RESEARCH REGARDING VARIATION OF MUSCULAR FIBRE DIAMETER AT RAINBOW TROUT DIFFERENTIALY FEED

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Abstract

Salmons' muscles are divided in myotomes assembled under form of letter W, delimited by myoseptes, which allow the muscular fibres to insert on bones. Orientation of muscular fibres in myotomes is complex and varies on all fish length function of the distance from vertebral column. To achieve the goals of the current paper were analysed lateral musculature, hypaxial and epaxial, for a number of 60 individuals of rainbow trout, 20 individuals for each age group (2nd summer, 3rd summer and 4th summer), determining the diameter of muscular fibres and also the density of muscular fibres per square unit. Trout from those six batches are from two salmon farms situated in Neamț County, and during our research those ones were feed with granulated fodder with different granulation and with different protein level. As a result of cytometric and hystometric determinations on musculature gathered from rainbow trout individuals from those six batches were obtained values between 56.82 and 89.20 µ for muscular fibres diameter, respectively between 157.40 and 193.35 m. f./mm^2 for density of muscular fibres.

Key words: musculature, rainbow trout, muscular fibres, epaxial, hypaxial

INTRODUCTION

Muscular tissue represents the most valuable part of fish meat, representing around 40-60% from the live mass of organism [15].

This one produces the necessary energy for swimming, being formed by trunk musculature, head musculature and fins musculature [11, 15].

Salmons' muscles are divided in myotomes assembled under form of letter W, delimited by myoseptes, which allow the muscular fibres to insert on bones. Orientation of muscular fibres in myotomes is complex and varies on all fish length function of the distance from vertebral column [14].

Fish meat is organised in myomeres, mainly formed by muscular fibers, conjunctive and adipose tissues [3, 4, 5, 12].

At rainbow trout red muscular fibres are superficial bring arranged in parallel with the bodies' longitudinal axis, in one or more superficial stripes, and white profound muscular fibres are oriented with an angle of 40º face to the fish longitudinal axis [1, 8, 17].

At trout length of muscular fibres increase linear with body length from less 1 mm, at trout of 5 cm to almost 8 mm at trout of 45 cm [9].

Diameter of muscular fibres at salmonids varies into very large limits, function of breed, waist and age, temperature regime, being between 47.67 µ and 116.2 µ [2a, 4, 6, 10a, 10b, 13].

MATERIAL AND METHOD

Biological material utilized for realization of the current paper was represented by 60 rainbow trout *(Oncorhynchus mykiss)* individuals of both sexes, but with different ages, which were reared in two trout fisheries from Neamț County. To reach the proposed goals, from the biological material which were subjected to the current study were settled up six experimental batches L_1, L_2, L_3, L_4, L_5 and L_6 each with 10 individuals per batch, on three age categories, 2nd summer (Pc_{1+}), 3rd summer (Pc_{2+}) and 4th summer (Pc_{3+}).

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During research trout from experimental batches were differentially feed, so rainbow trout from batches L_1, L_3, L_5 were reared in ground ponds with natural feed and also with artificial feed. Feeding of trout was manually made, with extruded granulated fodders, having a protein level between 40-45% raw protein, feed being administrated in one meal per day (10 o'clock in the morning), and the daily administrated feed, function of fish weight and water temperature, represented up to 1.2% from corporal mass of trout.

Individuals of rainbow trout from batches L_2, L_4, L_6 were reared in concrete ponds, consumption feed being represented by extruded granular fodders with a protein level between 40-45% raw proteins. Fodders were daily administrated in 2 meals, (at 10 am and at 16 pm). Daily quantity of administrated feed represented up to 1.8% from corporal mass of trout.

Gathering of musculature was made from lateral epaxial and hypaxial muscles, gathered samples being represented by muscular fragments with 5-10 mm length and a thickness of around 5 mm.

Samples processing was made in according with the modern histological techniques requirements in the UASVM Iaşi laboratories [2b, 10a, 11, 16].

Detailed and long term structural exam of muscular tissues sections was realised utilising permanent samples for microscope. Their obtaining supposes some working stages such as: gathering, fixation, washing, inclusion, sectioning, colouring, mounting and labelling.

So after removal of tegument were gathered stripes with 5-10 mm length and 1-5 mm thickness both from epaxial lateral muscles and also from hypaxial ones from rainbow trout individuals of different ages (2^{nd} summer, 3^{rd} summer and 4^{th} summer).

Fixation was made for strengthening of parts and to stop the vital phenomena from the tissues to be able to observe at microscope some certain histo-physiological aspects which existed at the gathering.

Washing consist in removing the fixative reagent (formalin 10%) and it is the moment of dehydration for inclusion and could be realised with water or with alcohol.

Waxing represents the immersion of parts in melted paraffin and solidification in block by cooling.

Sectioning of parts was realised using a microtome with vertical slider.

Colouring of sections included in paraffin was made using HEA colouration (haematoxylin, eosine and methylene blue).

Mounting of coloured samples was made in different environments, initially fluid but which in the majority of cases have a further solidification, assuring a good adherence for lamella.

Mount samples are left for 2-3 days on a plain surface, after mounting labelling is made.

Examination of histological samples and data interpretation were realised using a LEICA LAS DM 750 microscope equipped with a micro-photographing system.

After a previous calibration of microscope histological samples were studied, were enlightened the most finest samples, then were micro-photographed and measurements were realised with a computer using line measurement and area computation functions of software LEICA LAS SOFTWARE version V4.2 from 2012.

At the end were effectuated measurements regarding large and small diameter of muscular fibres, and their area on transversal section. The obtained data were utilised to calculate the mean diameter.

To calculate the muscular fibres density (nr. fibre/mm^2 muscle) was effectuated an exact counting of fibres at the level of each primary muscular fascicle, was calculated the transversal area of primary muscular fibres.

The obtained data were statistical processed, calculating: arithmetic mean variance, standard mean deviation, variability coefficient and difference signification was establish with Fischer test.

RESULTS AND DISCUSSIONS

To establish the finesse of muscular fibres were gathered samples from rainbow trout fresh musculature, which were subjected to cytometric and histometric measurements. Data obtained were used to evaluate the muscular fibres diameter and to determine the density of muscular fibres per area unit.

Corporal mass of rainbow trout individuals presented mean values between 165.33-172.57 g for 2nd summer individuals, between 249.53-264.01 g for 3rd summer individuals, respectively between 472.40-480.06 g for 4th summer individuals (table 1).

Table 1 Diameter of muscular fibre at rainbow trout epaxial musculature

Specification	Experimental batches	Corporal mass (g)	n	$\bar{X} \pm s_{\bar{X}}$ (μ)	V%	Min. (μ)	Max. (μ)
Pc_{1+}	L_1	172.57	10	63.62±1.46	7.27	52.47	69.79
	L_2	165.33	10	60.47±1.28	6.64	53.45	69.41
Signification between batches' mean			L_1 vs. L_2 = n.s; F(2.2255) < Fα(4.4138) for 1:18 GL				
Pc_{2+}	L_3	264.01	10	79.58±0.23	0.91	78.45	81.13
	L_4	249.53	10	76.65±1.32	5.46	68.14	83.32
Signification between batches' mean			L_3 vs. L_4 = *; F(5.1258) > Fα(4.4138) for 1:18 GL				
Pc_{3+}	L_5	480.06	10	89.20±1.37	4.86	81.13	96.41
	L_6	472.40	10	86.54±1.38	5.04	81.14	96.52
Signification between batches' mean			L_5 vs. L_6 = n.s; F(1.8692) < Fα(4.4138) for 1:18 GL				

Analysing the data from table 1 can be observed some differences in evolution of mean values for diameter of muscular fibres from epaxial musculature of studied trout, function of their waist. So the recorded mean values for 2nd summer trout were 63.62±1.46 μ for individuals from batch L_1, respectively 60.47±1.28 μ for trout individuals from batch L_2.

For rainbow trout of 3rd summer the obtained mean values were between 76.65±1.32-79.58±0.23 μ, and for individuals of 4th summer from batches L_5 and L_6 the obtained mean values for diameter of muscular fibres were 89.20±1.37 μ, respectively 86.54±1.38 μ.

Regarding the obtained variation coefficient for all six studied batches of rainbow trout, this one presented low values, less than 10%, which show a very high homogeneity inside batches.

Analysing from statistical point of view the obtained mean values for diameter of muscular fibres from epaxial musculature gathered from studied trout, were observed insignificant statistical differences between rainbow trout batches of 2nd summer and 4th summer and significant between the two batches of 3rd summer rainbow trout.

Regarding mean diameter of muscular fibres from hypaxial muscles, we can observe that the highest values were recorded at smaples gathered from rainbow trout individuals of 4th summer from batch L_5 (85.31±1.50 μ), and the lowest values (56.82±1.21 μ) were recorded for individuals of 2nd summer from batch L_2 (table 2).

Table 2 Diameter of muscular fibre at rainbow trout hypaxial musculature

Specification	Experimental batches	Corporal mass (g)	n	$\bar{X} \pm s_{\bar{X}}$ (μ)	V%	Min. (μ)	Max. (μ)
Pc_{1+}	L_1	172.57	10	59.58±1.42	7.52	51.63	66.45
	L_2	165.33	10	56.82±1.21	6.72	49.85	62.44
Signification between batches' mean			L_1 vs. L_2 = n.s; F(2.2097) < Fα(4.4138) for 1:18 GL				
Pc_{2+}	L_3	264.01	10	76.03±1.25	5.22	66.54	80.45
	L_4	249.53	10	74.67±1.52	6.43	65.41	81.16
Signification between batches' mean			L_3 vs. L_4 = n.s; F(0.4784) < Fα(4.4138) for 1:18 GL				
Pc_{3+}	L_5	480.06	10	85.31±1.50	5.56	77.56	92.45
	L_6	472.40	10	83.65±1.67	6.32	74.41	93.45
Signification between batches' mean			L_5 vs. L_6 = n.s; F(2.2255) < Fα(4.4138) for 1:18 GL				

Values obtained for variation coefficient did not over-pass 10%, which show a high homogeneity inside those six batches of studied rainbow trout.

The recorded statistical differences between those three pairs of batches were insignificant.

In according with our research regarding the density of muscular fibres per area unit, was observed that their number decrease with trout aging and mass increasing, so for epaxial muscles the highest density was 183.80±2.29 m.f/mm^2, recorded at rainbow trout individuals of 2nd summer from batch L_2. At the opposite pole were situated the individuals of 4th summer from batch L_6, at which the fibres density was 158.20±1.93 m.f/mm^2 (table 3).

Also in this case variation coefficient had values lower than 10% (V%=3.08-8.70), resulting a good homogeneity of the studied character inside those six batches of rainbow trout.

Table 3 Density of muscular fibres at rainbow trout epaxial musculature

Specification	Experimental batches	Corporal mass (g)	n	$\bar{X} \pm s_{\bar{X}}$ (m.f/mm^2)	V%	Min. (m.f/mm^2)	Max. (m.f/mm^2)
Pc_{1+}	L_1	172.57	10	169.45±1.65	3.08	162.00	179.00
	L_2	165.33	10	183.80±2.29	3.94	171.00	198.50
Signification between batches' mean			L_1 vs. L_2 = ***; F(25.8363) > Fα(15.3793) for 1:18 GL				
Pc_{2+}	L_3	264.01	10	159.05±4.38	8.70	145.50	194.00
	L_4	249.53	10	173.40±3.46	6.31	152.50	196.00
Signification between batches' mean			L_3 vs. L_4 = *; F(6.6127) > Fα(4.4138) for 1:18 GL				
Pc_{3+}	L_5	480.06	10	160.19±3.69	7.28	144.00	187.50
	L_6	472.40	10	158.20±1.93	3.87	143.00	164.50
Signification between batches' mean			L_5 vs. L_6 = n.s; F(0.2285) < Fα(4.4138) for 1:18 GL				

The recorded statistical differences were very significant between individuals from batches L_1 and L_2, significant between individuals from batches L_3 and L_4 respectively insignificant between batches L_5 and L_6.

Also in the case of hypaxial muscles was observed the fact that density of muscular fibres decrease with trout aging and weight increasing (table 4).

In the case of hypaxial muscles the highest density was 193.35±2.48 m.f/mm^2 which was recorded for rainbow trout individuals of 2nd summer from batch L_2, and the lowest density (157.40±2.42 m.f/mm^2) was recorded ath trout of 4th summer from batch L_6.

The studied character was also in this case very homogenous, fact enlightened by the low values of variation coefficient, which did not over-pass 10%.

Table 4 Density of muscular fibres at rainbow trout hypaxial musculature

Specification	Experimental batches	Corporal mass (g)	n	$\bar{X} \pm s_{\bar{X}}$ (m.f/mm^2)	V%	Min. (m.f/mm^2)	Max. (m.f/mm^2)
Pc_{1+}	L_1	172.57	10	176.75±2.22	3.98	167.00	187.00
	L_2	165.33	10	193.35±2.48	4.06	178.00	202.50
Signification between batches' mean			L_1 vs. L_2 = ***; F(24.8215) > Fα(15.3793) for 1:18 GL				
Pc_{2+}	L_3	264.01	10	164.05±4.22	8.13	147.50	195.00
	L_4	249.53	10	177.70±3.74	6.65	156.50	196.50
Signification between batches' mean			L_3 vs. L_4 = *; F(5.8603) > Fα(4.4138) for 1:18 GL				
Pc_{3+}	L_5	480.06	10	162.60±3.44	6.69	143.50	185.50
	L_6	472.40	10	157.40±2.42	4.87	142.50	169.50
Signification between batches' mean			L_5 vs. L_6 = n.s; F(1.5259) < Fα(4.4138) for 1:18 GL				

The recorded statistical differences were very significant between individuals from batches L_1 and L_2, significant between individuals from batches L_3 and L_4 respectively insignificant between rainbow trout individuals of 4th summer from batches L_5 and L_6.

In according with the date cited in literature, diameter of muscular fibres at rainbow trout varies in very large limits, function of weight and mass, being between 47.67 μ and 116.2 μ [2a, 4].

The obtained data regarding diameter and density of muscular fibres from rainbow trout meat, for all those six experimental batches, are in according with the data mentioned in literature for this fish breed [4, 2a, 2b, 7, 10a, 10b, 11, 13].

CONCLUSIONS

So it can be concluded that muscles with the finesse texture were hypaxial muscles while epaxial muscles presented a rough texture.

Analysis of rainbow trout meat under histological way show the fact that diameter of muscular fibre is changing with fish aging.

Trout age had a direct influence on diameter and density of muscular fibres, and indirectly on establishment of the optimal moment for capitalization of rainbow trout.

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