio
Carbohydrate level						
410 g kg^{-1}	249.87 ± 25.14 a	238.95 ± 25.21 a	1.55 ± 0.14 b	1.99 ± 0.21 a	2.60 ± 0.08 a	2.88 ± 0.28 b
460 g kg^{-1}	250.58 ± 19.39 a	239.62 ± 19.49 a	1.35 ± 0.19 a	2.00 ± 0.16 a	2.61 ± 0.07 a	3.36 ± 0.54 a
510 g kg^{-1}	200.77 ± 31.88 b	189.98 ± 31.89 b	1.37 ± 0.13 ab	1.58 ± 0.26 b	2.42 ± 0.14 b	3.27 ± 0.30 ab
Lipid level						
40 g kg^{-1}	240.35 ± 26.44	229.49 ± 26.46	1.48 ± 0.13	1.91 ± 0.22	2.57 ± 0.09	3.01 ± 0.29
80 g kg^{-1}	222.52 ± 42.42	211.60 ± 42.35	1.36 ± 0.20	1.76 ± 0.35	2.50 ± 0.16	3.32 ± 0.50
Effects (F-value; ANOVA)						
Carbohydrate (CHO)	10.03**	9.92**	4.56*	9.92**	7.94**	3.94*
Lipid (LIP)	1.67^{NS}	1.68^{NS}	3.89^{NS}	1.68^{NS}	2.17^{NS}	4.29^{NS}
CHO × LIP interaction	1.88^{NS}	1.86^{NS}	0.80^{NS}	1.86^{NS}	1.43^{NS}	1.20^{NS}
Coefficient of variation (%)	10.61	11.16	10.48	11.16	3.94	11.43

Different letters in a column indicate a significant difference according to the Tukey test (P < 0.05).
^{NS} = non- significant.
*=P < 0.05 (Tukey test).
**=P < 0.01 (Tukey test).

Table 3
Body composition (mean ± sd, in natural matter percentage; N = 4) and corresponding F-values of juvenile tambaqui fed the experimental diets.

Factors	Moisture (%)	Crude protein (%)	Ether extract (%)	Crude energy (MJ kg^{-1})	Ashes (%)
Carbohydrate level					
410 g kg^{-1}	62.29 ± 1.20	14.41 ± 1.00	16.06 ± 2.08	10.93 ± 0.72	3.48 ± 0.45
460 g kg^{-1}	61.44 ± 1.63	14.08 ± 0.75	17.15 ± 1.45	11.46 ± 0.62	3.34 ± 0.32
510 g kg^{-1}	61.25 ± 0.93	13.86 ± 0.30	17.49 ± 0.77	11.52 ± 0.32	3.51 ± 0.22
Lipid level					
40 g kg^{-1}	62.56 ± 0.98	14.42 ± 0.68	15.85 ± 1.45	10.92 ± 0.53	3.47 ± 0.30
80 g kg^{-1}	60.72 ± 0.81	13.82 ± 0.72	18.02 ± 0.77	11.71 ± 0.38	3.41 ± 0.38
Effects (F-value; ANOVA)					
Carbohydrate (CHO)	7.67*	1.21^{NS}	9.28*	11.98*	0.48^{NS}
Lipid (LIP)	48.98*	4.12^{NS}	53.87*	44.84*	0.18^{NS}
CHO × LIP interaction	3.76*	0.25^{NS}	10.52*	3.96*	0.01^{NS}
Coefficient of variation (%)	1.07	5.10	4.26	2.68	10.82

Different letters in a column indicate a significant difference according to the Tukey test (P < 0.05).
^{NS} = non- significant.
*=P < 0.05 (Tukey test).

CHO, moisture, ether extract and crude energy were not affected by an increase in diet LIP (Table 5).

3.3. Nutrient and energy retention efficiency

CHO levels affected all the variables related to nutritional and energy retention efficiency. PPV and CP_{WG} were lower in fish fed diets containing 510 g kg^{-1} CHO, higher in fish fed diets with 410 and 460 g kg^{-1} CHO and similar between the two last treatments. Lipid levels did not affect nutrient and energy retention efficiency. The interaction effect of CHO and LIP was observed only for EPV and EE_{WG} (Table 4).

Mean EPV was lower in fish fed a diet with a CHO/LIP ratio of 510/80 g kg^{-1}, a variable that was not affected by CHO levels in fish fed diets with the lowest LIP level (40 g kg^{-1}). In fish fed 80 g kg^{-1} LIP, the higher the diet CHO levels, the lower the EE_{WG} value, and the highest EE_{WG} was obtained for those fed 410/80 CHO/LIP. In fish fed diets with 40 g kg^{-1} LIP, the highest EE_{WG} was obtained using 460 g kg^{-1} CHO (Table 5).

3.4. Digestibility assay

No interaction effect of CHO and LIP was observed on the ADC of the nutrients and energy evaluated. Diet LIP levels affected the ADC of protein, energy, ether extract and starch, which were higher (P < 0.05) for diets with 40 g kg^{-1} LIP. In addition, the ADC of ether extract and starch decreased with an increase in diet CHO (Table 6).

4. Discussion

Diet CHO and LIP levels had a direct effect on tambaqui development. The performance of fish fed 460 g kg^{-1} CHO was better than that of fish fed diets with higher CHO levels (510 g kg^{-1}). Moreover, the addition of the lowest LIP level (40 g kg^{-1}) produced lower carcass fat deposition.

The results obtained showed that the lowest feed intake in fish fed a diet with 510 g kg^{-1} CHO decreased weight gain. The mechanisms regulating food intake are not well known, and the amount of diet energy is one of the main factors affecting it (Fletcher, 1984). According to Tran-Duy et al. (2008), because of excess energy, high CHO levels can limit food intake before the animal meets its nutritional requirements. In the present study, however, only CHO levels affected food intake and, therefore, the amount of digestible energy was similar among the diets. Thus, the response to the significant decrease in food intake likely resulted from the higher starch content of this treatment. According to Arnesen and Krogdahl (1993) and Venou et al. (2003), the higher digestibility of gelatinized starch can increase the amount of available energy, thereby reducing food

Table 4
Nutrient and energy retention efficiency (mean ± sd, in natural matter percentage; N = 4) and corresponding F-values of juvenile tambaqui fed experimental diets.

Factors	PPV (%)	EPV (%)	CP_{WG} (%)	EE_{WG} (%)
Carbohydrate level				
410 g kg^{-1}	35.90 ± 3.65 a	32.66 ± 5.91	13.41 ± 1.42 a	15.76 ± 3.63
460 g kg^{-1}	35.18 ± 3.71 a	34.26 ± 3.27	13.14 ± 1.44 a	17.14 ± 1.83
510 g kg^{-1}	28.36 ± 5.29 b	27.13 ± 4.14	10.47 ± 2.06 b	13.08 ± 1.99
Lipid level				
40 g kg^{-1}	34.70 ± 4.52	31.05 ± 4.62	12.95 ± 1.76	15.05 ± 2.79
80 g kg^{-1}	30.80 ± 5.91	31.29 ± 6.43	11.43 ± 2.30	15.44 ± 3.41
Effects (F-value; ANOVA)				
Carbohydrate (CHO)	7.77**	7.04**	7.77**	7.50**
Lipid (LIP)	3.62^{NS}	0.25^{NS}	3.61^{NS}	0.63^{NS}
CHO × LIP interaction	1.02^{NS}	4.80*	1.02^{NS}	6.88**

Different letters in a column indicate a significant difference according to the Tukey test ($P < 0.05$).
^{NS} = non- significant.
*=$P < 0.05$ (Tukey test).
**=$P < 0.01$ (Tukey test).
PPV = protein production value.
EPV = energy production value.
CP_{WG} = crude protein participation in weight gain.
EE_{WG} = ether extract participation in weight gain.

ingestion, or prolonging gastrointestinal transit time. Excess gastrointestinal transit time decreases animal performance because the space that could be filled by additional food is unavailable (Meurer et al., 2002), thereby decreasing the consumption of nutrients, including proteins.

Lower protein intake leads to the protein sparing effect, but if the consumption of this nutrient is excessively low, fish growth may be compromised. Even though exhibiting low feed intake, fish fed diets with 510 g kg^{-1} CHO used diet protein more efficiently, showing a high protein efficiency and feed conversion ratio, similar to that exhibited in treatments with 460 g kg^{-1} CHO (Table 2). In a test on pacu (*Piaractus mesopotamicus*) fed diets containing 410, 460 and 500 g kg^{-1} CHO, Abimorad and Carneiro (2007) also found a better protein efficiency ratio and feed conversion in the groups fed the two diets with the highest CHO levels. Accordingly, in a study testing six levels of corn starch (60, 140, 220, 300, 380 or 460 g kg^{-1}) in diets for hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) Wang et al. (2005) observed that growth was not affected by the highest levels evaluated, but that fish fed diets with the lowest supplementations (60 and 140 g kg^{-1}) used a higher protein content, consumed as an energy source rather than for tissue deposition.

In the present study, excess non-protein energy, with a high energy:protein ratio, decreased feed intake and promoted body fat accumulation (Table 3). This was also observed by Abimorad et al. (2007) in an assessment of two digestible protein levels (200 and 230 g kg^{-1}), two LIP levels (40 and 80 g kg^{-1}) and three CHO levels (410, 460 and 500 g kg^{-1}) in juvenile pacu levels. In the present study, the proportion of body fat was greater in fish fed diets with a higher LIP content (Table 5), corroborating other studies on tambaqui, *Colossoma macropomum* (Van der Meer et al., 1997), Nile tilapia, *Oreochromis niloticus* (Ali and Al-Asgah, 2001), surubim, *Pseudoplatystoma coruscans*, (Martino et al., 2002), rainbow trout, *Oncorhynchus mykiss* (Gumus and Ikiz, 2009) and Japanese seabass, *Lateolabrax japonicus* (Xu et al., 2011).

On the other hand, low-energy diets can increase protein use by fishes an energy source (Cho, 1990). They may also increase ammonia excretion, thereby posing a water pollution risk (Boscolo et al., 2005). In the present study, a protein sparing effect was observed in fish receiving 460 g kg^{-1} CHO because they showed the highest

Table 5
Mean body composition and efficiency of nutrient and energy retention in juvenile tambaqui (interaction effect between diet carbohydrate and lipid levels).

	Lipid (g kg^{-1})	Carbohydrate (g kg^{-1})		
		410	460	510
Moisture (%)	40	63.49 ± 0.19 aA	62.67 ± 0.57 aAB	61.75 ± 1.09 aB
	80	61.38 ± 0.57 bA	59.80 ± 0.65 bA	60.75 ± 0.40 aA
Ether extract (%)	40	14.24 ± 1.00 bB	16.03 ± 0.21 bA	17.28 ± 0.65 aA
	80	17.88 ± 0.52 aA	18.64 ± 0.68 aA	17.69 ± 0.92 aA
Crude energy (MJ kg^{-1})	40	10.27 ± 0.43 bB	10.98 ± 0.23 bAB	11.34 ± 0.33 aA
	80	11.42 ± 0.41 aA	12.10 ± 0.10 aA	11.71 ± 0.19 aA
EPV (%)	40	29.11 ± 3.40 aA	35.74 ± 3.67 aA	28.31 ± 2.95 aA
	80	37.41 ± 5.35 aA	32.29 ± 1.28 aAB	25.96 ± 5.25 aB
EE_{WG} (%)	40	13.48 ± 1.89 bB	18.21 ± 1.61 aA	13.44 ± 1.54 aB
	80	18.79 ± 3.16 aA	15.72 ± 0.92 aAB	12.73 ± 2.56 aB

Different lowercase letters in a column and uppercase letters in a row indicate significant difference according to the Tukey test ($P < 0.05$).
EPV = energy production value.
EE_{WG} = ether extract participation in weight gain.

Table 6
Apparent digestibility coefficients (ADC) of nutrients and energy (mean ± sd; N = 4) and corresponding F-values of juvenile tambaqui diets containing different carbohydrate and lipid levels.

Factors	Crude protein ADC	Crude energy ADC	Ether extract ADC	Starch ADC
	(%)	(%)	(%)	(%)
Carbohydrate level				
410 g kg^{-1}	87.76 ± 2.25	86.78 ± 1.60	87.27 ± 5.46 a	86.89 ± 4.12 a
460 g kg^{-1}	86.27 ± 1.26	85.60 ± 1.61	84.80 ± 3.55 ab	82.83 ± 4.95 b
510 g kg^{-1}	86.85 ± 2.06	86.86 ± 2.17	81.91 ± 2.23 b	83.26 ± 2.58 ab
Lipid level				
40 g kg^{-1}	88.01 ± 1.56 a	87.58 ± 1.50 a	86.34 ± 5.30 a	86.89 ± 3.84 a
80 g kg^{-1}	85.82 ± 1.64 b	85.10 ± 1.10 b	83.07 ± 2.96 b	81.63 ± 2.95 b
Effects (F-value; ANOVA)				
Carbohydrate (CHO)	2.04^{NS}	2.01^{NS}	3.84^{*}	4.70^{*}
Lipid (LIP)	12.34^{*}	22.86^{*}	4.68^{*}	18.14^{**}
CHO × LIP interaction	0.94^{NS}	1.26^{NS}	1.73^{NS}	1.05^{NS}
Coefficient of variation (%)	1.77	1.44	4.36	3.48

Different letters in a column indicate a significant difference according to the Tukey test (P < 0.05).
^{NS} = non- significant.
*=P < 0.05 (Tukey test).
**=P < 0.01 (Tukey test).

protein efficiency ratio (Table 2), protein productive value (PPV) and crude protein impact on weight gain (CP_{WG}) (Table 4). The treatment with 510 g kg^{-1} CHO also showed a high protein efficiency ratio, but due to the lower food intake of this group, mean PPV and CP_{WG} were also reduced. The improved growth and protein sparing effect observed in fish fed diets with 460 g kg^{-1} may be related to the importance of glucose as metabolism fuel for glucose-dependent tissues, such as red cells and nervous tissues. As such, diet CHO can reduce gluconeogenesis, diverting amino acids from the oxidative route (Cowey et al., 1977).

A similar protein sparing effect was reported by Mohapatra et al. (2003) in a study on juvenile rohu (*Labeo rohita*) fed 450 g kg^{-1} of gelatinized starch. Indian carp (*Catla catla*) fries spared more protein for growth when the amount of diet CHO was higher (350 g kg^{-1}); However, growth decreased and they lost weight when diet LIP was increased from 40 to 120 g kg^{-1} (Seenappa and Devaraj, 1995).

In the present study, an increase in diet LIP did not affect growth or nutrient and energy retention efficiency. A higher LIP content (80 g kg^{-1}), however, increased body fat, except for fish feeding 510 g kg^{-1} CHO (Tables 3 and 4).

Despite their nutritional importance, diets with high LIP content can affect fish metabolism and carcass composition, causing undesired accumulation of body fat in fish (Ribeiro et al., 2008). This response was also reported by Martino et al. (2002) after increasing LIP levels from 60 to 180 g kg^{-1} in surubin diets (*Pseudoplatystoma coruscans*). They also observed an increase in carcass fat despite improved growth and protein efficiency. The findings of the present study indicate that an LIP diet should only be increased in the diet with highest CHO levels (510 g kg^{-1}) given that body fat deposition was higher in fish fed diets containing 410 and 460 g kg^{-1} CHO (Table 5).

A CHO increase in the diets did not affect crude protein and crude energy digestibility, but reduced ether extract and starch digestibility. An LIP increase, in turn, reduced the ADC of all the nutrients tested and of energy, but an interaction effect of the factors evaluated was not detected (Table 6). Abimorad and Carneiro (2007) observed that diet CHO levels did not affect protein digestibility in pacu, but they also found that energy digestibility increased proportionally with an increase in diet CHO, whereas an increase in LIP from 40 to 80 g kg^{-1} reduced crude protein ADC but did not affect crude energy ADC. According to Wang et al. (2014), when LIP levels are above the nutritional requirements, the excess is deposited in the liver. If the fat liver is overloaded, apparent digestibility is compromised.

In addition to the energy and nutrient levels in the diet, a number of studies have shown that food processing is an important factor for digestibility in fish. Honorato et al. (2015), in a study on the effects of two levels of CHO inclusion (400 and 500 g kg^{-1}) and two types of diet processing (extruded and pelletized) in the digestive physiology of pacu, observed an increase in crude protein ADC in fish fed extruded diet and high CHO levels (500 g kg^{-1}). This is likely a result of starch gelatinization, which occurs during extrusion. Gelatinization is the combination of changes that include rupture of the granular structure of starch molecules, their expansion, hydration and solubilization, increasing their susceptibility to digestion by gastrointestinal amylases (Denardin and da Silva, 2009). In the present study, all the diets were extruded, which may have increased diet ADC values and improved nutrient utilization by tambaqui.

Amirkolaie et al. (2006) evaluated the effects of gelatinization and two starch inclusion levels (180 and 300 g kg^{-1}) on digestibility by Nile tilapia. The inclusion levels did not affect protein and starch ADC. However, an increase in starch inclusion levels resulted in higher energy and LIP ADC. By contrast, the increase in LIP and CHO levels in the present study reduced ether extract and starch ADC. The difference among species may be related to the relative amylolytic activity in the digestive tract, showing an association with eating habits and its considerable influence on digestion and absorption processes due to the anatomic and physiological differences among fish (Lee et al., 2002).

Based on the results obtained, CHO are better used as a primary non-protein energy source for juvenile tambaqui. The fish did not respond efficiently to the increase in dietary lipid levels. The productive performance and efficiency of nutrient and energy retention were not affected by an increase in dietary LIP levels. It did, however, promote an accumulation of body fat and reduce ADC in all the nutrients evaluated. In addition, the protein sparing effect was observed in tambaqui fed diets containing 460 g kg^{-1} CHO. Thus, the inclusion of 460 g kg^{-1} CHO and 40 g kg^{-1} LIP is recommended for juvenile tambaqui weighing between 10 and 250 g.

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