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# The compensatory growth of skeletal muscle cells in Amazonian catfish (*Pseudoplatystoma reticulatum* female x *Leiaris marmoratus* male)

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**Abstract.** The growth characteristics and morphological pattern of skeletal muscle tissue of Amazonian catfish (*Pseudoplatystoma reticulatum* female x *Leiaris marmoratus* male) from fingerlings until juvenile stage submitted to different restriction levels during compensatory growth were evaluated. There were four groups (G) submitted to different levels feed restriction: Group 1 (G1) animals that were fed for 120 days nonfasting (5% of body mass); Group 2 (G2) animals submitted to partial fasting for 30 days (2% of body mass) and after 90 days nonfasting; Group 3 (G3) animals submitted the partial fasting for 30 days (0.5% of body mass) and after 90 days nonfasting; Group 4 (G4) animals submitted to fasting for 30 days (0.0% of body mass) and after 90 days nonfasting. The results showed that a reduction in the diet of 2% and 0.5% of body mass during a period of fasting stimulated in G2 the total compensatory growth with 15 days of nonfasting through the muscle cells hypertrophy, G3 with 60 days and G4 with 90 days, being the last one a partial compensatory growth. Moreover, didn't affect the hyperplastic growth of muscle cells in nonfasting, although it has stimulated an upward hyperplasia of muscle cells of G4 from the period of 60 days. The strategy of compensatory growth to the Amazonian catfish in the juvenile stage proved to be efficient with 30 days off feeding and 90 days of nonfasting inducing the mechanism of muscle hypertrophy of Amazonian catfish. Therefore to evaluate the performance of the muscle cells associated with compensatory growth technique it can be helpful in the production system of fish farms.

**Keywords:** fasting, refeeding, hypertrophy, hyperplasia, muscle tissue, fish farming.

## Introduction

The success of fish farming depends of techniques development related to adequate feed and breeding management to reduce the cost of production, with maximum yield (Lovell, 1998). Therefore, there is a demand for studies related to different species with production potential that satisfies the market requirements (Salario et al., 2003). Among these species we can highlight the Amazonian catfish (*Pseudoplatystoma reticulatum* female x *Leiaris marmoratus* male), animal that has an excellent flavor meat, clear appearance, no intramuscular fishbones, firm texture and very appreciated and with high market value. Beyond, the specie fits easily to the omnivorous fish's diet and has higher carcass yield, which has been stimulating interest among fish farmers (Queirozet et al., 2012).

A known technique is a compensatory growth, which has been widely reported in farm fish for cost reduction in a production (Gaylord & Gatlin, 2001; Ali et al., 2003). This physiological process is characterized by growth acceleration in animals following a period of

growth depression because of reduced feed intake in comparison with their normally growing counterparts (Hornicket et al., 2000; Ali et al., 2003; Jobling, 2010). The fish growth is an integrated process that depends of many factors, such as, the availability of nutrients. In the nature, because of the high variation of availability of nutrients the fishes pass by different fasting levels during their lives, resulting in variations in the growth rates (Madrid et al., 2001). Long periods of fasting can induce a reduction in protein synthesis and consequently a slower growth. On the other hand, the refeeding after a period of fasting may result in the rapid development of growth (Hagen et al., 2009).

Ali et al. (2003) says the compensatory growth technique can be classified into four types: partial, full, overcompensation and the under compensation. There may be a single period of fasting (Rueda et al., 1998; Tian & Qin, 2003) or periods of fasting and refeeding alternated in cycles (Hayward et al., 1997; Wu et al., 2002; Nikki et al., 2004; Zhu et al., 2004). This ability to survival during these periods is well developed and

evident in many fish species (Dave et al., 1975; Chappazet al., 1996; Lawrence et al., 2002). It was found by Gaylord & Gatlin (2000) and Kim & Lovell (1995) that studied the channel catfish (*Ictalurus punctatus*), Ituassu et al., (2004) by assessing juvenile tambaqui (*Colossomacropomum*) and Skalskiet al., (2005) with hybrid Robalo (whitebass *Morone chrysops* x stripedbass *M. saxatilis*), these fishes were in fasting and when refeeding they got a fast growth with an improvement in the efficiency feed close to those fed normally. In addition, the development of new feeding strategies could improve the personnel time management, water quality as well as reducing feed and labour costs (Gaylord & Gatlin, 2001).

Despite the compensatory growth occurs in several species, the mechanism that controls the compensatory responses still little known (Won & Borsky, 2013). There is a degree of particularity for each species regarding hormonal action and other factors involved in the compensatory growth, especially in muscle growth where the satellite cells are influenced by different molecules, such as hormones, growth and transcription factors, resulting in its activation, proliferation and cell differentiation (Hawke & Garry, 2001; Aguiar et al., 2008; Won & Borski, 2013).

Fish skeletal muscle tissue makes up between 40 to 75% of total mass and is distributed in red, intermediate and white compartments or layers. The red or superficial muscle layer is under the dermis, and consists of fibers with oxidative metabolism and abundant myoglobin. The white or deep muscle layer represents most of the muscle mass, and consists of fibers with reduced oxidative activity and little myoglobin. Between the white and red layers is the smaller intermediate layer with intermediate characteristics (Kilariski, 1990; Johnston, 1999).

Skeletal muscle growth in fishes occurs by both hypertrophy and hyperplasia from undifferentiated myoblasts or myosatellite cells (Johnston, 1999). Myoblast proliferation and differentiation is controlled by myogenic regulatory factors (Watabe, 2001), they can enlarge existing fibres by fusion, thus adding new nuclei resulting in fibre hypertrophy. In hyperplasia, the undifferentiated myoblasts aggregate to the surface of existing fibres forming myotubes which then separate giving rise to new muscle fibres. Therefore when hyperplasia takes place, a mosaic of different diameter fibres (large and small associated fibres) is often seen in white muscle (Johnston, 2001).

Muscle growth in the larval and juvenile phases of commercial fish species is very important for final fish size. The hyperplasia and hypertrophy in muscle growth have been studied in many species. In fast growing fishes, which reach larger sizes and include many commercial aquaculture species, hyperplastic growth continues for longer (Kiessling et al., 1991; Valente et al., 1999; Rowleron & Veggetti, 2001). In small fishes, however, hyperplasia stops earlier and hypertrophy is a more effective growth mechanism (Veggetti et al., 1993; Koumans & Akster, 1995).

Muscle growth regulatory factors, responsible for myoblast activation and differentiation, can be influenced by many variables such as nutrition (Brodeuret al., 2003). Feeding can increase the concentration of available amino acids, increasing metabolic rate and aiding muscle tissue growth (Brown & Cameron, 1991a, b; Houlihan et al., 1995). Other factors such as temperature and oxygen consumption can stimulate this phenomenon, which is known as specific dynamic action (SDA) (Jobling, 1994).

The plasticity is the main feature of this tissue, allowing the tissue alters its morphological features, metabolic and function in response to different stimulus (Acosta et al., 2005). Johnston, (2006) says that this characteristic of the muscle tissue is important to the adaptation of organisms to their habitat. In the case of fish, this versatility needs to be higher because the environment can profoundly affects the development and muscle tissue growth.

The contribution of hypertrophy and hyperplasia to the muscle tissue growth can vary with the species and stage of growth (Brooks & Johnston, 1993). Fish species of tropical regions tend to lose body mass and the compensatory growth can be influenced by various environmental factors such as temperature, water salinity, feed restriction, stocking density and parasites that can change the number of muscle fibers recruited different from the cold water fish (Wang et al., 2000; Palma et al., 2010).

Few species have their physiological and productive behavior studied during compensatory growth (Ali et al., 2001). Therefore, the introduction of new techniques can contribute to the understanding of the muscle behavior during the compensatory growth becoming a valuable tool to maximize production and lower cost.

This study aimed to analyze the growth characteristics and morphological pattern of skeletal muscle tissue of Amazonian Catfish submitted to different feed restriction levels during compensatory growth.

## Methods

### *Animals and feeding procedure*

The experiment was performed at Delicious Fish Pisciculture in Sorriso-MT and in the Morphology Laboratory in Sinop-MT, Brazil, using Amazonian catfish (*Pseudoplatystoma reticulatum* female x *Leiarius marmoratus* male). The fingerlings were obtained from the reproduction laboratory of Delicious Fish Pisciculture and were transferred to outside tanks ( $n=4$ ) with 3.0 m length, 2.0 m wide, 1.80 m high under natural conditions. Fingerlings ( $n=100$ ) were placed in each tank and were kept for 15 days in the adaptation period. The initial body mass and standard length (LT), were  $10.00 \pm 2.00$  g and  $12.00 \pm 1.00$  cm (mean  $\pm$  SD,  $n=100$ ) respectively. Water quality was monitored daily was totally recirculated every 15 hours. The mean morning (06:00 hours) and afternoon (17:00 hours) water temperatures were 26.5 and 27.4 °C respectively. The oxygen content was 5.70(mg/L) and the pH was

around 6.70. Fish were divided into four groups G1 to G4 as follows: Group 1 (G1) animals that were fed for 120 days nonfasting (5% of body mass); Group 2 (G2) animals submitted to the partial fasting for 30 days (2% of body mass) and after 90 days nonfasting; Group 3 (G3) animals submitted to the partial fasting for 30 days (0.5% of body mass) and after 90 days nonfasting and Group 4 (G4) animals submitted to the fasting for 30 days (0.0% of body mass) and after 90 days nonfasting.

During the experimental period fish were fed with commercial feed pellets with 40% PB twice a day (6:00h and 17:00h)(Table I). During the experimental period of 120 days seven fish removals were made, which were collected 10 animals per group. Up to the period of 60 days the fish removal were held every 15 days and thereafter it were made every 30 days. The animal biometrics was realized in all periods to update the amount of feed supplied according to their growth.

**Table 1.** Diet composition nutritional fed to use in the experiment.

Nutrients	%
Humidity	10
Protein	40
Lipid	4
Fibre	6
Calcium	4
Phosphate	1,5
Metionine	0,90
Lysine	2,2
Asparticacid	0,5
Sodium	1,85

#### *Sacrifice and extract of muscle samples*

Prior to the beginning of each muscle tissue collect were measured weight and standard length using up a precision scale and a precision caliper to evaluate the production performance respectively. There after animals were euthanized by the method of spinal cord section being previously anaesthetized with clove oil with the concentration of 50 mg/L, the clove oil was diluted in ethanol (1:20) for 10 minutes. Longitudinal fragments of the muscle tissue were removed from the media dorsal region comprising white muscle fibers. The fragments were fixed in 10% buffered formaldehyde for further processing.

This procedure was approved by the committee of ethics in animal research with record number 23108.780205/12-0.

#### *Morphometric analysis*

In this phase, it was used the semi-automatic rotary microtome equipment(Leica model 2245) with the purpose of severing the material to transversal sections of 3  $\mu\text{m}$ , cut into a glass knife and collected on glass blades. The stained blades were used for morphometric analysis by measuring the areas in of muscle approximately 250 cells for each animal using an image analysis system (Moticam 2.0). Images were photographed on 4 different points of standard manner for all animals and recorded on the computer for later measurement of areas in square micrometer of each muscle cells in transversal section. Thereafter muscle cells were distributed into classes according to their cell area. This methodology allows to evaluate the degree of growth hypertrophic and hyperplastic muscle cells.

#### *Statistical analysis*

For cell morphometric analysis and number of cells was used a mixed model with effects period, class, group and its interactions and as random effects were

considered animal nested in interaction period and class. It was used the logarithmic transformation for correction of normality presuppositions and homogeneity of variances for both variables in the comparison of means, however the figures are presented in the original scale of the data for better understanding the results. In all analyzes was submitted to one-way ANOVA and used PROC MIXED application of SAS (SAS, 2004) by adopting the significance level of 5%. The comparison among levels of treatment were performed by Tukey Kramer test ( $p < 0.05$ ).

## **Results and discussion**

For the evaluation of hypertrophic growth was observed the number of cells divided into 1,2,3,4 and 5 classes according to their size for the comparison of means among groups. The evaluation of cell morphometry showed different hypertrophic growth among the groups in the partial fasting and nonfasting period.

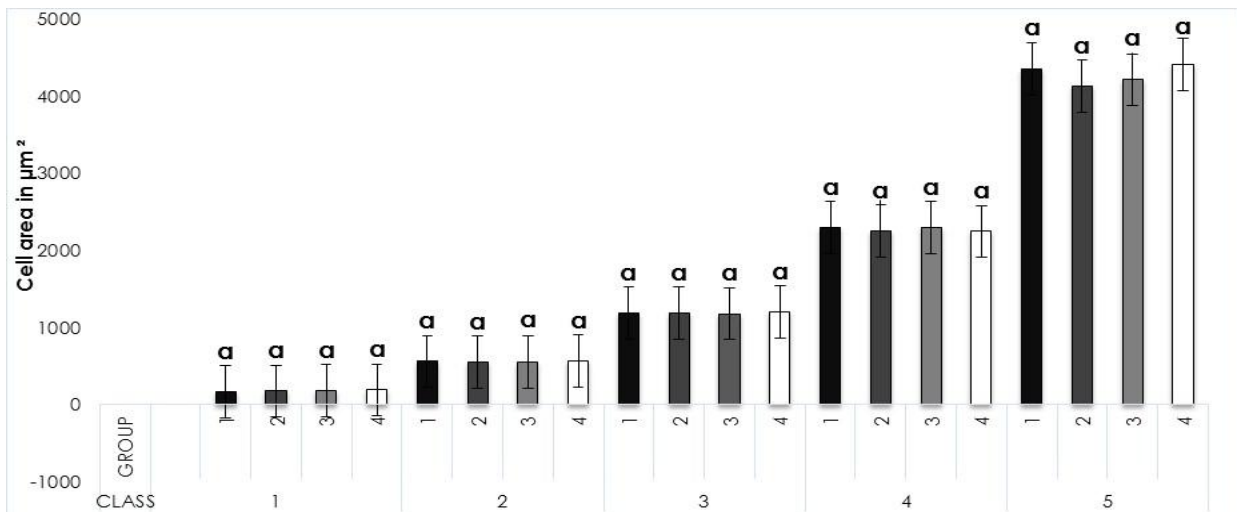
In the partial fasting period, in 15-day there was no difference in cell size distributed in classes. However, in 30-day period, there were differences in the size of class 5 of cells whose represent cells with areas greater than 3077.3  $\mu\text{m}^2$  among groups. In this class, G1 showed cells with increased cell area (5260.80  $\mu\text{m}^2$ ) different from the other groups ( $p < 0.05$ ). G2 (with 4515.83  $\mu\text{m}^2$ ) and G3 (with 4065.07  $\mu\text{m}^2$ ) groups were not different from each other ( $p > 0.05$ ). The G4 showed cells with smaller cell area (with 3761.05  $\mu\text{m}^2$ ) different from the other groups but not different from G3 ( $p < 0.05$ ). Therefore, in 15-day period, no change in cellular hypertrophic growth of small, medium and large cell among groups, although there was a trend toward a hypertrophy of the large cell in G1. However, in 30-day period of partial fasting, larger

cells of class 5, they had lower hypertrophic growth for G2, G3 and G4, the latter being the lowest growth rate ( $p < 0.05$ ). (FIGURE 1,2,3)

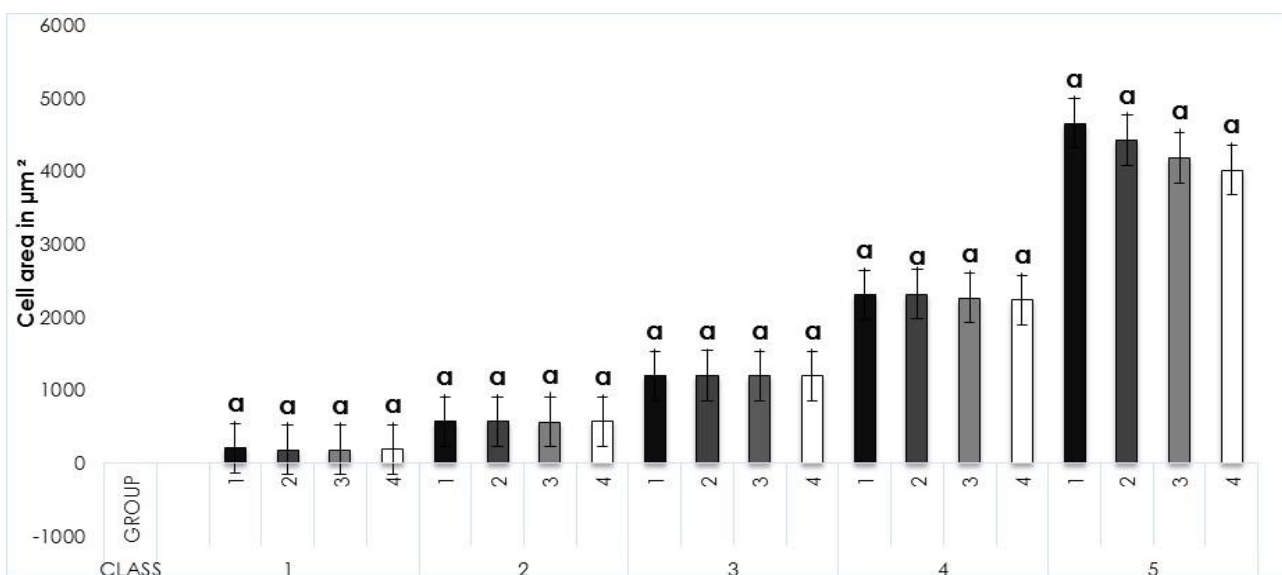
In nonfasting period, after 30-day, it was observed in 45, 60, 90 and 120-day period there was no difference in cell size distributed in the classes 1, 2, 3 and 4 which represent cells with lower areas among  $3077.3 \mu\text{m}^2$  groups ( $p > 0.05$ ). Note, again, that cellular hypertrophy occurred mainly in larger cells, class 5, although it was given a considerable increase in the supply of feed. In the 45-day period, G1 (with  $5181.15 \mu\text{m}^2$ ) and G2 (with  $5084.25 \mu\text{m}^2$ ) showed increased cellular area and were not different from each other, while the G3 (with  $3905.50 \mu\text{m}^2$ ) and G4 (with  $3904.05 \mu\text{m}^2$ ) showed cells with smaller cell area and were not different from each other. In 60-day period, G1 (with  $6610.05 \mu\text{m}^2$ ) and G2 (with  $5839.08 \mu\text{m}^2$ ) showed cells with larger cell area and were not different ( $p > 0.05$ ), while G3 (with  $4859.12 \mu\text{m}^2$ ) and G4 (with  $4514.60 \mu\text{m}^2$ ) showed cells with smaller cell area and were not different ( $p > 0.05$ ). Only in Class 2 there was a difference among groups but it was not maintained in subsequent periods. In 90 days the G1 (with  $6851.43 \mu\text{m}^2$ ), G2 (with  $7234.20 \mu\text{m}^2$ ) and G3 (with  $7504.57$

$\mu\text{m}^2$ ) showed cells with larger cell area and were not different from each other, while the G4 (with  $5260.18 \mu\text{m}^2$ ) showed cells with smaller cell area. In this period is clear the compensatory growth in G3 through the hypertrophy reached the same cell measurement values of G1 and G2. On the other hand, G4 continues to show a hypertrophic growth for compensatory growth, however, with lower values than the other groups. In 120 days the G1 (with  $6674.18 \mu\text{m}^2$ ), G2 (with  $5905.57 \mu\text{m}^2$ ) and the G3 (with  $5914.49 \mu\text{m}^2$ ) showed cells with a large cell area and were not different from each other ( $p > 0.05$ ) while G4 (with  $5443.56 \mu\text{m}^2$ ) showed cells with smaller cell area compared to G1 but similar to G2 and G3. Note that the hypertrophic growth in G1, G2 and G3 remains similar. The compensatory growth in G2 and G3 didn't progress beyond the G1 but they just remains. During this period the hypertrophic growth in G4 reaches the G2 and G3. The results indicate that is necessary 90 days after fasting so that the compensatory growth to reach desired values (Figures 4, 5, 6, 7).

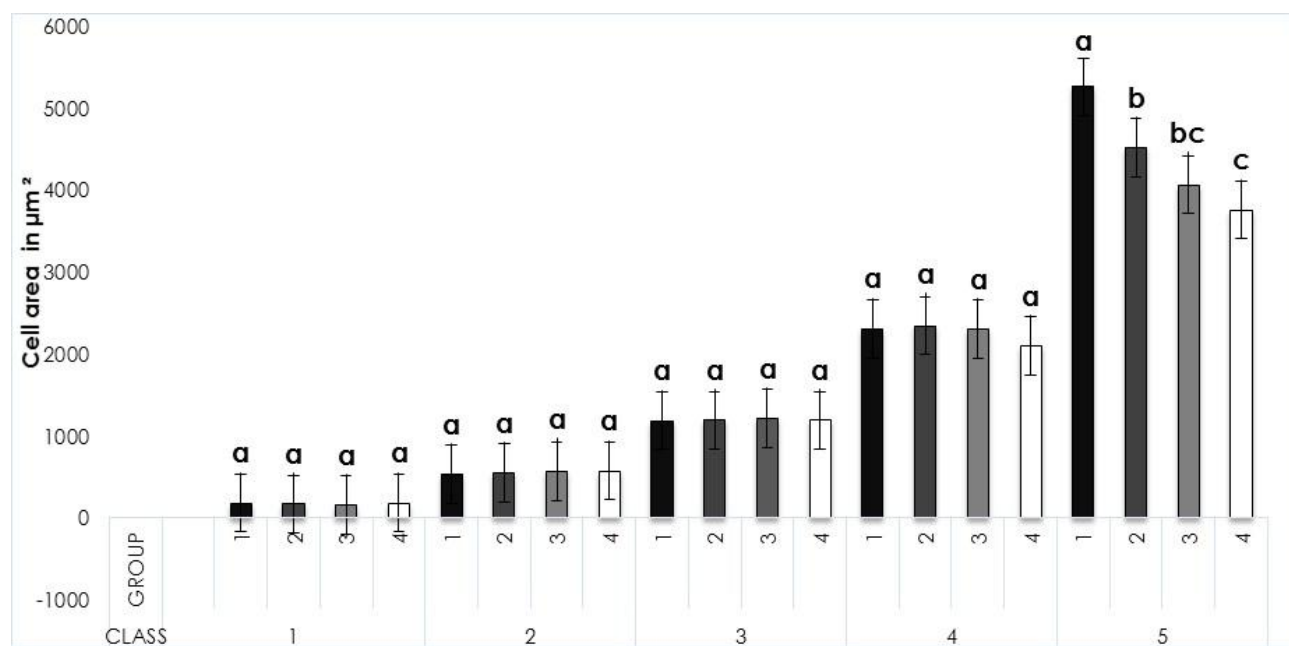
According to the below figures is possible to note the comparison of mean of cell area among classes in each group



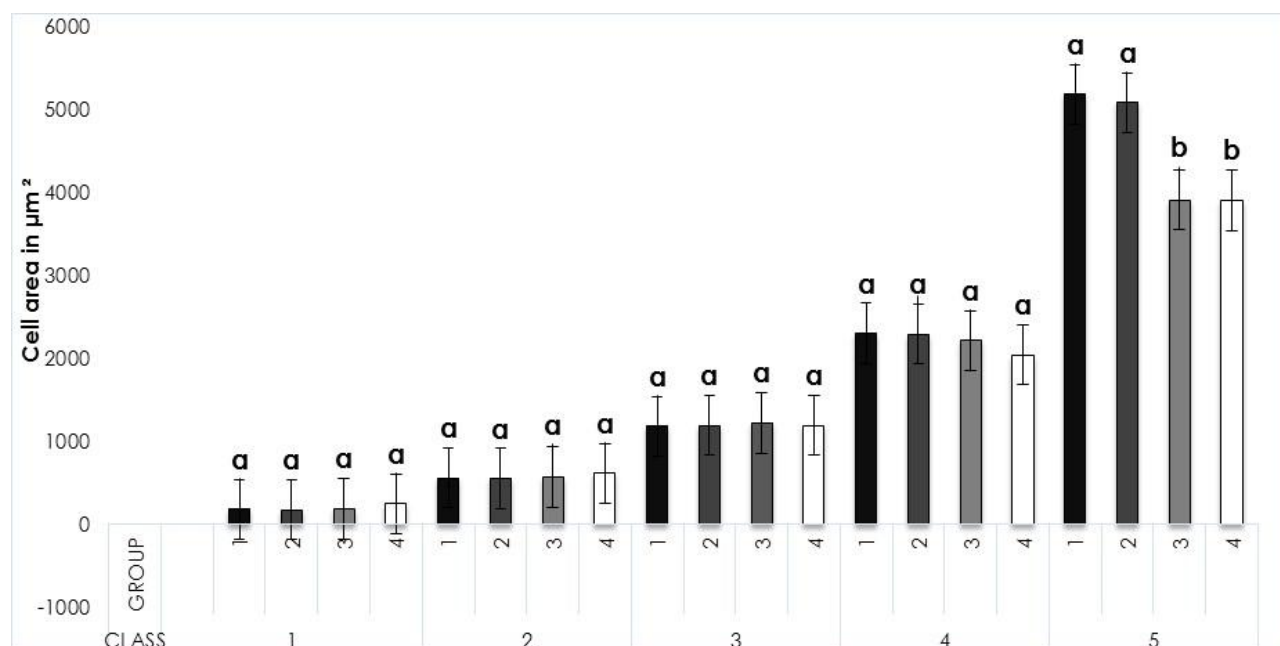
**Figure 1.** Mean the cellular area and standard error per class within each group - Period 0. Means with the same letter within the classes do not differ by Tukey test ( $p < 5$ ) CLASS 1;  $\leq 336.1 \mu\text{m}^2$  CLASS 2;  $> 336.1 \leq 831.3 \mu\text{m}^2$  CLASS 3;  $> 831.3 \leq 1612.6 \mu\text{m}^2$  CLASS 4;  $> \leq 3077.3 \mu\text{m}^2$  CLASS 5;  $> 3077.3 \mu\text{m}^2$ .



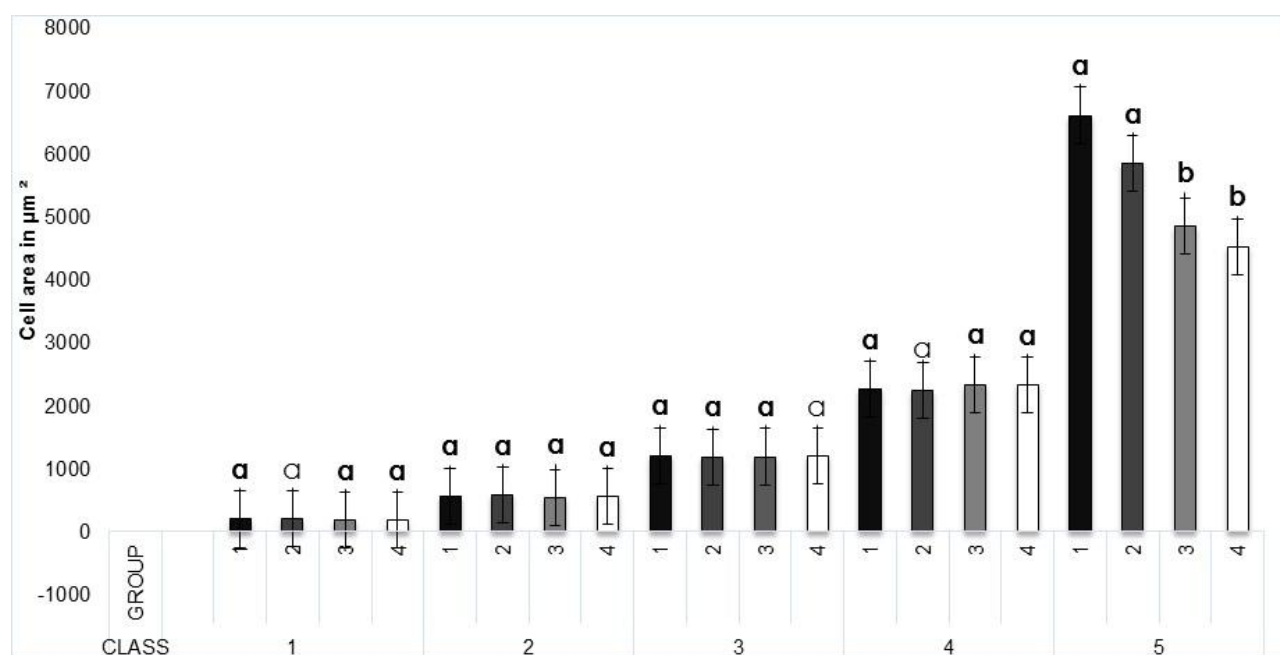
**Figure 2.** Mean the cellular area and standard error per class within each group - Period 15. Means with the same letter within the classes do not differ by Tukey test ( $p < 5$ ) CLASS 1;  $\leq 336.1 \mu\text{m}^2$  CLASS 2;  $> 336.1 \leq 831.3 \mu\text{m}^2$  CLASS 3;  $> 831.3 \leq 1612.6 \mu\text{m}^2$ , CLASS 4;  $> \leq 3077.3 \mu\text{m}^2$  CLASS 5;  $> 3077.3 \mu\text{m}^2$ .



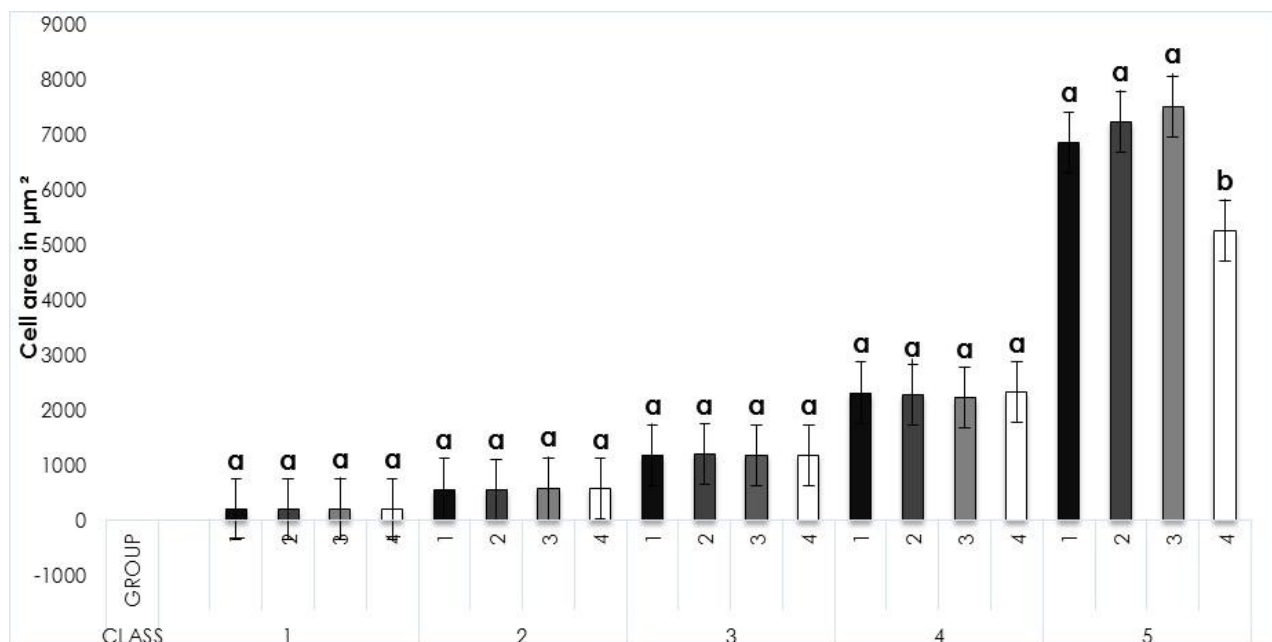
**Figure 3.** Mean the cellular area and standard error per class within each group - Period 30. Means with the same letter within the classes do not differ by Tukey test ( $p < 5$ ) CLASS 1;  $\leq 336.1 \mu\text{m}^2$  CLASS 2;  $> 336.1 \leq 831.3 \mu\text{m}^2$  CLASS 3;  $> 831.3 \leq 1612.6 \mu\text{m}^2$ , CLASS 4;  $> \leq 3077.3 \mu\text{m}^2$  CLASS 5;  $> 3077.3 \mu\text{m}^2$ .



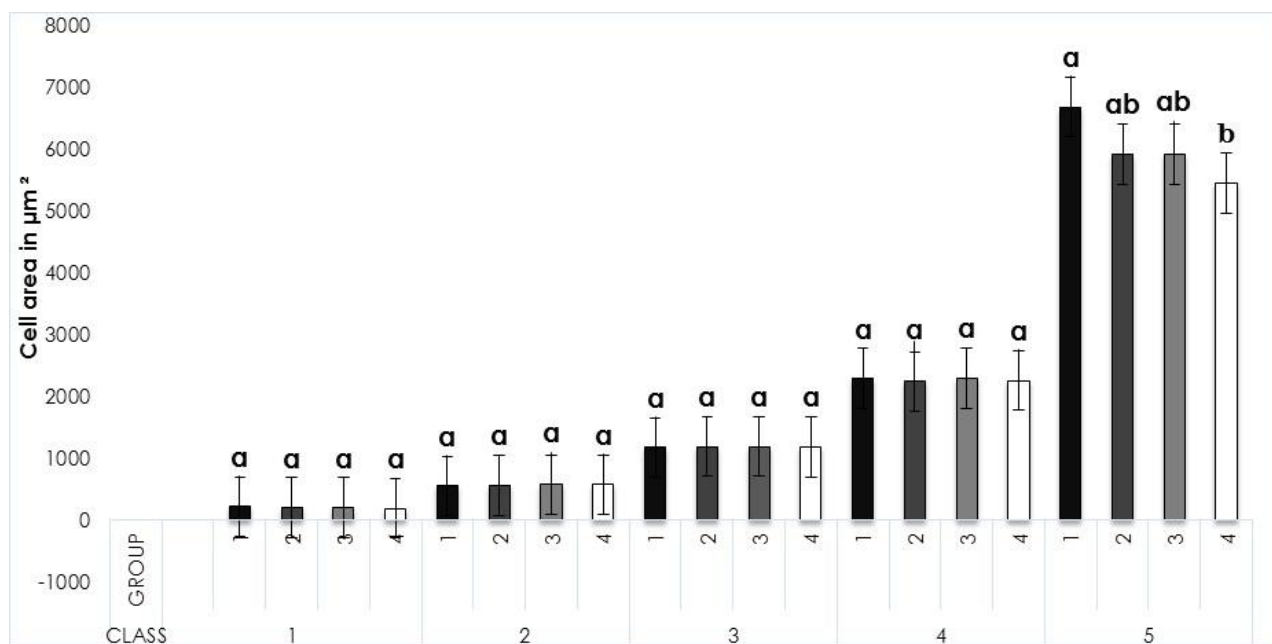
**Figure 4.** Mean the cellular area and standard error per class within each group - Period 45. Means with the same letter within the classes do not differ by Tukey test ( $p < 0.05$ ) CLASS 1;  $\leq 336.1 \mu\text{m}^2$  CLASS 2;  $> 336.1 \leq 831.3 \mu\text{m}^2$  CLASS 3;  $> 831.3 \leq 1612.6 \mu\text{m}^2$ , CLASS 4;  $> \leq 3077.3 \mu\text{m}^2$  CLASS 5;  $> 3077.3 \mu\text{m}^2$ .



**Figure 5.** Mean the cellular area and standard error per class within each group - Period 60. Means with the same letter within the classes do not differ by Tukey test ( $p < 0.05$ ) CLASS 1;  $\leq 336.1 \mu\text{m}^2$  CLASS 2;  $> 336.1 \leq 831.3 \mu\text{m}^2$  CLASS 3;  $> 831.3 \leq 1612.6 \mu\text{m}^2$ , CLASS 4;  $> \leq 3077.3 \mu\text{m}^2$  CLASS 5;  $> 3077.3 \mu\text{m}^2$ .



**Figure 6.** Mean the cellular area and standard error per class within each group - Period 90. Means with the same letter within the classes do not differ by Tukey test ( $p < 5$ ) CLASS 1;  $\leq 336.1 \mu\text{m}^2$  CLASS 2;  $> 336.1 \leq 831.3 \mu\text{m}^2$  CLASS 3;  $> 831.3 \leq 1612.6 \mu\text{m}^2$ , CLASS 4;  $\leq 3077.3 \mu\text{m}^2$  CLASS 5;  $> 3077.3 \mu\text{m}^2$ .



**Figure 7.** Mean the cellular area and standard error per class within each group - Period 120. Means with the same letter within the classes do not differ by Tukey test ( $p < 5$ ) CLASS 1;  $\leq 336.1 \mu\text{m}^2$  CLASS 2;  $> 336.1 \leq 831.3 \mu\text{m}^2$  CLASS 3;  $> 831.3 \leq 1612.6 \mu\text{m}^2$ , CLASS 4;  $\leq 3077.3 \mu\text{m}^2$  CLASS 5;  $> 3077.3 \mu\text{m}^2$ .

For the evaluation of hyperplastic growth was observed the numbers of cells and divided into 5 classes according to their size for comparison among groups.

In the partial fasting period the number of small and large cells did not differ among the groups in 15-day period ( $p > 0.05$ ), however in 30-day period the number of small and medium-sized cells, classes 1,2,3 and 4 were higher than G4 ( $p < 0.05$ ). It is possible to observe, that the number of large cells were lower only

for the G4 compared to the other groups. The groups G2 e G3, although receiving lower amount of feed compared to G1 in the same period, grown through a hyperplastic growth of muscle cells similar that observed in G1 (FIGURE 8,9,10).

In 45-day nonfasting period, there was a decrease in number of small cells Class 1 in G4, compared to G1 and G2 and a smaller number of large cells in G4 remains the same compared to 30 days. Although no

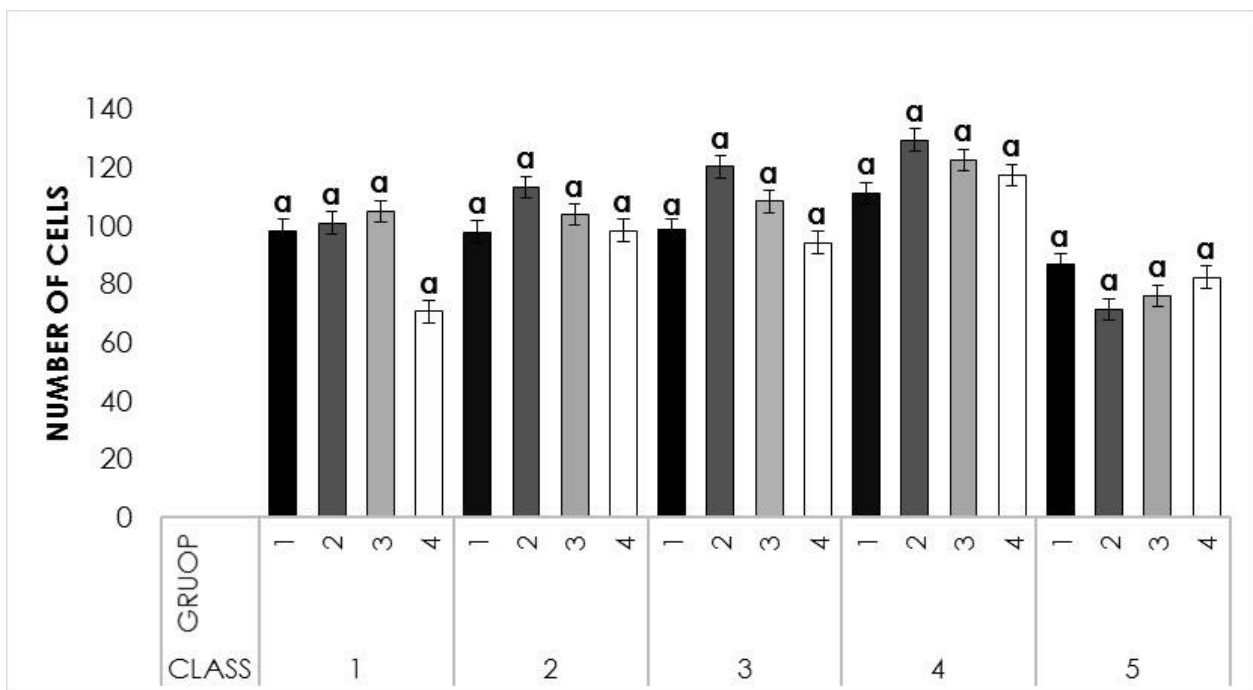


different from the other groups in 60 days, G4 increased the number of small and large cell. In 120 days it is clear that the hyperplastic growth mechanism remains increasing. In the present study occurred hyperplasia, although this growth mechanism has not influenced the difference between the groups G2 and G3, but only G4. A reduction of 2% and 0.5% body weight in the diet, during a partial fasting period didn't affect the hyperplastic growth in the nonfasting period but stimulated hyperplasia in G4 in 60-days period. Hyperplasia in the juvenile phase is important to establish the pattern of hypertrophic growth will occur throughout the animal's life, however it doesn't have major role to the animal's weight gain in younger stages (Figure 11, 12, 13, 14).

According to the below figures is possible to note the comparison of mean of number of cells among classes in each group.

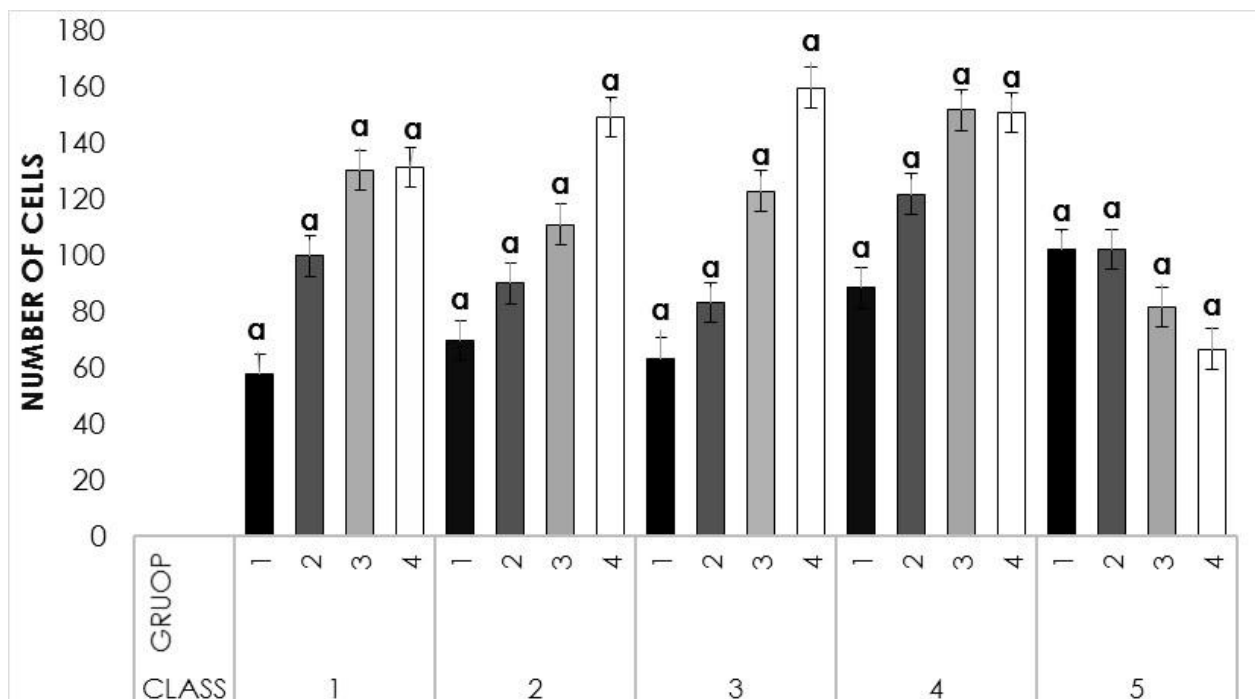
Our study showed that muscle growth in juvenile Amazonian Catfish after feed restriction was predominantly by cell hypertrophy, because the animal tried to store as much energy. The hypertrophy occurs mainly in larger cells and due the prolonged partial fasting period of 30 days, the growth mechanism was delayed, as observed in G2 and G3 that received less

feed and more critically in G4 that they do not were fed in this period. However, in the animals of G1, hypertrophy of the largest cells maintained its normal growth. The number of small and medium-sized were higher than G4 ( $p < 0.05$ ) in 30-day period. It is possible to observe, that the number of large cells were lower only for the G4 compared to the other groups because that severe dietary restriction induced a hyperplastic cell growth but not an increase in cell size due to food shortages and inability to build proteins for hypertrophy. The contribution of hypertrophy and hyperplasia in the growth of muscle tissue can vary with the species and the growth phase studied (Brooks & Johnston, 1993). The proportion small area cells in skeletal muscle depends on final fish size and is a relevant factor in comparing hyperplastic growth in large sized species of economical benefit (Kiesling et al., 1991; Fauconneau et al., 1997). Johnston (2001) said that for small fish species the hypertrophy is the main form of post-larval muscle growth since hyperplasia occurs during the early stages of embryogenesis. In big fish species, the new muscle fibers development occurs continuously in all stages of growth, but dietary restriction may affect these growth mechanisms as observed in our study.

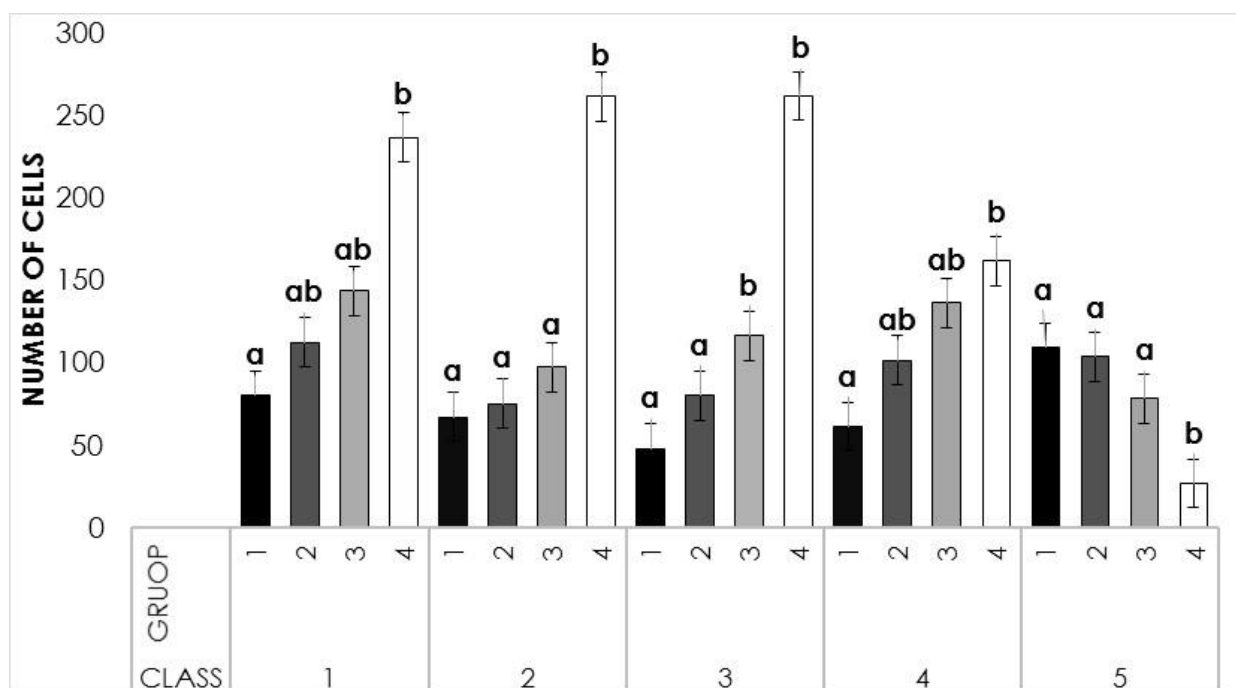


**Figure 8.** Mean the number cells and standard error per class within each group - Period 0. Means with the same letter within the classes do not differ by Tukey test ( $p < 5$ ) CLASS 1;  $\leq 336.1 \mu\text{m}^2$  CLASS 2;  $> 336.1 \leq 831.3 \mu\text{m}^2$  CLASS 3;  $> 831.3 \leq 1612.6 \mu\text{m}^2$ , CLASS 4;  $> \leq 3077.3 \mu\text{m}^2$  CLASS 5;  $> 3077.3 \mu\text{m}^2$ .

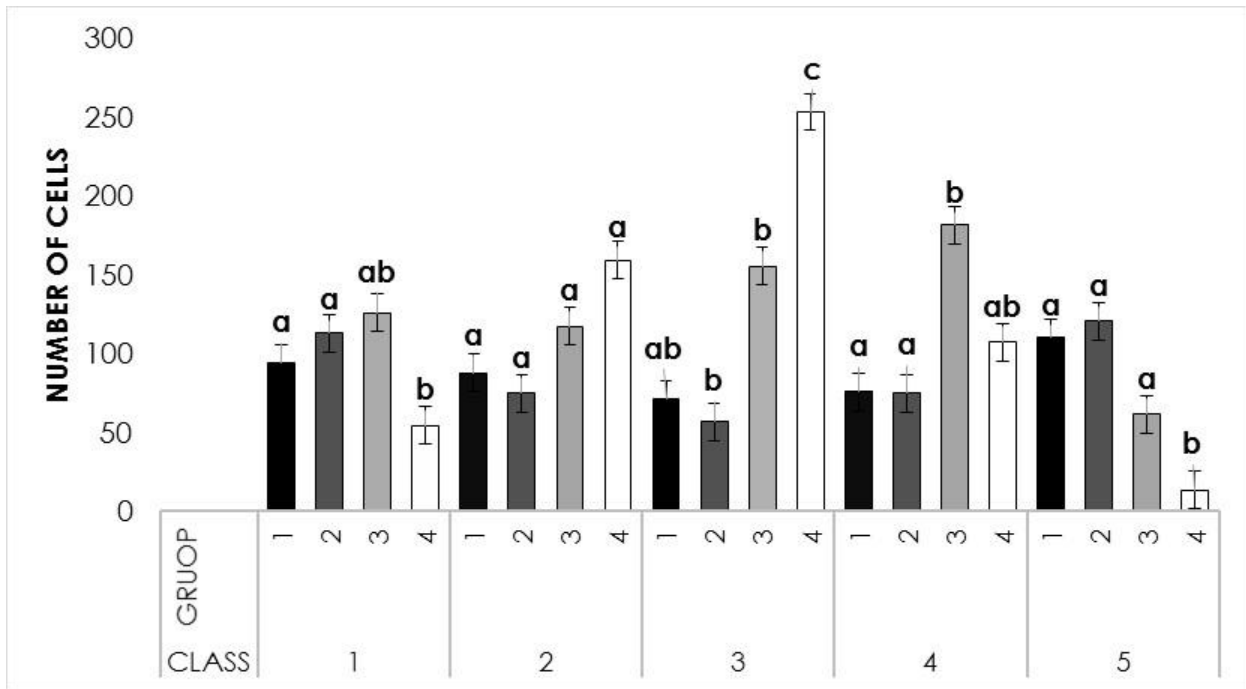




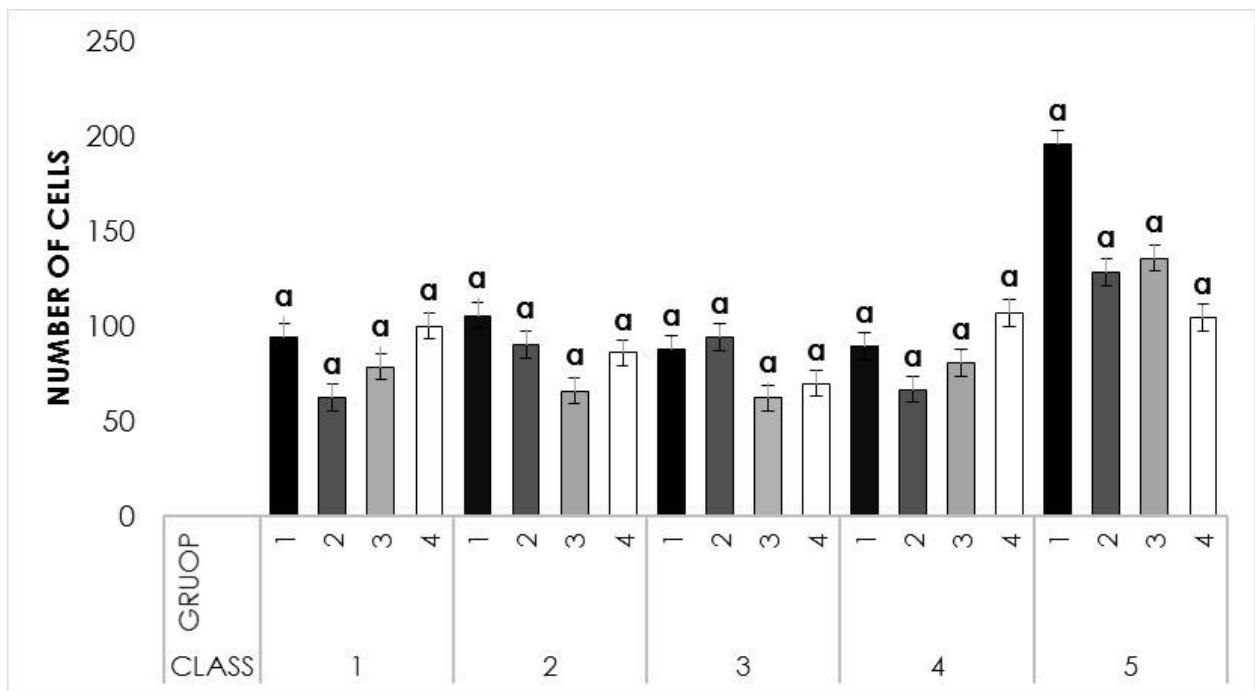
**Figure 9.** Mean the number cells and standard error per class within each group - Period 15. Means with the same letter within the classes do not differ by Tukey test ( $p < 0.05$ ) CLASS 1;  $\leq 336.1 \mu\text{m}^2$  CLASS 2;  $> 336.1 \leq 831.3 \mu\text{m}^2$  CLASS 3;  $> 831.3 \leq 1612.6 \mu\text{m}^2$ , CLASS 4;  $\leq 3077.3 \mu\text{m}^2$  CLASS 5;  $> 3077.3 \mu\text{m}^2$ .



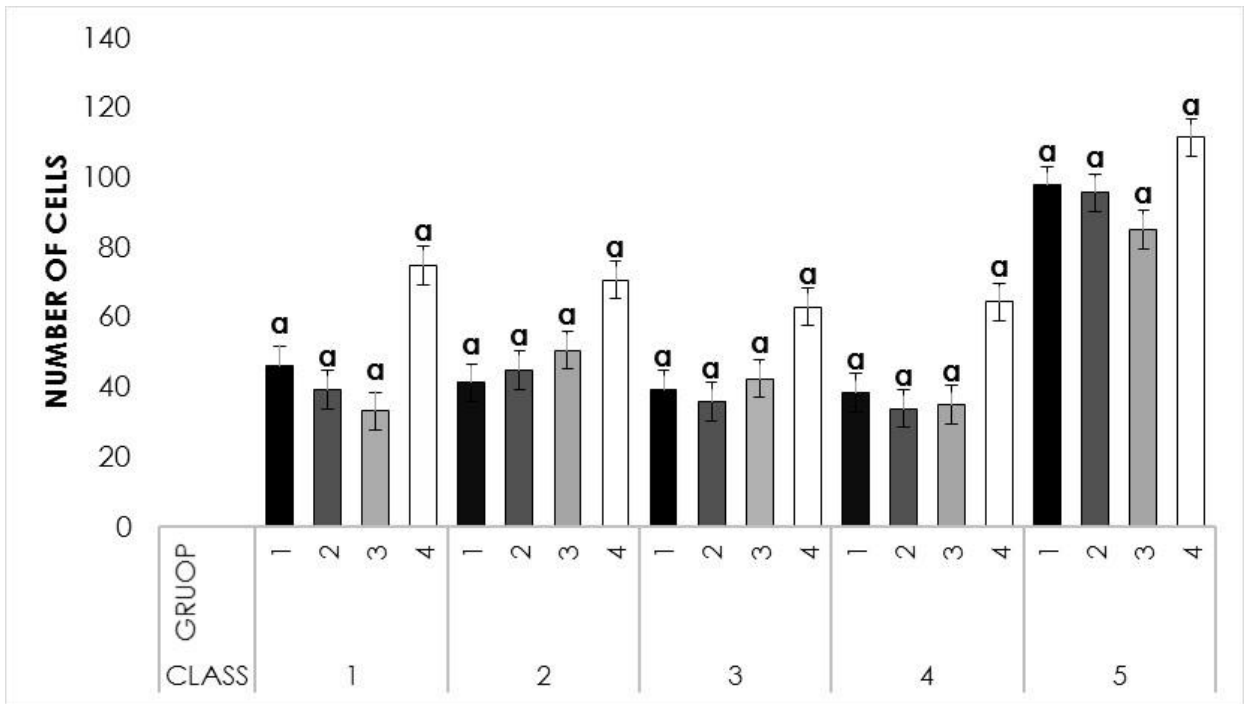
**Figure 10.** Mean the number cells and standard error per class within each group - Period 30. Means with the same letter within the classes do not differ by Tukey test ( $p < 0.05$ ) CLASS 1;  $\leq 336.1 \mu\text{m}^2$  CLASS 2;  $> 336.1 \leq 831.3 \mu\text{m}^2$  CLASS 3;  $> 831.3 \leq 1612.6 \mu\text{m}^2$ , CLASS 4;  $\leq 3077.3 \mu\text{m}^2$  CLASS 5;  $> 3077.3 \mu\text{m}^2$ .



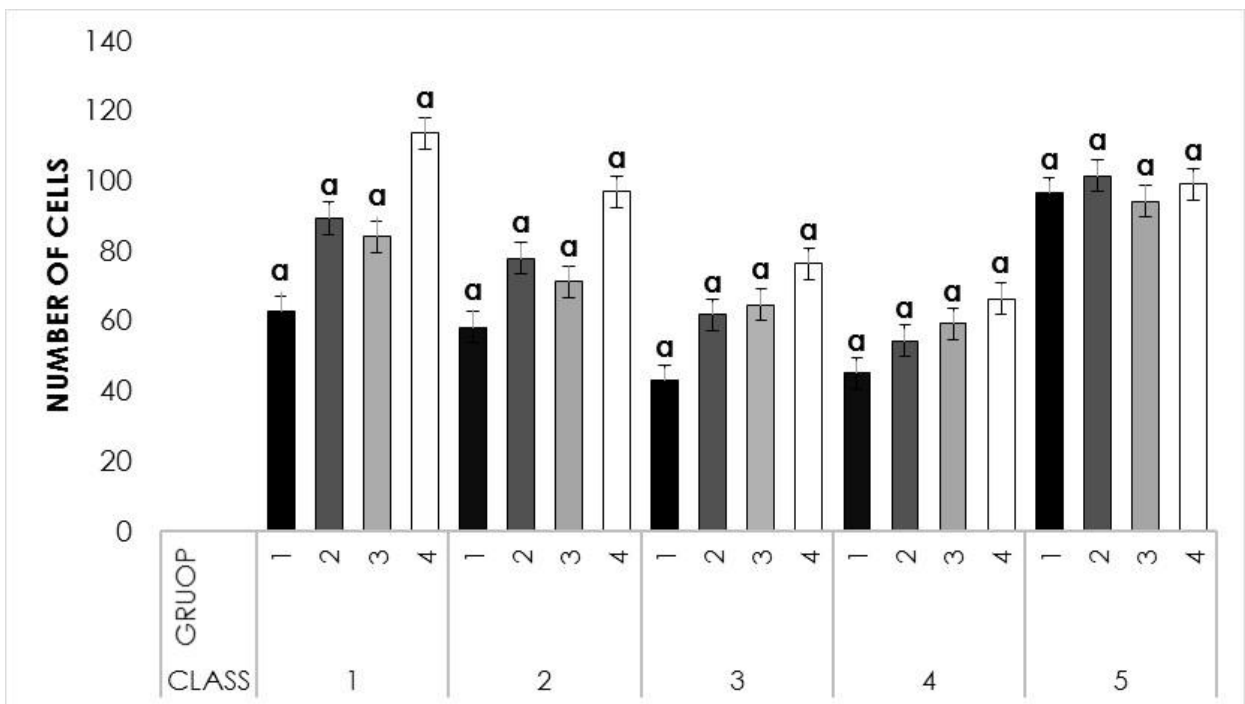
**Figure 11.** Mean the number cells and standard error per class within each group - Period 45. Means with the same letter within the classes do not differ by Tukey test ( $p < 5$ ) CLASS 1;  $\leq 336.1 \mu\text{m}^2$  CLASS 2;  $> 336.1 \leq 831.3 \mu\text{m}^2$  CLASS 3;  $> 831.3 \leq 1612.6 \mu\text{m}^2$ , CLASS 4;  $> \leq 3077.3 \mu\text{m}^2$  CLASS 5;  $> 3077.3 \mu\text{m}^2$ .



**Figure 12.** Mean the number cells and standard error per class within each group - Period 60. Means with the same letter within the classes do not differ by Tukey test ( $p < 5$ ) CLASS 1;  $\leq 336.1 \mu\text{m}^2$  CLASS 2;  $> 336.1 \leq 831.3 \mu\text{m}^2$  CLASS 3;  $> 831.3 \leq 1612.6 \mu\text{m}^2$ , CLASS 4;  $> \leq 3077.3 \mu\text{m}^2$  CLASS 5;  $> 3077.3 \mu\text{m}^2$ .



**Figure 13.** Mean the number cells and standard error per class within each group - Period 90. Means with the same letter within the classes do not differ by Tukey test ( $p < 5$ ) CLASS 1;  $\leq 336.1 \mu\text{m}^2$  CLASS 2;  $> 336.1 \leq 831.3 \mu\text{m}^2$  CLASS 3;  $> 831.3 \leq 1612.6 \mu\text{m}^2$ , CLASS 4;  $> \leq 3077.3 \mu\text{m}^2$  CLASS 5;  $> 3077.3 \mu\text{m}^2$ .



**Figure 14.** Mean the number cells and standard error per class within each group - Period 120. Means with the same letter within the classes do not differ by Tukey test ( $p < 5$ ) CLASS 1;  $\leq 336.1 \mu\text{m}^2$  CLASS 2;  $> 336.1 \leq 831.3 \mu\text{m}^2$  CLASS 3;  $> 831.3 \leq 1612.6 \mu\text{m}^2$ , CLASS 4;  $> \leq 3077.3 \mu\text{m}^2$  CLASS 5;  $> 3077.3 \mu\text{m}^2$ .

After partial fasting period, note again, that cellular hypertrophy occurred mainly in larger cells, class 5, although it was given a considerable increase in the supply of feed. We observed that

after partial fasting period stimulated the compensatory growth in G2 with 15 days because its cells has hypertrophy growth and reached the size of G1 cells. The same occurred in G3 and G4,

however, not reaching the size of G1 and G2 cells. It is possible to observe the progress of compensatory growth, although the hypertrophic framework has been similar on 45-day period because there was hypertrophy of muscle cells. It is likely that in this period the physiological conditions that control the course of hypertrophic growth were being restored, as observed in G4 that despite have been subjected to a severe fasting, showed in this period, the same size of G3 cells. In the period, G4 was with more showy coloration and skin mucus, unlike the pale and rough aspect observed in fasting period. In this period is clear the compensatory growth in G3 through the hypertrophy reached the same cell measurement values of G1 and G2. On the other hand, G4 continues to show a hypertrophic growth for compensatory growth, however, with lower values than the other groups.

The compensatory growth was reached more rapidly as there was a lower the food restriction rate during partial fasting period. Nebo, (2011) concluded that periods of 5 and 10 days of fasting followed by the nonfasting period has not affected the growth and the morphology in juvenile tilapia do nilo (*Oreochromis niloticus*). However, Nebo (2015) related the after the nonfasting period, the fish that were submitted to fasting showed higher amount of fibers with larger size and a reduction of frequency of smaller size classes featuring the hypertrophy, as reported by Veggetti et al, (1993); Zimmermann & Lowery (2000); Johnston, (2006). Nebo (2015), reports high frequency of cell small, during the fasting treatments, characterized muscle hyperplasia. The hyperplasia was evident within the first week of the experiment in the fish by observation several small fibers with central nuclei. This morphological feature characterizes skeletal muscle development in mosaic hyperplasia, with small fibers containing central nuclei surrounding big fibers throughout the myotome (Johnston et al., 2008; Valente et al., 2013). Johansen & Overtuf (2006) analyzing nonfasting and fasting period in rainbow trout (*Onchorhynchus mykiss*), found that the fasting of 30 days and nonfasting resulted in changes in metabolism that affected the molecular control of muscle growth genes that control hypertrophy. The same author noticed that after 14 days of nonfasting there was not increasing in hyperplasia in the white muscle fibers.

Few studies in the literature that evaluated the performance of the muscle cells associated with compensatory growth.

We conclude that In G2 the total compensatory growth occurred with 15 days of nonfasting through the muscle cells hypertrophy, G3 with 60 days and G4 with 90 days, being the last one a partial compensatory growth. A reduction in the diet of 2% and 0.5% of body weight during a period of fasting didn't affect the hyperplastic growth of muscle cells in nonfasting, although it has stimulated an upward hyperplasia of muscle cells of G4 from the period of 60 days. The strategy of compensatory growth of the

Amazonian catfish in the juvenile stage proved to be efficient with 30 days of fasting and 90 days of nonfasting not affecting the mechanism of muscle hypertrophy of Amazonian catfish. Therefore, the compensatory growth technique can be used in the production system of fish farms.

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