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Influence of temperature and exercise on growth performance, muscle, and adipose tissue in pacus (*Piaractus mesopotamicus*)

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ABSTRACT

The aim of this study was to evaluate the effects of temperature and swimming exercise on fish growth in pacus (*Piaractus mesopotamicus*). Pacus weighing 0.9 – 1.9 g and 2.7 – 4.2 cm in standard length were cultivated at an initial density of 120 fish m^{-3} in 3 recirculation systems containing 6 water tanks at a volume of 0.5 m^3 each at temperatures of 24, 28 and 32 °C. At each temperature, three tanks were modified to generate exercise activity in the specimens and force the fish to swim under a current speed of 27.5 cm s^{-1}. At the end of the experiment, the following metrics were evaluated: fish performance, morphometry (length, width, height and perimeter in different body positions), and the diameter and density of muscle and subcutaneous ventral adipose tissues. At 28 °C, pacus were both heavier and had greater weight gain after 240 days of cultivation. Additionally, exercise improved the feed conversion. An increase of 4 °C (30 °C) did not provide any improvement in the performance of the fish. However, swimming exercise improved the performance of pacus, providing increases of 38% and a 15% improvement in feed conversion. Both temperature and exercise influenced the body morphology (especially in the caudal region) and the cellularity of white and red muscle fibers and adipocytes.

1. Introduction

Rearing environments affect the growth and quality characteristics of farmed fish, and this means that various management strategies can be used to influence and optimize production. The pacu (*Piaractus mesopotamicus*) is an important fish in Brazilian aquaculture. It is omnivorous, rustic, and easily adapted to artificial diets (Nunes et al., 2013). The pacu is considered a fatty fish due to deposition of subcutaneous fat, mainly in the ventral region. The deposition of fat in animals occurs by hypertrophy of adipocytes and is an energetically expensive process (Grant and Helferich, 1991); furthermore, fatty tissues are not desirable for human consumption.

During growth, the animals not only increase in size and weight but also change the proportions in which tissues are deposited (hyperplasia and hypertrophy), accompanied by changes in body morphometry (Santos et al., 2008).

To increase the productivity and quality of meat in this species, both temperature control and employing swimming exercises are significant factors that may be used in culture systems. According to Johnston (2006), various environmental factors (such as temperature and flow rate), individually or in combination, affect the entire physiological system of organisms, including skeletal muscle in teleost fish.

Temperature is a major factor that affects the growth, physiology, reproduction, and metabolism of fish (El-Sayed and Kawanna, 2008). Different temperatures alter skeletal muscle cellularity (number and size of fibers) by the rate of proliferation and differentiation of myogenic cells, and this influence varies with species and the seasonal period studied (Vieira and Johnston, 1992; Usher et al., 1994; Johnston, 1999; Johnston, 2006).

Forced exercise in fish is a powerful stimulus of the hypertrophy of red and white muscle fibers (Johnston and Moon, 1980; Sänger, 1992), which increases the growth of salmonids and other groups (reviewed by Khan et al., 2014; Palstra et al., 2015), including pacus (Nunes et al., 2013). In fish, the relative contributions to hyperplasia and

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hypertrophy of muscle growth is variable depending on the species, growth stage, and type of muscle (Johansen and Overturf, 2005; De Almeida et al., 2008, 2010).

The muscle is the edible part of fish, and the adoption of technologies for the growth of this tissue is important for farm development. In addition, few studies have been conducted with adipose tissue growth in fish (Fauconneau et al., 1991; Fauconneau et al., 1995). The objective of this study was to evaluate the influence of exercise and temperature on both performance and muscle and adipose tissue growth in pacu. So, it was found whether swimming exercise has different effects on growth performance at different temperature.

2. Materials and methods

2.1. Location, biological material, and facilities

The experiment was carried out at Polo Regional Alta Sorocabana, Agência Paulista de Tecnologia dos Agronegócios (APTA), in Presidente Prudente city, SP – Brazil.

Pacus weighing 1.3 g (0.26) and 3.41 cm (0.28) in standard length were cultivated at an density of 120 fish m^{-3} in 3 independent recirculation systems containing 6 circular tanks (1.15 m diameter and 0.5 m deph) at a volume of 0.5 m^3 each, with biological filtration and sterilization by ultraviolet rays. Constant water flow was maintained at a rate of 3000 L h^{-1} in each tank. In three tanks, the water flow was manipulated to form a circular current (with centripetal force) with a velocity of 27.5 cm s^{-1}, forcing the fish to swim against the flow. For the remaining three tanks, flow was not manipulated.

Within the recirculating systems, the water temperature was kept at 24 °C, 28 °C (ambient), and 32 °C. These temperatures are naturally experimented in pacu cultivation in farms. Dissolved oxygen and temperature were monitored daily. Ammonia, nitrite, nitrate and pH were monitored weekly.

Pacus were fed three times (at 8:00, 12:00 and 16:00 o′clock) a day with the same commercial feed, specific to each growth stage, with the amount supplied in accordance with the biomass of the tank. During feeding, the recirculating water system was turned off for a period of 30 min to allow feed consumption by the fish. Fish were fed in group (tank) and the amount of the supplied ration was weighed to determine the apparent feed conversion. The feed conversion (FC) was given by relation between biomass gain (BG) and ration supplied (RS) in the total period (240 days): $FC = BG \times RS^{-1}$.

On the 240th day, 24 fish (8 fish/tank) were sampled after a 24 h fasting period. Fish were stunned by thermal shock (by water with ice), slaughtered (by disruption of the cervical spine), and individually weighed. Performance measures, such as weight, feed conversion and specific growth rate (SGR) were then determined. The SGR ($\% \times d^{-1}$) was calculated as $[(\ln Wf - \ln Wi) \times d^{-1}] \times 100$, where Wf and Wi are the final and initial weight and "d" are the days of cultivation, respectively. Body morphometric measurements (cm) were also determined, including standard length, height, and width; perimeter of the middle portion of the body; and perimeter of the tail (Santos, 2004).

2.2. Histological techniques

At the beginning of cultivation and on the 240th day, muscle tissue samples were removed from four fish of each tank at the median portion of the body, below the dorsal fin, and at the tail for further analysis of white and red fibers, respectively (Fig. 1). These regions contain the greatest concentrations of white and red muscle fibers, respectively (Sanger and Stoiber, 2001). Samples were also removed from ventral subcutaneous adipose tissue. Tissues were fixed in buffered formalin, preserved in 70% alcohol and then embedded in historesin (according to instructions from the Leica Historesin Embedding Kit (Heidelberg, Germany)). Adipose tissue was dehydrated in a series of acetone dilutions before embedding in historesin. Cross sections of 5 µm were stained with hematoxylin-eosin and mounted on microscope slides for morphological analysis.

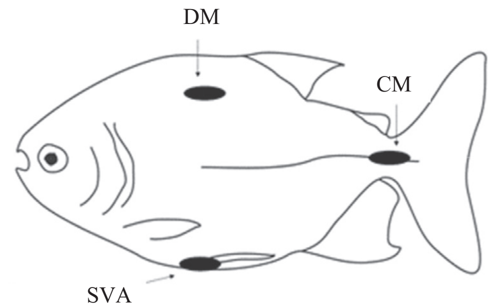

Fig. 1. Tissue samples for morphologic analysis. DM, muscle sample in the region below the dorsal fin to analysis white fibers. CM, muscle sample in caudal fin to analysis red fibers. SVA, sample to analysis the subcutaneous ventral adipose tissue taken in the pectoral region.

All measurements were made using a microscope coupled to an image analysis system. Under the microscope, representative areas of the muscle sections were obtained for each sample to determine the density of fibers (fibers mm^{-2}). Assuming that the fibers have a circular shape, and knowing the magnification of the image, the "diameter" of approximately 200 fibers was determined using the following formula: $D = 2A^{0.5}\pi^{0.5}$, where D is the diameter (µm), A is the cell area (µm^2), and π is the number 3.1416 (Valente et al., 1999). The fiber diameters were subsequently grouped into classes, and the frequency was determined as the number of fibers in each size class relative to the total number of fibers measured.

2.3. Data analysis

The experiment was completely randomized design with treatments in a factorial (two-way ANOVA) 2 × 3 (exercise × temperature) using 3 replicates (tanks) for performance analysis and 12 replicates (fish) for morphometric measurements and histology. Analyses of normality and homogeneity of variance were also performed. Variance analysis was performed on the data and supplemented with Tukey's multiple comparisons tests, using an alpha of 5%. The diameter of 200 cells (muscle and fat) was separated into classes and plotted on a histogram by frequency. The classes of cell diameter frequencies from fish cultivated according to exercise treatment (with exercise or without exercise) were compared using a *t*-test with an alpha of 5%.

3. Results

3.1. Performance and morphometry

There was significant temperature x exercise interaction for final weight and feed conversion ($P < 0.05$). The unfolding of the interaction indicated that the swimming exercise has various effects at different temperature conditions. The exercise influenced the feed conversion of pacus at the three growing temperatures (24, 28 and 32 °C).

Table 1 shows the means of weight, feed conversion and SGR of pacus on the 240th day of cultivation. The cultivation at 24 °C caused lower final weight and lower SGR than the 28 °C or 32 °C treatments. Feed conversion was lower in fish grown at 24 °C without exercise (1.56) than in fish grown at 28 °C or 32 °C (1.83 and 1.89, respectively). At heated water, exercise improved feed conversion of pacus ($p < 0.05$).

Morphometric differences were observed in pacus grown at different temperatures with exercise compared to the group not exercised on the 240th day of cultivation ($p < 0.05$). These differences were primarily visible in the caudal region (Fig. 2).

The pacus that were exercised during the experiment had higher