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Variation of muscular fiber diameter in trout, depending on species and age

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Abstract. To assess the quality of rainbow and brook trout meat we considered that its opportune to make some histological investigations about muscle fiber diameter. The research was done using fish of one, two and three years old. Muscle was sampled systematically and differentiated by age and body mass and the results were statistically analyzed. The comparative statistical analysis reveals significant differences between the two species of trout and according to age is found a different evolution of body mass and implicitly of muscle fiber diameter. We also calculated the phenotypic correlation coefficients (r) between the two traits and the obtained values indicated a correlation degree with different intensity depending on body weight. Based on histological examination of rainbow and brook trout muscles the conclusion that emerges is whatever of species, the muscle fiber fineness has a significant trend according to the dynamics of growth and age.
Key Words: muscular fiber diameter, rainbow trout, brook, muscle.

Rezumat. În vederea aprecierii calitative a cărnii de păstrăv curcubeu şi fântânel, am considerat oportun să facem câteva investigaţii histologice care au vizat diametrul fibrei musculare. Cercetările s-au efectuat pe materialul biologic de păstrăv curcubeu şi fântânel în vârstă de un an, doi ani şi respectiv trei ani. Probele de muşchi au fost prelevate diferenţiat la cele două specii de păstrăv, pe categorii de vârstă şi masă corporală iar rezultatele au fost analizate din punct de vedere statistic. Analiza statistică comparativă în ceea ce priveşte diametrul fibrei musculare relevă astfel diferenţe majore între cele două specii de păstrăv şi, în funcţie de vârstă, se constată evoluţia diferită a masei corporale şi implicit a diametrului fibrei musculare. De asemenea am calculat coeficienţii de corelaţie fenotipică (r) dintre cele două însuşiri la cele două specii de păstrăv iar valorile obţinute indică un grad de corelare cu intensitate diferită în funcţie de masa corporală. În urma cercetărilor histologice efectuate la carnea de păstrăv curcubeu şi fântânel se desprinde o concluzie importantă şi anume că, indiferent de specie, fineţea fibrei musculare are o evoluţie semnificativă în funcţie de dinamica de creştere şi de vârstă.
Cuvinte cheie: diametrul fibrei musculare, păstrăv curcubeu, fântânel, muşchi.

Introduction. Most of fish muscle is represented by side muscle which is placed under the skin and supports the spine (Nicolae 2002). Morphological and functional characteristics of muscle fiber differ according to species and the stage of evolution (Patruno et al 1998). The studying and understanding the mechanism of muscle growth of fish is particularly important and relevant in the intensive farming of species for human consumption (Dal Pai-Silva et al 2003). The muscle fiber diameter analysis offered the opportunity to appreciate some features such as texture, which is an important variable of meat quality and is a constant concern for the aquacultural sector (Dunajski 1979; Bjørnevik et al 2003). The research in this area points out that in piscicultural farms the rearing and feeding conditions are factors which determine the muscle fiber development (Bjørnevik et al 2003; Dal Pai-Silva et al 2003) and also the texture of meat. To point out how the texture of meat is dependent of muscle fiber diameter, in this paper, we analyzed the muscle samples taken from rainbow

(*Oncorhynchus mykiss*) and brook (*Salvelinus fontinalis*) trout, one, two and three years age and the results were presented on species.

Material and Method. For the histological analysis of rainbow and brook trout flesh we collected samples of fresh biological material (Figure 1). The biological material originating from Remeți trout farm (Bihor county) and was fed with the same pelleted feed.

Samples were taken from lateral muscle, separately on species, age and body mass. The muscle strips of 5-10mm long and 1-5mm thick were placed in labeled containers in a formalin solution (Figure 2). The samples processing was performed at the Faculty of Veterinary Medicine Cluj-Napoca in Laboratory of Histopathology. The analyst was not let to know details of the sampling evidence, ensuring the quantification objectivity. Muscle fiber diameter was determined by inclusion technique in paraffin and staining with hematoxylin-eosin. Semi-automatic measurement of fiber diameter (4 rows of 100 measurements) was made with the DP.Soft OLYMPUS 5.0 program and preparations shooting (Figure 3) was performed with a BX 51 Olympus digital camera. The data were statistically analyzed (Graph Pad Instat 3) and were calculated the dispersion indices (mean and standard error of the mean X±sx and coefficient of variation V%). The mean values (n=106) were comparatively analyzed by using Tukey test. We also calculated the phenotypic correlation coefficients (r) between body mass and muscle fiber diameter.

Results and Discussion. Based on the measurements we calculated the average values and statistical indices for muscle fiber diameter (Table 1) according to species, age and body mass. Analysis of these results indicated very different values of the two species of trout, noting that the smallest muscle fiber diameter was recorded at one year age for both species. Thus after the first 12 months of growth the muscle fiber diameter was 47.67±1.95 μ in rainbow trout and 67.67±2.67 μ in brook trout. If we compare these data with the values reported by other authors (Bud et al 2004; Nag 1972) on muscle fiber fineness in different species of fish we can see that the rainbow trout average score obtained by us falls within the reference limits. Regarding the brook trout, the bibliographical data are few and our results can be reference in the texture of meat and qualitative assessment of Brooke trout meat in this regard. In the three age classes analyzed, our results indicate the higher values of the muscle fiber diameter compared with the other fish species.

Table 1

Muscular fiber diameter (μ) in rainbow and brook trout

Species	*Body weight (g)*	*X±sx*	*V%*
O. mykiss[1]	205	47.67±1.95	42.05
S. fontinalis[2]	235	67.67±2.67	40.59
O. mykiss[3]	330	48.65±1.66	35.15
S. fontinalis[4]	395	76.25±3.48	47.06
O. mykiss[5]	1200	59.14±2.72	47.39
S. fontinalis[6]	800	94.34±4.23	46.14

[1]*O. mykiss* - 12 months; [2]*S. fontinalis* - 12 months; [3]*O. mykiss* - 24 months; [4]*S. fontinalis* - 24 months; [5]*O. mykiss* - 36 months; [6]*S. fontinalis* - 24 months.

These results show that the texture is most consistent for meat of brook trout and we believe that such result appear mainly due to lower degree of genetic improvement in this direction of brook trout compared with the meat of fish species studied by other authors.

Furthermore, the values we obtained in muscle fiber diameter in the two species of trout highlights the changes that appear in texture of meat according to evolution of age and body mass. Thus, in rainbow trout, it can be seen a progressive increase in

muscle fiber diameter from the average of 48.65±1.66 μ at two years age to 59.14± 2.72 μ at three years old. To brook trout can be seen the same increase in muscle fiber diameter according to age but with a greater amplitude. So, if at one year age the average was 67.67±2.67 μ, at two years old was higher with about 8.5 μ, and in three years reached 94.34±4.23 μ. Summarizing, we can observe that the muscle fiber diameter increases until the three years age with about 24% for rainbow trout and with 39% in the case of brook trout. The comparative statistical analysis by muscle fiber diameter revealed major differences between the two species of trout (see Table 2). The values of measurements are not absolute because, as it is known, during the histological processing, fixation and staining, muscle fibers reduce their volume. However the differences we have noticed are accurate and true because all samples were processed at the same time as well. Based on this for each species of trout, according on age, we find a different evolution of body mass and consequently a different evolution of the muscle fiber diameter. The significant and highly significant statistically differences according to species, age and body mass can be observed in Table 2. The exception is noted in both species between one year and two years where the differences appear to be insignificant. The explanation may ascribe on account to smaller body mass accumulation by two years age. So if we follow the average body weight achieved at this age, we can see an accumulation of only 125 g for rainbow trout and 160 g for brook trout. In this context it is clear that for both species of trout meat texture changes with the age according to body mass and studies and research conducted by Stickland (1983), Weatherley (1988), Kiessling et al (1991), Bjørnevik et al (2003) showed an intense correlation between body weight and muscle fiber diameter. Based on these assumptions, we also calculated the phenotypic correlation coefficients (r) between the two traits in two species of trout and the values obtained (Table 3) indicate a degree of correlation with different intensity depending on body weight. Analyzing these values can be observed an intense and positive interdependence between the two traits in rainbow trout with a 205 g body mass ($r=0.602$) and 330 g ($r=0.532$), but in the case of fish at 1200 g the intensity decreases greatly and thus indicates a weak correlation ($r<0.2$). For the brook trout it is clearly a strong and positive correlation ($r=0.739$) to 395 g body mass and a lower degree of correlation ($r=0.136$) with the same effect to 235 g. Also the correlation intensity is reduced in conditions that increase body weight ($r=0.096$). Phenotypic correlation estimated by correlation coefficients calculated indicate therefore that, with age and thus the body mass accumulation, the degree of correlation with muscle fiber diameter is also smaller and this highlights the changing texture of meat to rainbow and brook trout as body mass increases. At the three years age and over the 800 g weight, the muscle fiber diameter of rainbow and brook trout meat increases and the texture becomes more fibrous. Therefore the correlation intensity between body mass and muscle fiber diameter to rainbow and brook trout is evident from an accumulation of body mass between 200 g and 400 g. We can say that muscle mass evolution is more pronounced in the early life, or up to two yers age and is mainly on account the muscle fibers hypertrophy.

Conclusions. The analysis of rainbow and brook trout flesh indicate that the muscle fiber diameter varies by species and change as the fish is older. Based on these results it appears that the texture of the meat is a variable of quality that depends, we believe, increase the production of trout and choice of species depending on consumer preferences. Species, age and origin are directly influences the fineness and density of muscle fibers, and these factors may be the basis for determining the optimal timing of capitalization the trout. Following histological research carried out to rainbow and brook trout meat an important conclusion emerges, namely that, whatever the species, the muscle fiber fineness has a significant trend according to the dynamics of growth and age. Also, making the comparison between the two species of trout in terms of muscle fiber fineness found to be significantly higher in rainbow trout due to a high degree of genetic improvement in this species of trout.

Table 2

Significance of differences concerning the muscular fiber diameter (μ)

Comparation	*X±sx*	*X±sx*	*Significance*
S_f12 vs O_m12	67.67±2.67	47.67±1.95	*** p≥0.001
S_f 12 vs O_m24	67.67±2.67	48.65±1.66	*** p≥0.001
S_f12 vs O_m36	67.67±2.67	59.14±2.72	** p≥0.050
S_f 24 vs O_m12	76.25±3.48	47.67±1.95	*** p≥0.001
S_f 36 vs O_m24	94.34±4.23	48.65±1.66	*** p≥0.001
S_f 36 vs O_m36	94.34±4.23	59.14±2.72	*** p≥0.001
S_f12 vs S_f24	67.67±2.67	76.25± 3.48	ns p<0.050
S_f12 vs S_f36	67.67±2.67	94.34±4.23	*** p≥0.001
O_m12 vs O_m24	47.67±1.95	48.65±1.66	ns p<0.050
O_m12 vs O_m36	47.67±1.95	59.14±2.72	*** p≥0.001
O_m24 vs O_m36	48.65±1.66	59.14±2.72	*** p≥0.001

S_f12= *S. fontinalis* 12 months; S_f24= *S. fontinalis* 24 months; S_f36= *S. fontinalis* 36 months; O_m12=*O. mykiss* 12 months; O_m24=*O. mykiss* 24 months; O_m36= *O. mykiss* 36 months. ***= extremely significat; ** = very significant; ns=not significant

Table 3

Correlation degree (r) with body weight and muscular fiber diameter

Correlated features	*Body weight (g)*					
	O_m 205	S_f 235	O_m 330	S_f 395	O_m1200	S_f 800
Muscular fiber diameter μ	0.602	0.136	0.532	0.739	0.097	0.096

O_m=*Oncorhynchus mykiss*; S_f=*Salvelinus fontinalis*

Figure 1. Appliance preparing for muscle sample collecting.

Figure 2. Collecting of the muscle samples.

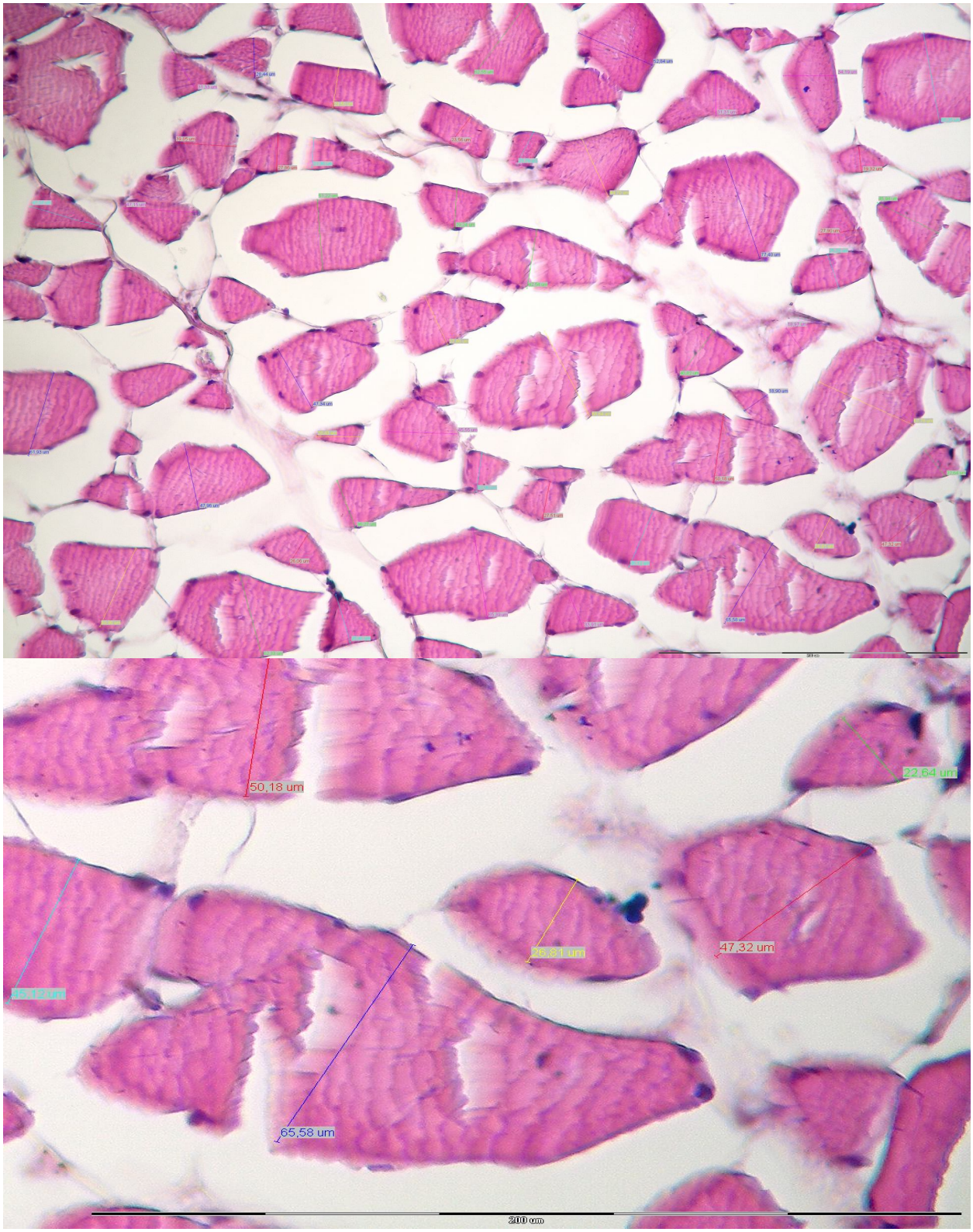

Figure 3. Measurement of muscle fiber diameter.

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