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Research Article

Effect of pond fertilization on growth performance of pirarucu (*Arapaima gigas*) during grow-out phase

Fabricio Pereira Rezende[1] & **Adriana Ferreira Lima**[1]
[1]Embrapa Pesca e Aquicultura, Loteamento Água Fria, Palmas, Tocantins, Brasil
Corresponding author: Adriana Ferreira Lima (adriana.lima@embrapa.br)

ABSTRACT. Fertilization of aquaculture ponds has been pointed out as an efficient practice to improve fish feed conversion and growth performance. In this way, the present study aimed to assess the effect of pond fertilization on the growth performance, body composition, and water quality of juvenile pirarucu *Arapaima gigas* reared in earthen ponds (600 m²) during the grow-out phase. Two fertilized and two non-fertilized ponds were stocked with 208 juvenile pirarucus (19.9 ± 3.6 g and 15.0 ± 0.9 cm) and reared for 100 days. All four ponds were limed before the study. Ponds were fertilized before fish were stocked and weekly until the end of the experiment, and fish offered feed until apparent satiation. Pirarucu reared in fertilized ponds presented higher final weight (20%) and biomass (19%), further to lower feed intake and feed conversion rate (1.31) than fish reared in non-fertilized ponds (1.61). Pond fertilization did not affect the fish coefficient of variation, which was reduced over the culture cycle. The body composition of fish reared in fertilized ponds presented higher water content, protein, and energy retention rates and lowered crude fat and energy. Fertilized ponds presented lower transparency levels and higher pH from the 50th day of culture, whereas ammonia concentration was higher in the fertilized ponds in the first 25 days. Therefore, pond fertilization is a recommended practice for pirarucu during the grow-out phase.

Keyword: *Arapaima gigas*; growth; natural food; yield; fertilized ponds; aquaculture

INTRODUCTION

In Brazil, fish are mainly farmed in earthen ponds (Valenti et al. 2021), a production system in which the culture environment can be managed to increase yield (Boyd et al. 2020). In earthen ponds, the increased fish yield is closely related to the increased availability of natural food, resulting from the nutrient supply, usually organic or inorganic fertilizers (Boyd 2018, Otieno et al. 2021). Accordingly, such increased yield results from dietary supplementation with natural food for some fish species, especially in the early stages of development (Feiden & Hayashi 2005, Asano et al. 2010, Filbrun & Culver 2014, Narimbi et al. 2018). In this way, properly managing the pond's natural productivity is an efficient means of improving the food conversion ratio, reducing production costs, and increasing business profitability (Bhakta et al. 2004, Narimbi et al. 2018). Additionally, plankton growth in the pond water prevents undesired problems with macrophytes, and if well managed, it can be positive for water quality and yield (Boyd & Lichtkoppler 1979, Biswas et al. 2017, Duodu et al. 2020).

Pirarucu, *Arapaima gigas* (Schinz, 1822) is a native fish species with great potential for aquaculture in Brazil, especially due to fast growth, aerial breathing, and tolerance to low water oxygen levels (Cavero et al. 2004, Lima et al. 2015a, Valladão et al. 2018, Lima 2020, Valenti et al. 2021). Although pirarucu farming is still limited, it has great potential and is gradually increasing, reaching 2000 t in 2020 (IBGE 2020), and the majority (71%) reared in earthen ponds (Rebelatto-Junior et al. 2015). Nevertheless, pond fertilization is a procedure rarely adopted by pirarucu farmers, mainly due to the lack of information on its effect on fish growth performance. Despite the carnivorous feeding

Corresponding editor: Eduardo Ballester

habit, pirarucu feeds on plankton in the early developmental stages. Occurrence of microcrustaceans in the stomach of wild pirarucu of up to 50 cm total length (Queiroz 2000) and high relative abundance of planktonic organisms in the stomach of 43 cm farmed pirarucu (700 g) (Lima et al. 2018) support the possibility of pond fertilization increasing yield of the species. Since feed may account for 56% of the production cost (Pedroza-Filho et al. 2016), natural food in the pond could improve both feed conversion and profitability by complementing the formulated diet, especially because there is not yet specific diets meeting the pirarucu nutritional requirements (Valenti et al. 2021). Therefore, the present study aimed to assess the effect of pond fertilization on juvenile pirarucu growth performance reared in earthen ponds during the grow-out phase.

MATERIALS AND METHODS

Preparation and experimental design

The study was carried out at Fazenda Acácia, Porto Nacional, Tocantins, Brazil (10°30'25.065"S, 48°20'38.788"W) between January and May 2015. The region is classified as Köppen Aw, i.e. tropical climate with dry winter, 1622 mm annual rainfall. The trial was conducted during the rainy season.

The study was conducted in a completely random design with two treatments and two replicates: fertilized (CF) and non-fertilized ponds (SF). The reduced number of replicates was due to the high number of juvenile fish needed to simulate commercial farming densities practiced by farmers in Brazil.

Two hundred and eight 50 days old juvenile pirarucu *Arapaima gigas* (19.9 ± 3.6 g and 15.0 ± 0.9 cm) were stocked in 600 m^2 earthen ponds (0.35 fish m^{-2}). Juveniles were purchased from a commercial farm in the state (Piscicultura Alvorada, Alvorada/TO, Brazil). Juvenile pirarucu of 10-20 cm is commonly stocked in commercial grow-out farms.

Twenty days prior to the trial, ponds were prepared, i.e. drained, water supply and draining systems checked, vegetation removed from the bottom and sides of the ponds, the anti-bird system installed, pond limed (calcitic limestone 2 t ha^{-1}), and fertilized. One week before the trial, ponds were filled, and subsequently, water loss due to evaporation was compensated to maintain the same level throughout the study. Only the CF treatment ponds were fertilized with one initial preparation dose and weekly maintenance doses (Table 1). The study was conducted for 100 days, correspon-ding to the first growing out phase in commercial proprieties (Lima et al. 2018).

Feeding

In the first week of trial, fish were fed twice per day (08:00 and 16:00 h) an extruded feed for juvenile carnivorous fish (45% crude protein - CP, 1.5. mm, Aquaxcel, Purina, Cascavel Paraná, Brazil) *at libitum*. From the second week, fish were fed twice per day (08:00 and 16:00 h) a commercial extruded feed (45% CP, 2.6 mm until day 75, 45% CP, 4 mm until day 90, and 40% CP, 6 mm until day 100, Laguna, Socil, Descalvado, São Paulo, Brazil) *at libitum*. Feed intake and morphometric measurements were monitored to assess the feed conversion ratio.

Experimental monitoring

Fish growth was assessed by morphometric measurements taken every 25 days. Fish were captured with a dragnet, and 30 fish per experimental unit were randomly sampled for total length and weight measurement, with ichthyometer (1 mm precision) and electronic scale (1 g precision), respectively. Water temperature, transparency, and pH were monitored daily with a thermometer, a Secchi disc, and a commercial colorimetric kit (Alfakit, Florianópolis, SC, Brazil), respectively. Water concentrations of total ammonia, nitrite, dissolved oxygen, carbon dioxide, alkalinity, and hardness were measured every three days with the aid of a commercial colorimetric kit (Alfakit, Florianópolis, SC, Brazil).

Fish growth performance was assessed based on weight gain (WG = initial weight - final weight), biomass gain (BG = initial biomass - final biomass), apparent feed conversion ratio (FCR = total feed intake / total weight gain), and survival. Additionally, fish weight coefficient of variation (CV weight = 100 × standard deviation / mean weight) and apparent protein / energy retention rate (%) = ([(final biomass × final body protein/energy) - (initial biomass × initial body protein/energy)] / total protein/energy intake × 100) and survival = (100 × (final number of fish / initial number of fish) were assessed. Eight fish were sampled at the beginning of the trial for fish carcass composition, and four fish per experimental unit were sampled at the end of the study. Fish feed and carcass samples were analyzed according to the methods described by AOAC (1990).

Statistical analysis

Data normality and homogeneity of variance were assessed by Shapiro-Wilk and Bartlett tests, respec-tively. Data were subjected to one-way analysis of variance, and the difference between means was compared by the Tukey test (P < 0.05). Value is presented as mean ± standard deviation. Analysis was

Table 1. Fertilization scheme of the earthen pond for pirarucu (*Arapaima gigas*) reared for 100 days in the grow-out phase. Adapted from Lima et al. (2015b).

Fertilizer	Initial dose (kg) (Preparation)	Weekly dose (kg) (Maintenance)	Total in 100 days (kg)
Rice bran	20.0	6.0	104.0
Urea	5.0	2.0	33.0
Single superphosphate	5.0	1.5	26.0
Potassium chloride	0.0	0.5	7.0

run using R statistical analysis software (R Core Team 2016).

RESULTS

Pond fertilization had a positive effect on the growth performance of pirarucu *Arapaima gigas* in earthen ponds, with significantly higher growth in fish reared in fertilized ponds. Actually, from day 50, it was already possible to observe such a growth trend in fertilized ponds (Fig. 1). Accordingly, biomass gain was higher in fish reared in fertilized ponds (181.36 ± 5.56 kg) than in non-fertilized ponds (151.86 ± 7.94 kg) at the end of the study.

Feed intake was similar in both treatments until day 75 of the study, whereas between days 76 and 100, fish in the non-fertilized ponds presented higher feed intake than those in fertilized ponds (Fig. 2a). In general, daily feed intake varied between 2.3 to 3.1% of the estimated biomass in the ponds. Feed conversion was lower in the fertilized ponds from day 25 until the end of the study (1.31 in fertilized ponds and 1.61 in non-fertilized ponds) (Fig. 2b).

There was no significant difference in fish survival in non-fertilized (100%) and fertilized (99%) ponds. The CV for fish weight was similar between treatments and tended to decrease throughout the trial (Fig. 3).

Fish carcass composition at the end of the study was similar between treatments for ash and crude protein. However, moisture content and protein and energy retention rates were higher in fish reared in fertilized ponds, whereas crude fat and gross energy were lower (Table 2).

Water quality parameters (pH, transparency, and total ammonia concentration) are presented (Table 3). Water pH was significantly lower in the non-fertilized ponds from the beginning until the end of the 100 day study period. Water transparency decreased after day 50 of the study in the fertilized ponds. The other water quality parameters did not differ between treatments or during the study: mean water temperature was 29.3 ± 0.2°C, mean dissolved oxygen and carbon dioxide concentrations were 8.3 ± 0.3 and 0.51 ± 0.1 mg L^{-1}, respectively, mean nitrite, hardness, and alkalinity were 0.003 ± 0.009 mg L^{-1}, 8.6 ± 2.5 mg L^{-1} of $CaCO_3$ and 12.1 ± 3.7 mg L^{-1} of $CaCO_3$, respectively.

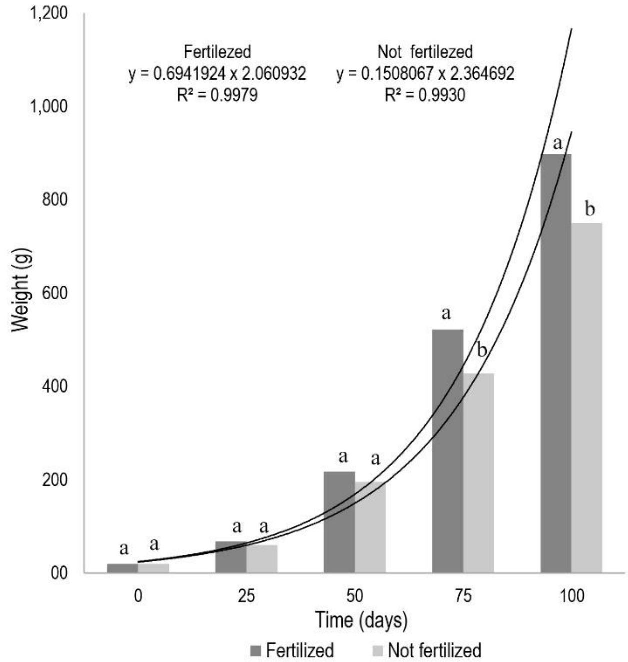

Figure 1. Growth curve for juvenile pirarucu *Arapaima gigas* reared in fertilized and non-fertilized ponds during grow-out phase. Different superscript letters indicate a significant difference between treatments (Tukey, $P < 0.05$).

DISCUSSION

During the grow-out phase trial, none of the water quality parameters presented critical values for pirarucu *Arapaima gigas* (Boyd & Lichtkoppler 1979, Cavero et al. 2004, Ono & Kedhi 2013). Water pH was lower in non-fertilized ponds, although ponds were equally limed as the fertilized ponds, which may be the result of more intense photosynthesis in fertilized ponds, with consequent carbon sequestration from the water and, therefore, higher water pH values (Boyd & Lichtkoppler 1979, Boyd 2012).

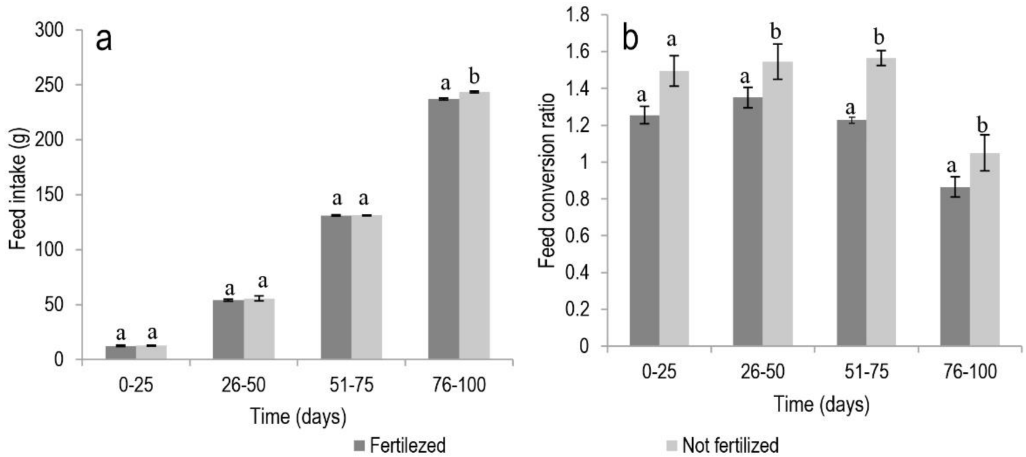

Figure 2. a) Feed intake and b) feed conversion ratio of pirarucu *Arapaima gigas* reared in fertilized and non-fertilized ponds during the grow-out phase. Different superscript letters indicate a significant difference between treatments (Tukey, $P < 0.05$).

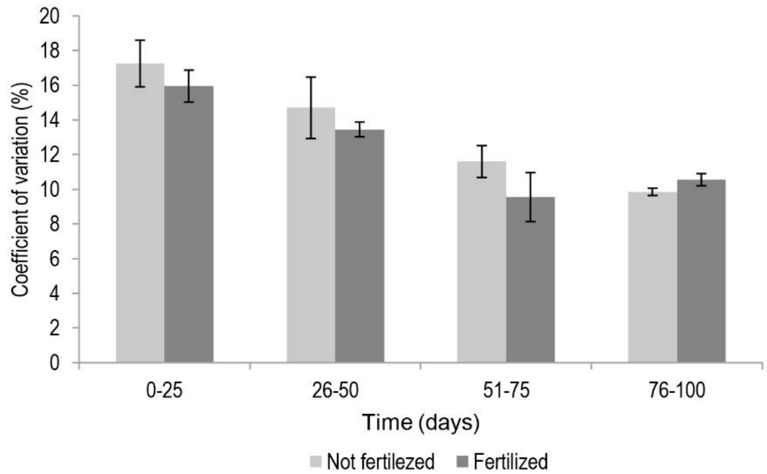

Figure 3. Coefficient of variation for the weight of juvenile pirarucu *Arapaima gigas* reared in fertilized and non-fertilized ponds during the grow-out phase. No significant difference was observed between treatments (Tukey, $P > 0.05$).

Water transparency did not differ among ponds in the first 50 days of the experiment, even with the weekly fertilization in the fertilized ponds, whereas in the second half of the study, water transparency was lower in fertilized ponds. The water transparency in fertilized ponds may have taken longer to be reduced because they were not used for a long time before the trial corroborating Boyd & Lichtkoppler (1979) that reported that new ponds without fertilization history require a higher amount of fertilizers for the establishment of the plankton community. Complementary, Boyd (2018) discussed that water and soil quality differences among ponds could affect fertilizer efficiency, promoting plankton production. In the same way, Khan & Bari (2019) observed three weeks to find differences in plankton communities in ponds fertilized or receiving commercial food. Additionally, Biswas et al. (2017) describe an increase in plankton community density along the production time when ponds are fertilized and receive formulated feed, corroborating the transparency decrease in fertilized ponds.

Total ammonia concentration was significantly higher in the fertilized ponds in the first 25 days of culture, possibly due to ammonia released by inorganic fertilizer (Soderberg 2012). Higher ammonia concentrations were also reported by Garg & Bhatnagar (2000) and Thakur et al. (2004) in the first days of *Cirrhinus mrigala* (Hamilton, 1822) and Nile tilapia *Oreochromis niloticus* culture, with ponds being fertili-

Table 2. Proximate carcass composition and protein and energy retention rates of juvenile pirarucu *Arapaima gigas* reared for 100 days in fertilized and non-fertilized earthen ponds during the grow-out phase. Different letters within the line indicate a significant difference between treatments (One-way ANOVA, Tukey, $P < 0.05$).

	Fertilized pond	Non-fertilized pond	*P*-value
Ash (%)	2.37 ± 0.09	2.36 ± 0.14	0.2597
Moisture (%)	77.71 ± 0.74 a	75.86 ± 0.44 b	<0.001
Crude protein (%)	14.88 ± 0.55	15.33 ± 0.73	0.1838
Crude fat (%)	4.36 ± 0.56 a	5.37 ± 0.41 b	0.0011
Gross energy (kJ kg^{-1})	4135.47 ± 19.72 a	4589.24 ± 24.33 b	0.0011
Protein retention rate (%)	30.39 ± 1.25 a	25.47 ± 1.18 b	<0.001
Energy retention rate (%)	22.69 ± 1.43 a	20.51 ± 0.75 b	0.0018

Table 3. Mean water pH, transparency, and total ammonia during the grow-out phase of pirarucu *Arapaima gigas* reared in fertilized and non-fertilized earthen ponds. Different capital letters in the same column indicate a significant difference between treatments, and different lower case letters in the same line indicate the difference between days of culture (One-way ANOVA, Tukey test, $P < 0.05$). Same values in different times mean total transparency in ponds.

Treatment	Days of culture			
	0-25	26-50	51-75	76-100
	pH			
Fertilized	6.3 ± 0.0 aA	6.5 ± 0.0 aA	6.9 ± 0.0 aA	6.6 ± 0.1 aA
Non-fertilized	5.4 ± 0.6 aB	5.5 ± 0.1 aB	6.4 ± 0.1 aB	6.1 ± 0.0 aB
	Transparency (cm)			
Fertilized	128.0 ± 17.0 aA	120.0 ± 17.0 aA	69.0 ± 4.9 bB	68.0 ± 8.5 bB
Non-fertilized	111.0 ± 6.4 aA	131.0 ± 5.7 aA	131.0 ± 4.9 aA	131.0 ± 4.9 aA
	Total ammonia (mg L^{-1})			
Fertilized	0.3 ± 0.0 aA	0.3 ± 0.1 aA	0.8 ± 0.4 aA	0.7 ± 0.2 aA
Non-fertilized	0.1 ± 0.0 aB	0.3 ± 0.1 aA	0.4 ± 0.2 aA	0.4 ± 0.1 aA

zed every two weeks and weekly, respectively. However, the ammonia concentrations were in the range found in commercial fish farms (Zhou & Boyd 2015) and below of reported by Pedrosa et al. (2019), Santana et al. (2020), and Paredes-López et al. (2021), in pirarucu studies. Survival did not differ between treatments, suggesting that the water quality alterations did not negatively affect pirarucu.

Pond fertilization resulted in better growth performance of juvenile pirarucu, a trend that could be observed from day 50 of the study and statistically significant from day 75 onwards. Accordingly, biomass gain was significantly higher in fertilized ponds after 100 days of culture. The positive effect of fertilization on yield has been observed in the production of several fish species (Boyd & Lichtkoppler 1979, Green et al. 1990, Garg & Bhatnagar 2000, Waidbacher et al. 2006, Tabinda & Ayub 2010, Narimbi et al. 2018). In general, it is because of the supplementary feeding provided by natural food intake in the pond (Chakrabarti & Jana 1991, Ribeiro et al. 2001, Rahman et al. 2008, Narimbi et al. 2018). Considering that pirarucu reared in earthen ponds feed on planktonic organisms until 700 g (43 cm) in size (Lima et al. 2018), better growth performance of fish reared in fertilized ponds may result from the natural food intake to meet the nutritional requirements (Conceição et al. 2010). In general, formulated feeds available in Brazil still do not meet the nutritional requirements of pirarucu (Valenti et al. 2021).

Feed intake was significantly lower in fish reared in fertilized ponds from day 76 of the trial (Fig. 2a), the same period when better growth performance was observed. Such a result suggests that plankton supplemented the diet and enhanced growth, as reported for Nile tilapia reared in earthen ponds (Waidbecher et al. 2006). Natural food, especially zooplankton organisms, the most consumed food item by juvenile pirarucu (Lima et al. 2018), contains higher levels of protein, fat, and energy (77%, 13%, and 23.86 kJ g^{-1}, respectively) on a dry matter basis, than commercial feeds (45%, 10%, and 17.16 kJ g^{-1}, respectively) (Blaxter 1989, Verga & Böhm 1992, Mitra et al. 2007), resulting in better growth with lower feed intake.

Additionally, protein and energy retention efficiency was 19 and 10%, respectively, higher in fish reared in fertilized ponds. Considering that the same compound feed was provided to both treatments and that natural food was not included in the calculations, higher protein and energy retention rates in fish reared in fertilized ponds may result from a higher natural food intake. A higher protein retention rate was also observed in Nile tilapia feeding natural food rather than formulated feed (Waidbecher et al. 2006, Asano et al. 2010).

Daily feed intake during the 100 days culture period varied between 2.3 and 3.1%, similar to values reported by Ono & Kedhi (2010) in feeding tables for 500 g and 1 kg pirarucu. FCR was better in fish reared in fertilized ponds already from day 26 of the study. Such better FCR has been reported for other species reared in fertilized ponds, e.g. tambaqui *Colossoma macropomum* (Cuvier, 1816) (Gomes & Silva 2009) and Nile tilapia (Diana et al. 1994) and the benefits of plankton in the initial stages have also been reported for carnivorous fish species such as dourado *Salminus brasiliensis* (Cuvier, 1816) (Mai & Zaniboni-Filho 2005). Waidbecher et al. (2006) reported that the access to natural food for Nile tilapia reared in fertilized ponds resulted in a higher protein: energy ratio of the ingested food, so protein was not a limiting factor for growth and, consequently, better fish growth performance. Differences in FCR from day 26 suggest that fish consumed plankton produced by fertilization management, despite no differences in transparency, has been observed, as pointed by Duodu et al. (2020). The characteristic of young pirarucu forage plankton more actively (Lima et al. 2018) added to Vakkilainen et al. (2004) and Brucet et al. (2010) observations that the presence of zooplankton predator fish has more influence in plankton density than nutrients availability by fertilization, can explain the absence of difference in transparences despite better FCR were observed on the same period.

Kang'ombe et al. (2006) reported that pond fertilization has a significant effect on fish carcass composition, i.e. higher moisture, crude protein, and fat content, because of the availability of natural food in the pond. Nevertheless, fish reared in fertilized ponds presented lower fat and higher moisture contents in the present study. Accordingly, Veverica et al. (2000) reported similar results when rearing *Clarias gariepinus* (Burchell, 1822) in fertilized ponds in semi-intensive systems. Higher availability of protein for fish reared in fertilized ponds in the form of natural food may have influenced fish carcass composition, mainly fat content, as possibly better energy: protein ratio may have resulted in lower lipid deposition (Portz et al. 2001). Additionally, the different final weights of fish from different treatments may also have influenced fish carcass composition. A variation of body composition in fish of different class weights has usually been reported for pirarucu (Fogaça et al. 2011) and other species, such as *O. niloticus* (Santos et al. 2012).

Despite higher final biomass, reduced feed intake, and better feed conversion ratio in fertilized ponds, other studies evaluating the economic impact of this management in production are necessary. In general, this management has a positive cost-benefit, as described by Bhakta et al. (2004) and Duodu et al. (2020), but it is important to evaluate the specific conditions for pirarucu production.

CONCLUSION

During the pirarucu grow-out phase, pond fertilization resulted in up to 20% enhanced fish growth performance compared to non-fertilized ponds. It also increased protein and energy retention rates. Additionally, fertilization resulted in higher final biomass, reduced feed intake, and better feed conversion ratio; therefore, a management practice indicated for the grow-out phase of pirarucu culture.

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