

Growth, lipogenesis and body composition of piracanjuba (*Brycon orbignyanus*) fingerlings fed different dietary protein and lipid concentrations

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Abstract

Piracanjuba (*Brycon orbignyanus*) is a Brazilian migratory fast-growing omnivore, very appreciated as a sport fish, which is threatened to extinction in Southern Brazil due to stock over exploitation and dam building. Therefore, efforts have been made to raise this fish in captivity for reintroduction and aquaculture purposes. In the present study, the effects of different dietary protein and lipid concentrations on piracanjuba fingerlings growth performance, feed utilization, body composition, hepatosomatic index (HSI) and activity of the lipogenic enzymes fatty acid synthetase (FAS), glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme (ME) were investigated using a 2×3 factorial experiment. Six casein–gelatin based diets were prepared combining two protein (30% and 32%) and three lipid concentrations (5.5%, 8.8% and 12.1%). Eleven fish, average weight 11.30 ± 0.1 g, were held in each of 18 100-l aquaria, supplied with recirculating freshwater. Each diet was randomly assigned to triplicate groups of fish and fed to apparent satiation, twice a day for 100 d. Piracanjuba fingerlings' daily weight gain (0.36–0.40 g), specific growth rate (1.43–1.51%), feed utilization and HSI were not influenced by dietary protein or lipid concentration. However, body composition was directly affected by dietary treatment. An increase in body fat and dry matter was observed as dietary lipid increased, for both dietary protein concentrations tested. The activity of FAS was depressed by increasing dietary fat levels but the G6PD activity did not differ among dietary treatments, although ME activity showed some regulation by dietary protein. These results indicate that an increase from 5.5% to 12.1% in the dietary lipid, at a dietary protein concentration of 30% or 32%, promotes body fat accumulation in piracanjuba fingerlings with no improvement in growth, suggesting that the lipid requirement for this species should be 5% or less, when raised for commercial purposes. However, the additional energy reserve from body fat accumulation could be desirable for piracanjuba fingerlings produced for stock enhancement.

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Résumé

Croissance, lipogenèse et composition corporelle d'alevins de piracanjuba (*Brycon orbignyanus*) nourris avec différentes concentrations de protéines et lipides. Le piracanjuba (*Brycon orbignyanus*) est un poisson migrateur et omnivore du Brésil, à croissance rapide ; il est très apprécié pour la pêche sportive mais menacé d'extinction due à une surexploitation et à la construction de barrages. Ainsi, des efforts ont été menés pour l'élever en captivité à des fins de réintroduction et d'aquaculture. Nous avons étudié les effets des teneurs en protéines et en lipides alimentaires sur les performances de croissance, l'efficacité alimentaire, la composition corporelle et le rapport hépato/somatique (HSI) d'alevins de piracanjuba, ainsi que sur l'activité des enzymes de la lipogenèse : acide gras synthétase (FAS), glucose-6-phosphate déshydrogénase (G6PD) et enzyme malique (ME) en utilisant une expérimentation à 2×3 facteurs. Six régimes, à base de caséine et gélatine, ont été préparés en combinant 2 taux de protéines (30 et 32 %) et 3 de lipides (5,5 ; 8,8 et 12,1 %). Onze poissons, de poids moyen de $11,30 \pm 0,1$ g, ont été élevés dans chacun des 18 bacs de 100 l, alimentés en eau douce et en circuit fermé. Chaque régime a été assigné, de façon aléatoire, à 3 groupes de poissons, nourris à satiété visuelle, 2 fois par jour durant 100 j. Pour les alevins de piracanjuba,

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le gain de poids journalier (0,36 à 0,40 g), le taux de croissance spécifique (1,4 à 1,5 %), l'efficacité alimentaire et HSI ne sont pas influencés par la teneur en protéines alimentaires ou en lipides. Cependant, la composition corporelle est directement affectée par le régime alimentaire. Les teneurs en matières sèches et en lipides corporels s'accroissent lorsque la teneur en lipides des aliments est augmentée et ce, pour les 2 concentrations en protéines testées. L'activité de FAS est inhibée par l'augmentation du taux de lipides alimentaires, l'activité de G6PD ne diffère pas entre les traitements alors que l'activité de ME montre une certaine régulation par le taux de protéines alimentaires. Ces résultats indiquent qu'un accroissement de 5,5 à 12,1 % des lipides dans l'aliment, pour une teneur en protéines de 30 ou 32 %, favorise l'accumulation de lipides corporels chez le piracanjuba, sans amélioration de croissance ; laissant supposer que le besoin en lipides pour cette espèce serait de 5 % ou moins quand il est élevé à des fins commerciales. Cependant, des réserves énergétiques supplémentaires, par accumulation de lipides, pourraient être souhaitables pour des alevins de piracanjuba produits à des fins d'empoissonnement.

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1. Introduction

Piracanjuba (*Brycon orbignyanus*) is a characin migratory fish, native to the Uruguai and Paraná River basins in Southern Brazil, which presents fast growth in captivity, omnivorous feeding habit and easy adaptation to artificial feeds. Due to dam building along the rivers and over exploitation of the stocks, this species is threatened to extinction. Therefore, diminishing natural populations and high appreciation by consumers has stimulated efforts to raise this species in captivity for stock enhancement or commercial aquaculture. Studies have been carried out on different aspects of piracanjuba biology and ecology (Goulding, 1980; Zaniboni-Filho and Resende, 1988), nutritional requirements (Esquivel, 1999; Sá and Fracalossi, 2002), and biochemical physiology (García-Carreño et al., 2002).

The development of cost effective and environmentally sustainable diets aims the maximum retention of dietary protein for growth (Nankervis et al., 2000). The optimal protein utilization is closely related to its concentration in the diet and the availability of dietary non-protein energy sources, such as lipids and carbohydrates (El-Sayed and Garling, 1988; Kaushik and Médale, 1994; Chou and Shiau, 1996; Nankervis et al., 2000; Watanabe et al., 2001). Fish, like other animals, control their feed intake to meet their energy requirements (Kaushik and Médale, 1994). Therefore, the protein and energy concentrations must be balanced for the maximum expression of the growth potential (Ellis and Reigh, 1991). Deficiencies in dietary non-protein energy sources result in the catabolism of proteins to generate energy, while excess energy can suppress appetite and reduce growth or increase fat deposition (Cowey and Sargent, 1979; NRC, 1993; Webster et al., 1995).

Recently, Sá and Fracalossi (2002) evaluated the performance of piracanjuba fingerlings fed isocaloric (12.5 kJ ME g⁻¹) experimental diets containing six protein concentrations (26–42%), at P:E ratios ranging from 33.7 to 19.4 kJ ME g⁻¹. Best growth performance was obtained at 29% protein and P:E ratio of 23.1. However, protein efficiency at dietary energy concentrations other than 12.5 kJ ME g⁻¹ has not been investigated to date for this species.

Many studies have demonstrated an improvement in growth rates and protein utilization when dietary energy concentration was increased by the addition of non-protein energy sources (Garling and Wilson, 1976; Hillestad and Johnsen, 1994; Dias et al., 1998; Nankervis et al., 2000), and it is well known that lipids are an important source of energy in fish diets (Lee and Putnam, 1973; Ojaveer et al., 1996; Martino et al., 2002). Therefore, the present study was carried out to determine the possible protein-sparing action of dietary lipid, and its effect on growth, lipogenesis and body composition of piracanjuba fingerlings.

2. Materials and methods

2.1. Experimental diets

Six experimental casein–gelatin purified diets were formulated to contain 30% or 32% crude protein (CP) and three lipid concentrations: 5.4%, 8.8% or 12.1% (Table 1). Diets were formulated based on the nutritional requirements established for the omnivorous channel catfish, *Ictalurus punctatus* (NRC, 1993). Increases in dietary lipid concentrations were achieved by increasing the level of cod liver oil and soybean oil (2:1) while reducing cellulose. Dry ingredients were blended, following the addition of oil and water. This mixture was passed through a food grinder (3 mm diameter dye). Resulting strings were broken, and pellets were stored in hermetically sealed plastic bags at –20 °C until fed (Fracalossi et al., 1998). Diet proximate analyses were determined according to procedures of the Association of Official Analytical Chemists (AOAC, 2000) and gross energy was measured using an automatic bomb-calorimeter (Table 1).

2.2. Fish and experimental procedure

The present study was carried out at the Freshwater Fish Biology and Aquaculture Laboratory (LAPAD), Federal University of Santa Catarina, Florianópolis, Brazil. Each diet was randomly assigned to triplicate groups of 11 piracanjuba fingerlings with average initial body weight of 11.30 ± 0.1 g stocked in 18 100-l fiberglass tanks (0.7 × 0.3 × 0.5 m), set in a water recirculation system supplied with a continuous flow

Table 1
Feed ingredients and proximate composition of the experimental diets (% dry weight)

| Ingredients | Protein (%) Lipid (%) | Diets | | | | | |
|---|--------------------------|-------|-------|-------|-------|-------|-------|
| | | 30 | | | 32 | | |
| | | 5.5 | 8.8 | 12.1 | 5.5 | 8.8 | 12.1 |
| Casein | | 22.34 | 22.34 | 22.34 | 23.83 | 23.83 | 23.83 |
| Gelatin | | 9.58 | 9.58 | 9.58 | 10.21 | 10.21 | 10.21 |
| Dextrin | | 36.38 | 36.38 | 36.38 | 34.15 | 34.15 | 34.15 |
| Cod liver oil | | 1.82 | 2.93 | 4.05 | 1.82 | 2.93 | 4.05 |
| Soybean oil | | 3.63 | 5.85 | 8.07 | 3.63 | 5.85 | 8.07 |
| Cellulose | | 17.15 | 13.82 | 10.48 | 17.26 | 13.93 | 10.59 |
| CMC ^a | | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Vitamin and mineral premix ^b | | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Choline chloride | | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| K ₂ SO ₄ | | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| MgSO ₄ | | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| NaH ₂ PO ₄ | | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| CaCO ₃ | | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| <i>Proximate composition</i> | | | | | | | |
| Moisture (%) | | 23.84 | 24.16 | 20.69 | 24.53 | 22.55 | 23.02 |
| Crude protein (%) | | 30.67 | 30.69 | 30.74 | 32.74 | 32.70 | 32.65 |
| Crude lipid (%) | | 5.58 | 8.64 | 12.25 | 6.01 | 8.92 | 12.36 |
| Ash (%) | | 4.67 | 4.41 | 4.24 | 4.34 | 4.32 | 4.33 |
| Crude fiber (%) | | 17.92 | 14.62 | 10.95 | 18.03 | 14.55 | 11.49 |
| Nitrogen free extract (%) ^c | | 41.16 | 41.64 | 41.82 | 38.88 | 39.51 | 39.17 |
| Gross energy (kJ g ⁻¹) | | 19.72 | 20.46 | 21.02 | 19.94 | 20.59 | 21.25 |
| Protein:gross energy ratio (mg kJ ⁻¹) | | 15.6 | 15.0 | 14.6 | 16.4 | 15.9 | 15.4 |

^a Carboxymethylcellulose.

^b Mineral–vitamin premix—units kg⁻¹ of premix: antioxidant 0.6 g; folic acid 250 mg; pantothenic acid 5000 mg; biotin 125 mg; niacin 5000 mg; vitamin A 1 000 000 IU; thiamin 1250 mg; cyanocobalamin 3750 mg; riboflavin 2500 mg; pyridoxine 2485 mg; ascorbic acid 42 000 mg; vitamin D₃ 500 000 IU; vitamin E 20 000 IU; vitamin K₃ 500 mg; cobalt 25 mg; copper 2000 mg; iron 13 820 mg; iodine 100 mg; manganese 3750 mg; selenium 75 mg and zinc 17 500 mg.

^c Nitrogen free extract = 100 – (ash + crude lipid + crude protein + crude fiber).

(0.7 l min⁻¹) and diffused aeration. Water temperature was measured daily and averaged 29.5 ± 1.4 °C during the 100-d growing period. Dissolved oxygen was measured daily, while pH, total ammonia–nitrogen and nitrite were measured once a week. Light was supplied by overhead fluorescent bulbs controlled by a timer to provide a 12-h photoperiod. Light bulbs were covered with black plastic to reduce light intensity to 4 lx, to prevent aggressive behavior among fish. Piracanjuba fingerlings were acclimated to experimental conditions for 1 week. During this period, they were fed a channel catfish reference purified diet (NRC, 1993) twice a day. At the beginning of the feeding trial, fish were anaesthetized (70 mg l⁻¹ MS222), individually weighted and measured. Piracanjuba fingerlings were hand fed to apparent satiety, twice a day (09:00 and 17:00 h) and feed intake was recorded.

2.3. Evaluation of growth and nutrient retention

Fish in each tank were group weighed every 2 weeks and individually weighed and measured at the end of the experiment. Samples of 23 fish of the initial stock and nine fish per dietary treatment were collected at the beginning and at the end of the feeding trial, respectively, for whole body composition analysis (AOAC, 2000) and gross energy determina-

tion. Fish were euthanized by hypothermia and stored (–20 °C) until analysis. At the end of the trial, liver samples from six fish per dietary treatment were also collected and immediately frozen in liquid nitrogen for later analysis of selected lipogenic enzyme activities. Livers of nine fish per dietary treatment were removed and weighed for hepatosomatic index (HSI) determination.

Performance and nutrient retention were evaluated considering the following parameters:

- Daily weight gain (g d⁻¹): DWG = [final weight (g) – initial weight (g)]/time in days.
- Specific growth rate (%): SGR = 100 × [(ln final weight [g] – ln initial weight [g])/days of the experiment].
- Daily feed consumption (%body weight d⁻¹): DFC = [feed intake (dry weight) (g)/(final weight [g] + initial weight [g]/2)]/time in days × 100.
- Feed conversion ratio: FCR = feed intake (dry weight) (g)/weight gain (g).
- Protein efficiency ratio: PER = weight gain (g)/protein intake (dry weight) (g).
- Apparent net protein retention: ANPR = [(final weight [g] × final body protein [%]) – (initial weight [g] × initial body protein [%])/total protein intake (dry weight) (g)] × 100.

- Apparent net energy retention: $ANER = [(final\ weight\ [g] \times final\ body\ energy\ [kcal]) - (initial\ weight\ [g] \times initial\ body\ energy\ [kcal]) / total\ energy\ intake\ (kcal)\ (dry\ weight)] \times 100$.
- Hepatosomatic index: $HSI = (liver\ weight\ [g] / whole\ body\ weight\ [g]) \times 100$.

2.4. Enzyme assays

Liver homogenates and activity assays for glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49), malic enzyme (ME; EC 1.1.1.40) and fatty acid synthetase (FAS; EC 2.3.1.38) were performed as described by Dias et al. (1998). Soluble protein content of liver homogenates was determined by the Bradford (1976), using bovine serum albumin (BSA) as the standard. Care was taken to ensure that initial rates were being measured in all assays. Control experiments established that the enzyme was stable in the buffer used during the time, and at the temperature required to perform the assay. All enzyme assays were performed in duplicate or triplicate. The enzymatic activity units (IU), defined as μmol of substrate converted to product per minute at assay temperature, were expressed per mg of hepatic soluble protein (specific activity).

2.5. Statistical analysis

A two-way analysis of variance was performed, taking into account the effects of dietary protein and lipid levels, and the interaction between the two factors on performance, lipogenesis and fish whole body composition. Tukey's Honestly Significant Difference was applied when significant differences were detected ($P < 0.05$) among dietary treatments.

3. Results

3.1. Growth performance and nutrient retention

Daily weight gain and specific growth rate varied between 0.36 and 0.40 g and 1.43% and 1.51%, respectively, and were not affected ($P > 0.05$) by dietary protein or lipid concentration (Table 2). No statistical differences ($P > 0.05$) were detected for daily feed consumption, nutrient retention, nor feed and protein utilization. Although there was no difference among groups ($P > 0.05$) for feed conversion ratio and protein efficiency ratio, there was a tendency for improvement of these indexes as dietary lipid concentration increased for both dietary protein concentrations tested (Table 2).

3.2. Whole body composition

The proximate body composition is shown in Table 3. Fish whole body protein, lipid and moisture contents were significantly affected ($P < 0.05$) by dietary lipid concentration, whereas dietary protein did not influence body composition ($P > 0.05$). Whole body lipid was positively related to dietary lipid concentration. Conversely, body moisture content decreased significantly from the lower (5%) to the higher (12%) dietary lipid concentration. Whole body protein, ash and HSI did not vary significantly among dietary treatments ($P > 0.05$).

3.3. Activities of lipogenic enzymes

The activity of FAS, G6PD and ME was determined to assess the effect of experimental diets on liver lipogenesis (Table 4). The activity of FAS was significantly reduced ($P < 0.05$) as dietary fat increased. The dietary protein con-

Table 2
Performance and nutrient retention of piracanjuba fingerlings fed two protein and three dietary lipid concentrations over 14 weeks

| Dietary factors | | DWG ^a | SGR ^b | DFC ^c | FCR ^d | PER ^e | ANPR ^f | ANER ^g |
|-----------------|-----------|------------------|------------------|------------------|------------------|------------------|-------------------|-------------------|
| Protein (%) | Lipid (%) | | | | | | | |
| 30 | 5.5 | 0.39 | 1.49 | 3.03 | 1.82 | 1.79 | 31.39 | 25.92 |
| 30 | 8.8 | 0.36 | 1.43 | 2.87 | 1.75 | 1.86 | 30.63 | 25.77 |
| 30 | 12.1 | 0.40 | 1.51 | 2.79 | 1.66 | 1.96 | 32.41 | 27.17 |
| 32 | 5.5 | 0.36 | 1.44 | 2.97 | 1.81 | 1.69 | 29.73 | 22.88 |
| 32 | 8.8 | 0.40 | 1.50 | 2.88 | 1.74 | 1.77 | 30.82 | 25.39 |
| 32 | 12.1 | 0.36 | 1.43 | 2.74 | 1.68 | 1.83 | 30.21 | 26.02 |

Analysis of variance

| | | | | | | | | |
|------------------------|-----------------|------|------|------|------|------|------|------|
| Protein | ns ^h | ns | ns | ns | ns | ns | ns | ns |
| Lipid | ns | ns | ns | ns | ns | ns | ns | ns |
| Protein \times lipid | ns | ns | ns | ns | ns | ns | ns | ns |
| Pooled S.E.M. | 0.04 | 0.22 | 0.10 | 0.07 | 0.07 | 0.07 | 1.25 | 1.66 |

Means of three replicate groups. Initial weight: 11.31 ± 0.06 g (mean \pm S.D.).

^a Daily weight gain (g d^{-1}).

^b Specific growth rate (%).

^c Daily feed consumption (% body weight d^{-1}).

^d Feed conversion ratio.

^e Protein efficiency ratio.

^f Apparent net protein retention (%).

^g Apparent net energy retention (%), based on gross energy).

^h No significant ($P > 0.05$).

Table 3
Whole body composition¹ and HSI of piracanjuba fingerlings fed two protein and three dietary lipid concentrations over 14 weeks

| Dietary factors | | Moisture (%) | Protein ² (%) | Lipid ² (%) | Ash ² (%) | HSI ³ (%) |
|-----------------|-----------|----------------------|--------------------------|------------------------|----------------------|----------------------|
| Protein (%) | Lipid (%) | | | | | |
| 30 | 5.5 | 68.14 ^a | 17.05 | 11.55 ^c | 3.23 | 1.55 |
| 30 | 8.8 | 67.61 ^{a,b} | 16.79 | 12.59 ^b | 3.01 | 1.30 |
| 30 | 12.1 | 66.42 ^b | 16.57 | 13.99 ^a | 3.03 | 1.34 |
| 32 | 5.5 | 68.86 ^a | 17.18 | 10.97 ^c | 2.99 | 1.36 |
| 32 | 8.8 | 67.50 ^{a,b} | 17.19 | 12.46 ^b | 2.88 | 1.27 |
| 32 | 12.1 | 67.04 ^b | 16.56 | 13.35 ^a | 3.05 | 1.29 |

Analysis of variance

| | | | | | |
|-----------------|-----------------|------|--------|------|------|
| Protein | ns ⁴ | ns | ns | ns | ns |
| Lipid | 0.0057 | ns | 0.0003 | ns | ns |
| Protein × lipid | ns | ns | ns | ns | ns |
| Pooled S.E.M. | 0.47 | 0.23 | 0.37 | 0.09 | 0.09 |

Means of pooled samples of three fish from each of three replicate groups. Values in the same column with different superscripts are significantly different ($P < 0.05$).

¹ Initial body composition (%): moisture 72.2; protein, lipid and ash (wet basis): 19.4, 5.2 and 3.1, respectively.

² Wet basis.

³ Means of three fish from each of three replicate groups.

⁴ No significant ($P > 0.05$).

Table 4
Specific activity of liver FAS, ME and G6PD of piracanjuba fingerlings fed diets at two levels of protein and three levels of lipid

| Dietary factors | | FAS ¹ | ME ² | G6PD ³ |
|-----------------|-----------|----------------------|----------------------|-------------------|
| Protein (%) | Lipid (%) | | | |
| 30 | 5.5 | 0.151 ^{a,b} | 0.073 ^{a,b} | 0.120 |
| 30 | 8.8 | 0.114 ^{b,c} | 0.071 ^{a,b} | 0.118 |
| 30 | 12.1 | 0.091 ^c | 0.082 ^a | 0.130 |
| 32 | 5.5 | 0.173 ^a | 0.074 ^{a,b} | 0.118 |
| 32 | 8.8 | 0.125 ^{b,c} | 0.059 ^b | 0.109 |
| 32 | 12.1 | 0.128 ^{b,c} | 0.063 ^{a,b} | 0.116 |

Analysis of variance

| | | | |
|-----------------|--------|------|-----------------|
| Protein | 0.01 | 0.02 | ns ⁴ |
| Lipid | 0.0001 | ns | ns |
| Protein × lipid | ns | ns | ns |
| Pooled S.E.M. | 0.02 | 0.01 | 0.01 |

Values in the same column with different superscripts are significantly different ($P < 0.05$).

¹ Fatty acid synthetase (mIU mg⁻¹ protein).

² Malic enzyme (IU mg⁻¹ protein).

³ Glucose 6-phosphate dehydrogenase (IU mg⁻¹ protein).

⁴ No significant ($P > 0.05$).

centration also affected FAS activity, reaching the lowest value in fish fed the 30% protein and 12.1% lipid diet. Both dietary protein and lipid had no significant effect on the specific activity of liver G6PD. However, a slight but significant effect was detected for dietary protein on ME activity.

4. Discussion

Lipids are important dietary energy sources for fish. Several studies have shown that they are efficiently metabolized by most fish species, resulting in increased feed performance and protein utilization (Lee and Putnam, 1973; Millikin,

1983; Stowell and Gatlin, 1992; Shiao and Lan, 1996; Nankervis et al., 2000; Martino et al., 2002). However, at too high levels, dietary fat may reduce fish growth, mostly due to reduction in fish consumption (Seenappa and Devaraj, 1995; Chou and Shiao, 1996; Weatherup et al., 1997; Silverstein et al., 1999; Regost et al., 2001b; Watanabe et al., 2001).

Piracanjuba fingerlings showed a 4.5-fold increase in weight in 100-d growing period. The specific growth rate observed in the present study was somewhat lower than the results verified for rabbitfish, *Siganus guttatus* (Parazo, 1990), red drum, *Sciaenops ocellatus* (Ellis and Reigh, 1991; Serrano et al., 1992) and surubim, *Pseudoplatystoma coruscans* (Martino et al., 2002). However, piracanjuba fingerlings showed a similar or even higher growth when compared with other fish species (Coloso et al., 1988; Tibaldi et al., 1996; Dias et al., 1998; Nankervis et al., 2000; Watanabe et al., 2001; Gélinau et al., 2002). The dietary protein and lipid concentrations tested did not result in significant differences ($P > 0.05$) in growth and nutrient utilization. However, a trend for decreasing feed consumption was evident, in both dietary protein concentrations, as the dietary lipid increased (Table 2), which improved feed conversion and protein efficiency ratio.

Similarly to our results, increasing dietary lipid concentration (15–21%) in diets (47% or 51% protein) for juvenile gilthead seabream, *Sparus aurata*, did not result in significant differences in weight gain (Santinha et al., 1999). However, better feed efficiency and nitrogen retention were obtained by fish fed diets containing the higher lipid concentration. Also, hybrid striped bass fingerlings, *Morone chrysops* × *M. saxatilis*, showed improvement in all feed efficiency parameters, except weight gain, in fish fed the higher dietary lipid concentration at the same protein level (Keembiyehetty and Wilson, 1998). No protein sparing effect by dietary lipid was verified for juvenile Asian seabass, *Lates*

calcarifer, with dietary lipid increasing from 5% to 15% (Catacutan and Coloso, 1995). Only dietary protein concentration had significant influence on fish performance. The best utilization of dietary protein was achieved at the lower concentration (35% CP) in comparison to the higher (50% CP), as reported for other species (Garling and Wilson, 1976; Parazo, 1990; Jantraratotai et al., 1998). In the present study, however, such effect of dietary protein concentration on fish performance was not observed probably due to the proximity of the two concentrations tested (30% or 32%).

Generally, studies carried out with other fish species, such as the Atlantic halibut, *Hippoglossus hippoglossus* (Berge and Storebakken, 1991; Helland and Grisdale-Helland, 1998), Nile tilapia, *Oreochromis niloticus* (Hanley, 1991); brown trout, *Salmo trutta* (Regost et al., 2001a), and turbot, *Psetta maxima* (Regost et al., 2001b), also showed that increasing dietary lipid did not improve growth and protein utilization, with no clear protein sparing effect of dietary fat. In the present study, fish whole body lipid and dry matter increased as dietary lipid concentration increased, although body protein and ash contents were not clearly affected by the diets. This is a common trend when body composition is compared on a wet weight basis (Shearer, 1994), and was also observed in experiments with milkfish, *Chanos chanos* (Coloso et al., 1988) and hybrid tilapia, *O. niloticus* × *O. aureus* (Chou and Shiau, 1996), among other species. From a product quality viewpoint, excessive fat deposition in fish is frequently deemed as undesirable, because this may affect negatively flesh shelf-life, organoleptic and physical properties (Millikin, 1983; Gjedrem, 1997). However, as stated by Winfree and Stickney (1981) and Parazo (1990), high fat levels in whole body may not be always undesirable, since this additional energy reserve may enhance survival of fingerlings when they are stocked in ponds with limited food availability. This aspect is especially relevant for the production of piracanjuba fingerlings for stock enhancement.

Piracanjuba fingerlings HSI were not influenced by dietary lipid concentration. Similar responses have also been reported for striped bass, *M. saxatilis* (Millikin, 1983), channel catfish (Stowell and Gatlin, 1992), and *Dentex dentex* (Tibaldi et al., 1996). Conversely, HSI increased with dietary lipid level in red drum (Serrano et al., 1992), and in juvenile Asian seabass (Nankervis et al., 2000).

The activity of FAS was clearly depressed with increasing dietary lipid level. This is in agreement with previously reported for juvenile European seabass, *Dicentrarchus labrax* (Dias et al., 1998), indicating that the rate of fatty acid synthesis in piracanjuba fingerlings is influenced by the diet and consistent with the general trend in fish (Henderson, 1996). Also a reduced dietary level of protein seems to produce a decrease in liver lipogenesis. In fish, the main carbon source for fatty acid synthesis is provided by amino acids derived from dietary protein (Henderson, 1996). Thus, a stronger effect would be expected if a wider range of dietary protein were used. However, no dietary effect was

found on hepatic G6PD specific activity. G6PD is one of the main enzymes involved in supplying reducing power in the form of NADPH for the biosynthesis of fatty acids (Alvarez et al., 1999), but is also needed to sustain the increased rate of cell growth to permit nucleotides synthesis involved in DNA metabolism (Barroso et al., 1994). Thus, a similar growth performance found in fish fed the experimental diets may suggest that NADPH produced by the pentose phosphate pathway was used to this end. The contribution of ME to NADPH production is variable among fish species (Segner and Bohm, 1994; Dias et al., 1998). In piracanjuba, ME activity represents about 50% of the G6PD activity, and seems more dietary regulated than G6PD. Since no changes were observed in the NADPH production by G6PD in the assayed diets, the apparent anomalous decrease in ME activity with the increased dietary protein could be a consequence of the slight increase in dextrin to counterbalance the energy ratio in the diets, as carbohydrates stimulate lipid biosynthesis not through delivery of carbon backbones but increasing the availability of cytosolic reducing equivalents (Hemre et al., 2002).

In conclusion, the present study demonstrated that piracanjuba fingerlings were able to store significant quantities of lipid in their body, but were not able to utilize this energy source to improve growth when dietary lipid increased from 5.5% to 12.1%. Likewise, no protein sparing effect was observed when the different lipid concentrations were fed in combination to 30% or 32% CP in the diet. Body fat accumulation is not desirable in food fish but can serve as energy store to fingerlings produced for stock enhancement, as may be the case for piracanjuba fingerlings.

Finally, as verified in other teleosts, the type and ratio of the dietary non-protein energy source may influence fish performance (Seenappa and Devaraj, 1995; Catacutan and Coloso, 1997; Erfanullah, 1998). Therefore, it would be worth investigating how far dietary carbohydrate concentration, as well as carbohydrate to lipid ratio can affect changes in lipid metabolism and performance of piracanjuba.

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