Journal of Agricultural Science and Technology B 2 (2012) 1195-1202
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Muscle Growth of Two Nile Tilapia (*Oreochromis niloticus*) Strains

Vander Bruno dos Santos[1], Eidi Yoshirara[1], Edson Assunção Mareco[2] and Rilke Tadeu Fonseca de Freitas[3]

1. Agencia Paulista de Tecnologia dos Agronegócios-APTA-Pólo Alta Sorocabana, Presidente Prudente, SP, 19015-970, Caixa Postal 298, Brazil

2. Instituto de Biociências, UNESP-Universidade Estadual Paulista, Departamento de Morfologia, Botucatu, SP 18618-000, Brazil

3. Departamento de Zootecnia, Universidade Federal de Lavras, Lavras, Minas Gerais 37200-000, Brazil

Received: August 7, 2012 / Published: November 20, 2012.

Abstract: The objective of this research was to evaluate the muscle growth of the Nile tilapias of Thai and supreme strain. Hyperplasia and hypertrophy of muscle fibers were studied by histology. The fish were cultivated in cages in a dam and as they grew they were weighed and measured in length. In tissue samples, it was evaluated the quantity of cells area^{-1} and the diameter of the white, pink and red muscle fibers. The supreme strain presented greater number of white and pink fibers mm^{-2} and smaller number of red fibers when compared to the Thai strain. 5-10 cm fish presented higher number of white, pink and red fibers mm^{-2} and smaller average diameter of the white and pink fibers, when compared to a 10-15 cm fish. The average diameter of the white and pink fibers increased in 10-15 cm fish, compared to 5-10 cm. White fibers smaller than 10 µm were found only in the 5-10 cm supreme strain. *Tilapias* of Thai and supreme strain presented different standard distribution of red, pink and white muscle fibers. Supreme strain, genetically improved, presented higher hyperplasia of white fibers than Thai strain, and this can indicate its higher potential growth.

Key words: Hypertrophy, hyperplasia, red fiber, supreme strain, Thai strain.

1. Introduction

The qualities of the tilapia flesh and its accelerated growth are the main factors that have called attention and increased producers and consumers' interest for this species. The search for tilapia strains of superior performance is more and more frequent by producers, because of the increasing demands for healthy food, among the world population. This had been demanded assessments of these fish cultured in different environments. The tilapia of Chitralada (Thai) strain and those derived from the Genetically Improved Farmed Tilapia (GIFT) program, are being widely distributed, as they have shown faster growth when compared to the wild population, and this can cause differences in the muscle and adipose tissue growth, affect the quality of carcass and flesh. In previous studies with these same strains, differences were found in growth between them, and the supreme strain, derived from the GIFT program presented 30% higher growth rate than the Thai [1]. As the skeletal muscle constitutes the edible part of the fish, knowledge of the mechanism of muscle growth is important for the development of fish farming [2].

The lateral muscles of fish mainly consist of white fibers, covered by a thin layer of red muscle fibers, and a layer of pink or intermediary muscle fibers between them. Silva et al. [3] identified these three fiber types in the Nile tilapia and observed increase in the size and number of fibers. In the longer length (28.3 cm) group studied, it was observed my oblasts and the association of small and large fibers diameters, which indicates the phenotypic plasticity of muscle,

Corresponding author: Vander Bruno dos Santos, Ph.D., research fields: aquaculture, fish growth, muscle growth, chemistry composition, morphometry, allometry, yield of processing and carcass characteristics. E-mail: vander@apta.sp.gov.br.

but nothing was reported about the end of recruitment in tilapia.

This study was conducted in order to evaluate the initial growth of Nile tilapias (*Oreochromis niloticus*), Chitralada (Thai) and Supreme strain, regarding muscle tissue morphology, characterizing and determining the degree of the occurrence of cells hyperplasia and hypertrophy in the cells in different fish sizes.

2. Materials and Methods

2.1 Fish Stocking and Culture

The experiment was carried out in a dam in Parque Ecológico Cidade da Criança in Presidente Prudente, SP-Brazil, from March to August, 2006.

Initially, 1,000 fish around 10 g were cultivated in four cages of 2.7 m^3. 500 fingerlings of the Thai (Chitralada) and 500 of the supreme strain, derived from a monosex male population (fed with 17 α-methyltestosterone) were used, each cage with 250 fish of used strains.

All tilapia were fed with the same commercial ration (32% crude protein), supplied according to cage biomass and the water temperature. The limnological conditions, average pH, alkalinity and transparency during growth period were 7.5, 166 mg L^{-1} and 54 cm, respectively. The temperature and oxygen level varied significantly during the experiment. The temperature ranged from 27 °C in March to 19.5 °C in July. The average oxygen level was 4.27 mg L^{-1} in the morning and 6.77 mg L^{-1} in the afternoon.

After 48 h of fasting, muscle samples of 40 fish (20 fish per cage) of each strain and each study period were collected. The fish were stunned by heat shock (water and ice). All fish were weighed and measured in length.

2.2 Histological Techniques

Tissue samples of 10 fish were collected in the size interval 5-10 cm (after 30 days of cultivation) and of 10 fish in the 10-15 cm (after 75 days of cultivation) of both strains. In each fish, the samples of muscle tissue were removed using a scalpel in two portions of the body: below the dorsal fin (MD) and caudal region (MC) as illustrated in Fig. 1.

All tissue samples were fixed in Bouin's fluid for 24 h at room temperature. After that time, they were transferred and stored in 70% ethanol for subsequent processing. The samples embedded in paraffin and transverse sections of 5 to 7 μm were stained with hematoxylin-eosin in microscopy slides, and then evaluated on the quantity (number of fibers/area) and size (mm) of the muscle fiber.

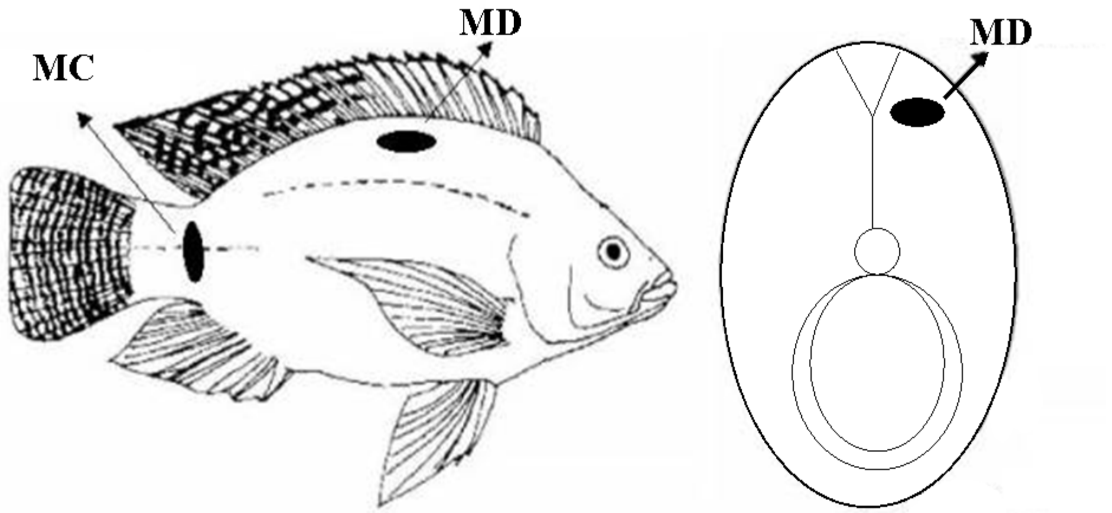
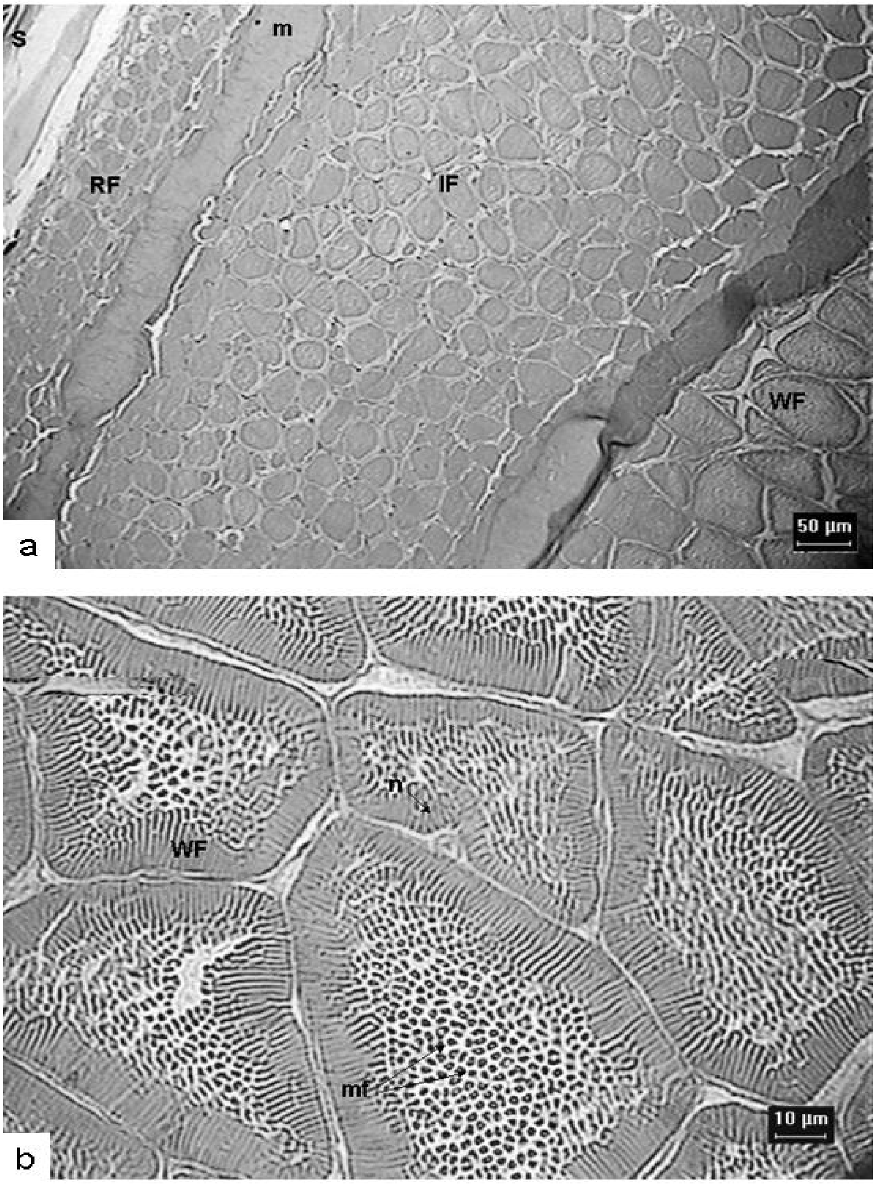


Fig. 1 Tissue samples location; on the left, longitudinal view, showing the muscle tissue sample in the median portion of the body, below the dorsal fin (MD) and in the caudal region (MC); in MD, only white muscle was analyzed and in the MC, the red and pink fibers only; on the right, cross-section of the body, showing the depth of the cut of MD.

All measures were taken using a microscope model Leica DMR, Germany coupled to a system of image analysis with the software Image-Pro Plus version 4.5, Media Cybernetics. Under the microscope, muscle fibers sections were located in each sample and five pictures were captured. Two areas of the muscle fiber in each picture were taken and the sizes of areas were determined, making it possible to count the cell number in each area. Approximately 50 cells of each slide were measured as perimeter regards (all the fibers in each picture). Assuming that the fiber have circular format, and knowing the image magnification, the diameters of each muscle fiber were determined (diameter = perimeter π^{-1}).

Images of transverse sections of the lateral muscle taken in the caudal region (MC), below the dorsal fin (MD) are presented in Fig. 2. In caudal region, different fiber types were observed, presumably white, pink or red fibers because of their morphometric characteristics and location. Furthermore, the mitochondrial density, degree of capillarization and volume of the subsarcolemmal cytoplasm also helped in the identification of the three fiber types according to Sänger and Stoiber [4].



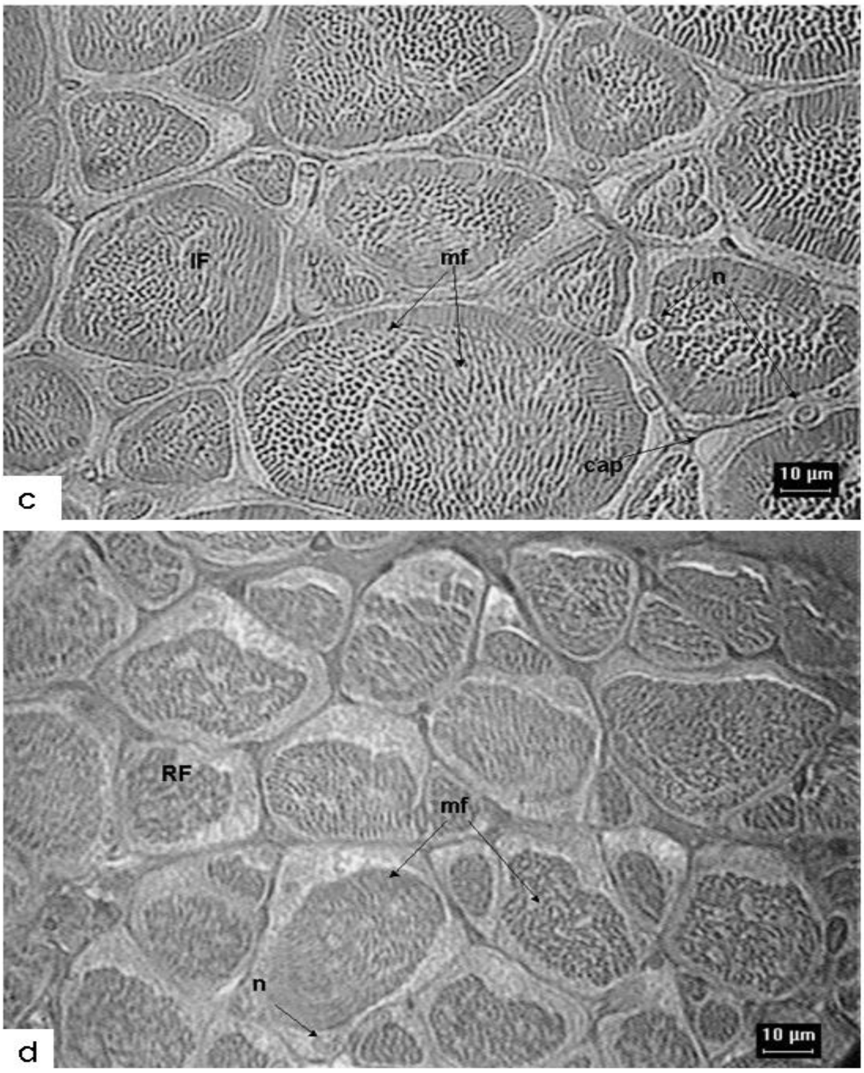


Fig. 2 Transverse section of different tissue samples: (a) lateral muscle in the caudal region; (b) white muscle; (c) intermediate muscle and (d) red muscle. Abbreviations: S, skin; WF, white fibers; IF, intermediate fibers; RF, red fibers; m, myosepta; n, nuclei; mf, myofibril; cap, capillaries.

In the muscle sample taken from the caudal region, it was possible to see all three types of fibers (white, pink and red) separated by myosepta (Fig. 2a). However, in this sample, only red and pink fibers were analyzed (Figs. 2c, 2d). The white fibers (Fig. 2b) were analyzed in sample taken below the dorsal fin where the white muscle is primarily located and red and pink fibers could not be seen due to the depth of the cut.

2.3 Statistical Analysis

The experiment was arranged in a 2 × 2 (strain × size class of the fish) factorial scheme in a completely randomized design, with 10 repetitions to evaluate the muscle tissue. The data were analyzed using the following statistical model:

$$Y_{ijk} = \mu + S_i + C_j + SC_{ij} + e_{ijk}$$

where

μ: general average;

Y_{ijk}: k observation in the i strain and j class-size;

S_i: effect of the i strain, and $i = 1, 2$;

C_j: effect of the class-size, and $j = 1, 2$;

SC_{ij}: effect of the interaction between the strain i and the class-size j;

e_{ijk}: error associated with each observation, which is NID $(0, \sigma^2)$ by assumption.

The different diameters of muscle fiber found were separated into classes of measures and plots in a frequency histogram (%) for each tilapia strain in the different sizes. The amount of cell by area and the average diameter of the fibers were compared between the strains following an *F* test at 5% of significance.

3. Results and Discussion

After 30 days of cultivation, the Thai strain were 20.78 ± 4.95 g and 8.32 ± 0.32 cm (mean ± standard error, $n = 10$) and the supreme strain were 22.73 ± 5.25 g and 8.78 ± 0.34 cm. In the 75 days, the Thai strain were 94.98 ± 4.95 g and 13.39 ± 0.32 cm and the supreme strain were 91.35 ± 4.70 and 13.27 ± 0.30 cm.

The variance analysis of the fibers number mm^{-2} showed significant effect ($P < 0.05$) for strain and fish size. Average values for each strain and each class of length are presented in Table 1. The supreme strain presented a greater number of white and pink muscle fibers mm^{-2} than the Thai one. The Thai strain showed a larger number of red muscle fibers mm^{-2}, compared to the supreme strain. Regarding the length, 5-10 cm fish also had a larger number of white, pink and red fibers mm^{-2} than 10-15 cm fish.

The variance analysis to the average size of the muscle fibers also showed effect ($P < 0.05$) of the fish length, indicating that those of the class of 5-10 cm have a lower average diameter of the white and pink fibers (Table 2) than fish of the class of 10-15 cm. For the average diameter of red muscle fiber, the variance analysis showed effect ($P < 0.05$) of the interaction between strain and fish size.

Table 1 Average fibers mm^{-2} results of the tilapia strains in different fish sizes (average ± standard error).

Fiber type	Strain	Fish size		Average*
		5-10 cm	10-15 cm	
White	Thai	434.92 ± 47.34	278.98 ± 47.34	356.95 ± 33.54 ^{b}
	Supreme	592.35 ± 50.31	346.71 ± 45.00	469.53 ± 33.75 ^{a}
	Average*	513.64 ± 34.57 ^{a}	312.84 ± 32.69 ^{b}	
Pink	Thai	1309.58 ± 91.58	757.50 ± 105.75	1033.54 ± 69.95 ^{b}
	Supreme	1414.24 ± 97.91	1110.11 ± 81.91	1262.17 ± 63.83 ^{a}
	Average*	1361.91 ± 67.03 ^{a}	933.80 ± 66.88 ^{b}	
Red	Thai	2796.48 ± 153.92	2304.53 ± 163.26	2550.51 ± 112.19 ^{a}
	Supreme	2438.23 ± 153.92	2035.20 ± 146.03	2236.71 ± 106.09 ^{b}
	Average*	2617.36 ± 108.84 ^{a}	2169.87 ± 109.52 ^{b}	

*Values in the same row or column with different superscript are significantly different ($P < 0.05$) following an *F* test for each fiber type.

Table 2 Diameter (μm) of the white, pink and red muscle fibers of the tilapia strains in different fish sizes (average ± standard error).

Fiber Type	Strain	Fish size		Average**
		5-10 cm	10-15 cm	
White	Thai	59.62 ± 3.15	75.19 ± 3.15	67.41 ± 2.23 ^{A}
	Supreme	53.78 ± 3.34	78.31 ± 3.15	66.04 ± 2.30 ^{A}
	Average*	56.70 ± 2.30 ^{b}	76.75 ± 2.23 ^{a}	
Pink	Thai	29.18 ± 1.89	36.83 ± 2.00	33.01 ± 1.38 ^{A}
	Supreme	30.40 ± 1.89	31.82 ± 1.79	31.11 ± 1.30 ^{A}
	Average*	29.79 ± 1.34 ^{b}	34.33 ± 1.34 ^{a}	
Red	Thai	17.45 ± 0.57 ^{Ab}	20.05 ± 0.65 ^{Aa}	18.75 ±0.43 ^{A}
	Supreme	18.71 ± 0.57 ^{Aa}	18.91 ± 0.54 ^{Aa}	18.81 ± 0.39 ^{A}
	Average	18.08 ± 0.40	19.48 ± 0.42	

*Values in the same row with different superscript lowercase are significantly different ($P < 0.05$) following an *F* test.

**Values in the same column with different superscript capital letters are significantly different ($P < 0 .05$) following an *F* test for each fiber type.

It was observed that the Thai strain, 10-15 cm class-size, shows an increase of the red muscle fibers average size, and this increase was approximately 13% over the 5-10 cm fish. This behavior has not been observed in the supreme strain, in which the increase of the fish length did not alter the average size of the red fibers.

The observations of a greater number of fibers mm^{-2}, as much as white and pink fibers, in the supreme strain, suggest that the genetic improvement program of this strain, with the objective of increasing the growth rates, provided a higher rate of muscle fibers hyperplasia. This is acceptable, since white and pink fibers occupy the greater part of the body of teleost fish. According to Sänger and Stoiber [4], the white fibers represent more than 70% of the mass of the myotomal muscle. On the other hand, the amount of pink (intermediate) muscle differs between species and fish development stage.

The red muscle fibers, being small in diameter (25-45 µm), represents usually less than 10% and not more than 30% of the muscle [5], and they are more present in the caudal region [6].

The reduction in the cell number mm^{-2} with the increasing of the fish size indicates the occurrence of the process of cellular hypertrophy, and this could be verified in the three types of muscle fibers (Table 1).

Increase of the muscle fiber size (Table 2) is a result of cellular hypertrophy. A 26% hypertrophy in the white fibers and 13% in the pink, indicates a great increasing capacity in the muscle fibers and a greater extensibility of the white and pink fibers in 10-15 cm fish, when compared with 5-10 cm fish.

The average size of red fibers remained unchanged with the growth of the supreme strain (Table 2). This may be an indication of a greater contribution to the process of fiber hyperplasia during the fish growth in the supreme, rather than in the Thai strain and, in contrast, a greater contribution to the hypertrophy process in the Thai than in the supreme strain.

The muscle fiber grows by hypertrophy throughout post-embryonic life until reaching a functional maximum diameter that is approximately of 100-300 µm for white fibers in most fish, but rather smaller for red fibers, which are much more dependent on the oxygen supply from adjacent capillaries [7, 8].

The hypertrophic growth rate will vary according to the somatic growth rate and in different life stages. The persistence of a hypertrophic growth throughout youth life to adult stages, even after the hyperplastic growth has ended, has also been described for a variety of fish [9].

In Fig. 3, the distribution of red, pink and white muscle fiber diameters in classes of fish length of 5-10 cm and 10-15 cm of the Thai and supreme strains was illustrated.

The distribution of red, pink and white muscle fiber diameters in the length classes of 5-10 cm and 10-15 cm of both Thai and supreme strain (Fig. 3) illustrates a mosaic appearance and shows the contribution of hyperplasia and hypertrophy process to the fish growth. Although the phase of hyperplastic mosaic growth is accepted as a distinct phase of hyperplasia, it has occurred concomitant with the process of hypertrophy.

Apparently, tilapias of the Thai strain, in the length classes studied, have hyperplasia only in red and pink fibers, and the largest contribution to the growth of white muscles occurs by the hypertrophy process. The absence of white fibers in the lower diameter class, in the two length classes of the Thai strain (Fig. 3e), indicates that the hyperplasia process has probably already been ceased as a contribution to the growth of this type of muscle. It was observed that there is also a reduction of the hyperplasia contribution of the red and pink fibers, with the increasing in the fish length. This reduction is 42% in the red and up to 50% in the pink fibers.

The hypertrophy of the different fibers types, in the Thai strain, can be easily observed by the appearance of the diameter class in red fibers from 30 to 40 µm, the classes of 70-80 µm, 80-90 µm and 90-100 µm in

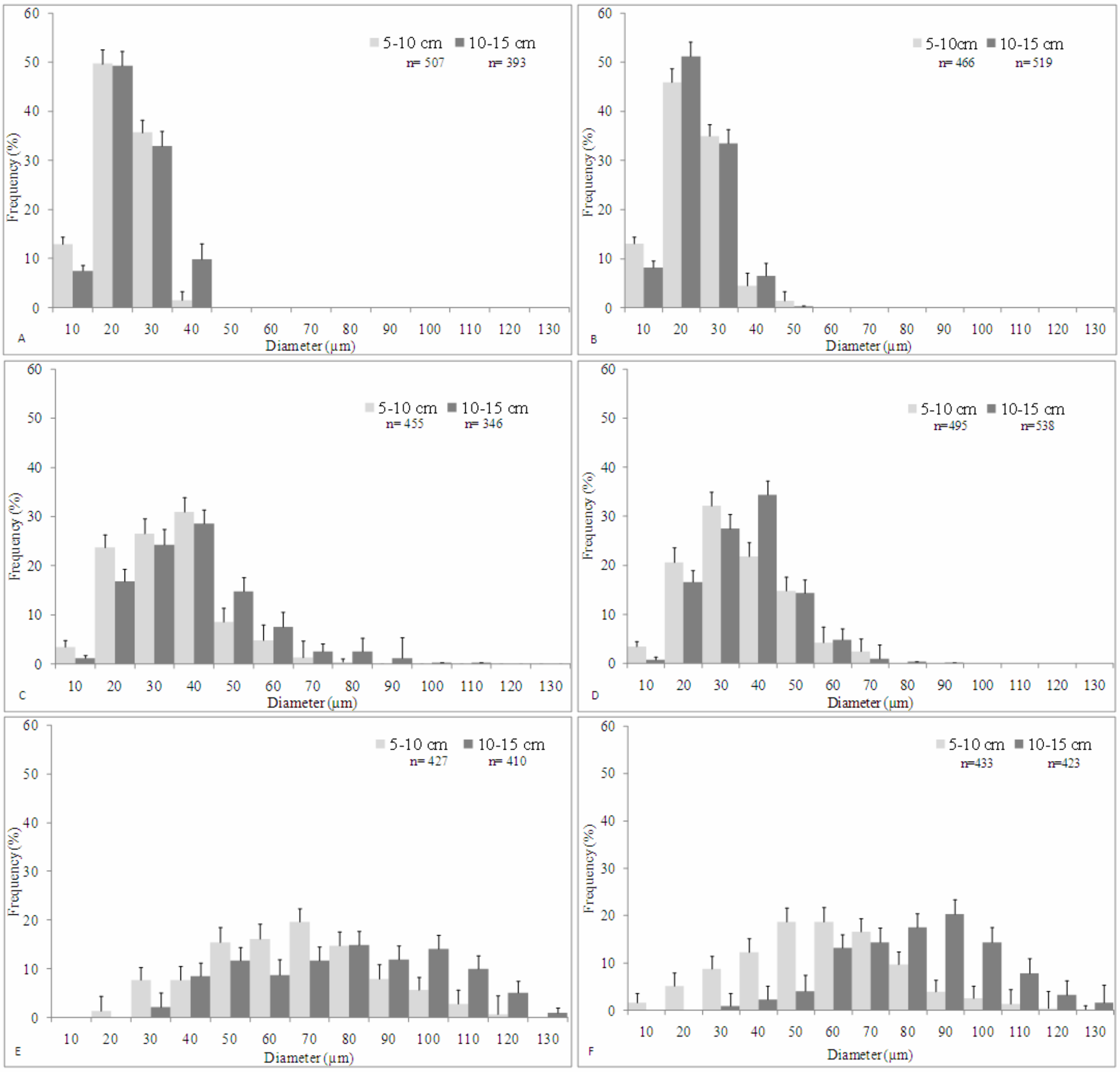


Fig. 3 Diameter distribution of muscle fibers from 5-10 and 10-15 cm tilapia strains. Red fibers in Thai strain (A) and Supreme strain (B). Pink fibers in Thai strain (C) and Supreme strain (D). White fibers in Thai strain (E) and supreme strain (F).

the pinks and the class of 110-120 µm in the white muscle. Moreover, we can see a decrease in the proportion of the fibers in the lower diameters classes, and an increase in the proportion of the fiber in the larger diameters classes.

Unlike the Thai strain, the supreme strain seems to have greater contribution of the process of hyperplasia for the white muscle growth, since that it was recorded the presence of the smaller diameter class of this type of fiber in 5-10 cm fish length. This, though, has not been observed in 10-15 cm fish and, interestingly the disappearance of 0-10 µm and 10-20 µm in the diameter classes, indicates the accelerated hypertrophy growth in this length class. The contribution of the red and pink fibers hyperplasia obtained similar behavior in the Thai strain, although the reduction of red fibers in the smaller diameter class has been of approximately 35%, or 17% less than the Thai.

Similar as in the Thai strain, a decrease in the proportion of the fibers in the lower diameters classes, and an increase in the proportion of the larger diameters fiber classes in the supreme strain was observed. However, in the pink and white fibers, this process seems to have occurred much more evidently in supreme strain.

Santos et al. [10] reported a higher precocity of the Thai strain than the supreme strain. Probably, the greatest precocity is associated with the transfer of energy to the gonadal development. Since the process of synthesis of new fibers (hyperplasia) has greater energy cost than the hypertrophy [11], the mobilization of energy for the muscle growth is probably higher in a strain with late maturing when compared with an early one.

Our results differ of results of Johnston et al. [12] with Atlantic salmon strains, in which the early population had higher growth performance associated with a longer period of fiber recruitment than the late population. However, it is not possible to ignore the differences in lifestyle and cultivation conditions of different fish species. Valente et al. [13], in study with rainbow trout, determined that the percentage of fibers with diameter below 25 μm is greater in the fish from fast- than the slow-growing strain.

4. Conclusions

Distribution of red, pink and white muscle fibers differs in tilapias of Thai and supreme strain. Genetically improved, supreme strain, presented higher hyperplasia of white fibers than Thai strain, and this can indicate higher growth potential. Future research is necessary in order to increase knowledge of the muscle growth process in tilapia and myostatin and myogenic regulatory factors gene expression.

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