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MORPHOMETRICAL AND HISTOCHEMICAL STUDY OF THE CROSS STRIATED SKELETAL MUSCLES OF PIGS

P. MAKOVICKÝ[1*], P. MAKOVICKÝ[2], V. KULÍŠEK[2], M. KAČÁNIOVÁ[2], S. PAVLIČOVÁ[2], P. HAŠČÍK[2]

[1]Czech University of Life Sciences, Prague, Czech Republic; [2]Slovak Agricultural University, Nitra, Slovak Republic

ABSTRACT

In this study morphological and histochemical parameters of the striated skeletal muscle of pigs of the Slovak large White breed was analyzed. New-born, one-day-old (1-day), three-day-old (3-day), eighteen-day-old (18-day), forty-eight-day-old (48-day), eighty-four-day-old (84-day), one-hundred-twenty-day-old (120-day), one-hundred-ninety-two-day-old (192-day) and finally one-thousand-fifty-five-day-old pigs (1055-day) were used for this purpose. Samples from the three muscles *m. triceps brachii* (MTB), *m. longissimus dorsi et lumborum* (MLD) and *m. rectus femoris* (MRF) were. The samples were collected by necropsy, fixed in liquid nitrogen and sliced on a freezing microtome. All the samples were stained with hematoxylin-eosin, toluidine blue, oil red "0", and succinate dehydrogenase (SDH) was used for differentiation of individual types of muscle fibres. Myogenesis is completed in the third day of the post-natal life of pigs. The creation of new muscle fibres is limited by the number of satellite cells which persist among muscle fibre populations. From birth up to eighteen days of post-natal growth, only red muscle fibres were detected, and white muscle fibres were manifested from the forty-eighth day (48-day) of post-natal growth. White muscle fibres were larger in diameter. The shape of muscle fibres changed with the growth in thickness from oval to angular shape. When pigs were growing, the proportion of interstitial connective tissue to muscle tissue remained in favour of muscle tissue. The results show the craniocaudal increase of muscle fibre thickness from MTB through MLD to MRF.

Key words: interstitial tissue, lipid cells, muscular fibre, pig, post-natal growth, skeletal muscle

INTRODUCTION

The muscles of farm animals represent 40 to 50% of their body weight. The greatest part of this mass consists of striated skeletal muscle. Cross striated skeletal muscle tissue develops from myotomes, which form the dorso-lateral part of somites. Myotomes change to myoblasts, which are connected in myotubes, and these differentiate into muscle fibres. Myogenesis can also continue in the post-natal period, with the formation of new muscle fibres being limited by the number of satellite cells. The elongation of muscle fibres takes place by the lengthening of sarcomeres or by an increase in the number of sarcomeres which create the individual myofibrils. The growth of muscle fibrils in thickness occurs through an increase in the quantity of contractile proteins in two ways. The newly synthesized actin and myosin filaments connect by Z–disc proteins, thereby forming new sarcomeres to create new myofibrils. A second method is a lengthwise fission of existing myofibrils into two or more „daughter" myofibrils, which is a result of the contraction mechanism (Mesires et al. 2002; Kalbe et al. 2005).

The present study describes the morphological and histochemical parameters of cross striated skeletal muscles in pigs. The first objective of this work was to evaluate the histological and histochemical changes in the selected skeletal muscles MTB (*m. triceps brachii*), MLD (*m. longissimus dorsi et lumborum*) and MRF (*m. rectus femoris*) at defined phases of post-natal development.

Correspondence: E-mail: pmakovicky@email.cz
Peter Makovický; Czech University of Life Sciences, Faculty of Agrobiology, Food and Natural Resources, Department of Veterinary Sciences, Kamýcká 129, 165 21 Prague 6 – Suchdol, Czech Republic

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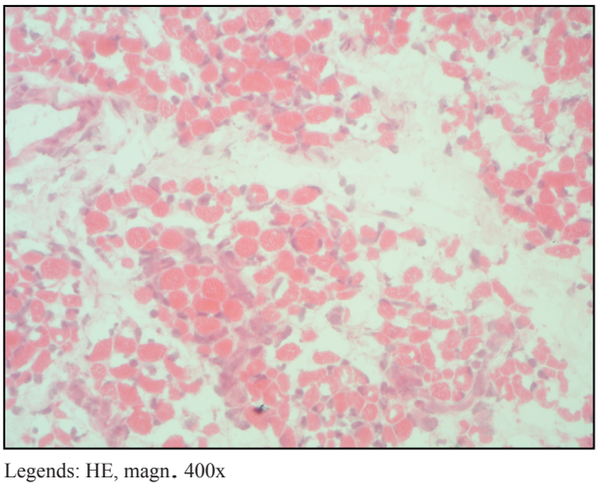
The second objective was the percentage evaluation of the proportion of individual muscle fibres and interstitial tissue, and the determination of the average thickness of muscle fibres, their individual types, and also the thickness of fat cells.

MATERIAL AND METHODS

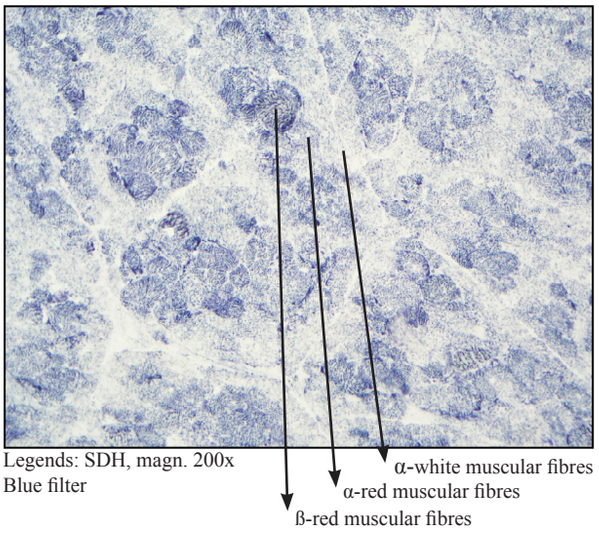
Slovak large White pigs of different ages were used for the morphological and histochemical study of skeletal muscles. The age categories were newborns, 1-day-old, 3-day-old, 18-day-old, 48-day-old, 84-day-old, 120-day-old, 192-day-old and 1055-day-old pigs (Figures 1, 2, 3, 4). Each age category was represented by a minimum of 5 animals (Table 1). The samples were taken necroptically, 30 minutes *post mortem*, from the three described muscles (Kulíšek et al., 2007). The samples were collected as per the Statute of the Slovak government which requires that animal protection be observed during experiments or for other scientific purposes (Statute 289/2003 § 11, clause 1 and 3).

The samples were taken from *m. triceps brachii* (MTB) latero-cranially from the muscle geometric centre, from *m. longissimus dorsi et lumborum* (MLD) at the level of the last rib from the muscle centre, and from *m. rectus femoris* (MRF) from its geometric centre. After marking, the samples were packed in foil and fixed in liquid nitrogen. These fixed samples were stored in a freezer box at a temperature of -30 ºC. The samples were cut on a freezing microtome (cryocut MTB) in 10 µm thick slides at temperatures -18 to -21 ºC. The muscle sections were then stained using histological and histochemical methods. The first set of samples was stained with transparent hematoxyline-eosine (HE) colours and toluidine blue. The second set, for the verification of neutral lipids, was stained with



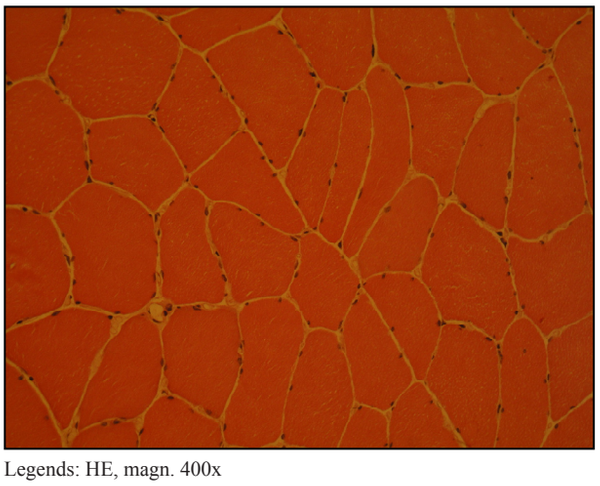
Legends: HE, magn. 400x

Fig. 1: Light-microscopic view about cross striated skeletal muscles of one-day-old pigs



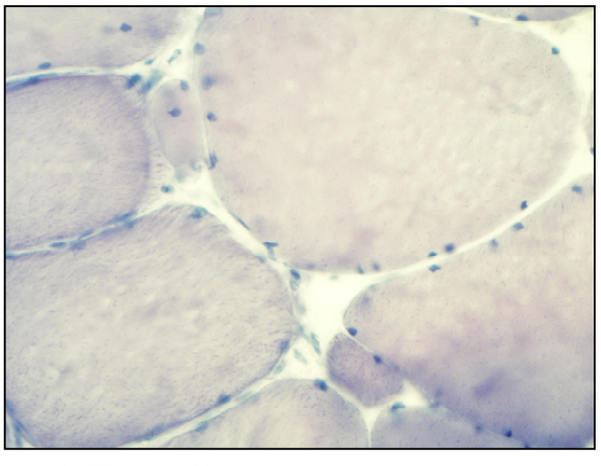
Legends: SDH, magn. 200x
Blue filter

Fig. 2: Muscles fibres of 48 old-days pigs



Legends: HE, magn. 400x

Fig. 3: Light-microscopic view about cross striated skeletal muscles of 192-days old pigs



Legends: HE, magn. 400x

Fig. 4: Light-microscopic view about cross striated skeletal muscles of 1055-days old pigs

oil red „0“. The determination of individual muscle fibre types was performed by the detection of succinate dehydrogenase (SDH) activity in accordance with the methods of Stein and Padykula (1962). The preparations were evaluated by objective morphometry using the light microscope Olympus Provis. Muscle fibre thickness, fat cell thickness, and the percentage area proportion of individual muscle fibre types and interstitial tissues were determined. Ten places were randomly selected for each preparation so that at least 500 fibres were evaluated during each preparation.

RESULTS

The average thickness of muscle fibres (Table 1) in the observed parts of MTB, MLD and MRF increased from birth (17.90 µm in MTB, 20.20 µm in MLD, 20.40 µm in MRF) up to the last observed stage (1055-day-old pigs), when thicknesses of 153.97 µm in MTB, 150.07 µm in MLD, and 151.07 µm in MRF were noted. The average thickness of muscle fibres at three days of age was similar to that at birth and almost equal among individual muscles (19.95 µm in MTB, 20.17 µm in MLD, 19.27 µm in MRF). A similar tendency was observed at the age of 84 days, when equal levels of average thickness were found (35.93 µm in MTB, 34.80 µm in MLD, 37.93 µm in MRF). On an average, the thickness of muscle fibres increased by the 84th day of age compared to the third day of age in MTB by about 15.98 µm, in MLD by 14.63 µm, and in MRF by 18.66 µm. A significant increase in the thickness of muscle fibres was reached at the age of 192 days (in MTB 73.30 µm, in MLD 79.03 µm, in MRF 86.61 µm). Approximately identical thicknesses of muscle fibres (153.97 µm in MTB, 150.07 µm in MLD and 151.07 µm in MRF) were found among individual muscles in the case of 1055-day-old pigs.

Table 1: The thickness of muscular fibers (µm)

Trait	N[1]	MTB[2]	MLD[3]	MRF[4]	Mean
New born	5	17.90	20.20	20.40	19.50
1-day	5	19.95	16.60	19.75	18.76
3-day	5	19.95	20.17	19.27	19.80
18-day	5	26.55	26.90	26.10	26.51
48-day	5	29.43	29.56	31.53	30.17
84-day	5	35.93	34.80	37.93	36.22
120-day	12	65.87	61.97	78.68	68.84
192-day	25	73.30	79.03	86.61	79.65
1055-day	3	153.97	150.07	151.07	151.70

[1]Number of pigs, [2]m. triceps brachii (MTB), [3]m. longissimus dorsi et lumborum (MLD), [4]m. rectus femoris (MRF)

The average thickness of individual types of muscle fibres (Table 2) was balanced up to the group of 84-day-old pigs. After the age of 120 days an increase in the thickness of muscle fibre type in MRF began with the exception of the 1055-day-old pigs. α-White fibres from the age of 48 days until the end of the observation period (1055 days) were thicker in the selected parts of MTB, MLD and MRF compared to ß-Red and α-Red muscle fibres.

From birth until the age of 3 days there was a decreasing tendency of the percentage of interstitial tissue at the expense (Table 3) of percentage increase of muscle tissue in all the observed parts. The percentage area representation of interstitial tissue and muscle fibres from the age of 18 to 1055 days is shown in Table 4. At birth the highest representation of interstitial tissue was in MTB (14.40 %) and the lowest in MLD (12.80 %).

Table 2: The thickness of individual types of muscular fibres (µm)

Trait	MTB			MLD			MRF		
	ß-red	α-red	α-white	ß-red	α-red	α-white	ß-red	α-red	α-white
New born	15.90	19.90	0.00	18.20	22.20	0.00	19.30	21.50	0.00
1-day	18.70	21.20	0.00	18.10	21.10	0.00	18.70	20.80	0.00
3-day	19.05	20.85	0.00	19.55	20.80	0.00	18.75	19.80	0.00
18-day	23.30	29.80	0.00	25.60	28.20	0.00	23.10	29.10	0.00
48-day	25.40	29.00	33.90	24.30	28.60	35.80	26.70	31.50	36.40
84-day	31.70	36.40	39.70	31.50	34.60	38.30	35.40	37.80	40.60
120-day	48.83	66.03	82.75	51.99	61.60	72.32	58.33	80.60	97.11
192-day	71.36	73.24	75.31	79.32	78.81	78.95	84.61	84.61	90.62
1005-day	119.60	156.50	185.90	121.10	147.60	181.50	125.20	141.10	186.90

Table 3: Percentage content of interstitial tissues and muscular fibres (µm)

Trait	MTB		MLD		MRF		Diameter	
	IT	Fibres	IT	Fibres	IT	Fibres	IT	Fibres
New born	14.40	85.60	12.80	87.20	13.40	86.60	13.50	86.50
1-day	12.40	87.60	12.60	87.40	13.20	86.80	12.70	87.30
3-day	11.20	88.80	12.40	87.60	12.00	88.00	11.87	88.13

IT- interstitial tissues

Table 4: Percentage content of interstitial tissues and muscular fibres (µm)

Trait	MTB				MLD			
	IT	β-red	α-red	α-white	IT	β-red	α-red	α -white
18-day	8.70	52.70	38.60	0.00	8.20	52.80	39.00	0.00
48-day	9.00	33.60	39.40	18.00	8.80	3420	37.00	20.00
84-day	9.70	30.60	38.50	21.20	8.30	30.50	39.40	21.80
120-day	7.80	23.20	25.50	43.50	8.90	19.90	25.20	46.00
192-day	2.92	26.12	15.07	55.89	3.51	17.68	27.01	51.80
1005-day	7.10	17.50	27.73	47.67	6.54	22.73	28.73	42.00
Traits	MRF				Diameter			
	IT	β-red	α-red	α-white	IT	β-red	α-red	α-white
18-day	8.00	53.60	38.40	0.00	8.30	53.00	38.70	0.00
48-day	8.80	32.80	38.80	19.60	8.90	33.60	38.30	19.20
84-day	8.80	31.40	37.40	22.40	8.93	30.83	38.43	21.81
120-day	8.50	26.16	29.56	35.78	8.40	23.09	26.75	41.76
192-day	3.80	24.68	31.52	40.00	3.41	22.83	24.53	49.23
1005-day	8.07	22.00	32.40	37.53	7.24	20.74	29.62	42.40

Table 5: The size of fat cells (µm)

Trait	MTB	MLD	MRF	Diameter
New born	0.00	0.00	0.00	0.00
1-day	0.00	0.00	0.00	0.00
3-day	0.00	0.00	0.00	0.00
18-day	12.15	11.60	14.50	12.75
48-day	15.75	21.25	14.50	17.16
84-day	13.50	12.00	12.15	12.55
120-day	21.30	25.00	22,15	22.82
192-day	39.70	41.10	36.70	39.17
1055-day	28.77	25.65	27.47	27.29

There was a similar tendency in 1055-day-old pigs, but only in the MLD muscle, where the lowest representation of interstitial tissue was 6.54 % and the highest was in MRF (8.07 %). The percentage area representation of interstitial tissues of 18-day-old pigs was found to be approximately at the same level. The percentage portions of muscle fibres in MTB, MLD and MRF changed with the growth of the pigs. At 1055 days the highest representation of β-Red fibres was found in MLD (22.73 %), of α-Red fibres in MRF (32.40 %), and of α-White fibres in MTB (47.67 %).

The average thickness of fat cells during the time of growth (Table 5) was more variable until the 84th day and reached levels similar to those at 18th day in all the parts observed. An increase in fat cell thickness

was noted until 192 days. Further growth and increasing age showed a tendency of the thickness of fat cells to decrease.

DISCUSSION

The myogenesis of pigs is *post partum* completed at the time of birth, as myotubes, i.e. muscle fibres with a centrally located nucleus remain present. The histological picture at this stage depicts the classic structure of skeletal muscles. These observations are also related to those of other authors (Zoecklein et al. 1994; Kimball, 2002). Animals are born with a given number of muscle fibres, which grow in length and thickness during their life. On the basis of our results it can be stated that sporadic myotubes with localization of nuclei near the sarcolemma are detectable even by the third day of post-natal development.

The muscle fibres are multi-nuclei at the time of birth, but an increase in the number of nuclei can be caused by mitotic activity of myoblasts which are called satellite cells (Ontel et al. 1982; Swatland, 1983).

Uhrín et al. (1984) studied pig skeletal muscles in order to determine the stages of differentiation and separation of individual muscle fibres. These authors stated that individual types of muscle fibres are not histochemically distinguishable at birth and shortly post-natal. This is in agreement with our results. All muscle fibres are characterized by the presence of a large number of mitochondria in the first day post-natal. It is possible to precisely determine only the red fibres. With increasing age, the number of mitochondria decrease and fibres with a low number of mitochondria increase, which corresponds to the findings of Herpin et al. (2002). Kaman (1995a) examined seven topographically and functionally different muscles in new-born wild, primitive and domestic pigs, ten per group. According to their reports, muscle fibres were morphologically and histochemically differentiated completely in new-born wild pigs. Other studies reported the distribution of muscle fibre types of new-born pigs on the basis of histochemical detection of ATP-ase activity, SDH reaction and distribution of glycogen (Kaman, 1995b). Their work concluded that the muscle fibres of new-born pigs are morphologically and biochemically adequately differentiated. All the three fibre types are distinguishable on the basis of ATP-ase, but SDH reaction renders impossible the objective identification of individual types of muscle fibres in pigs until one month of age. According to Pestov et al. (2001), individual muscle fibres are already distinguishable in new-born pigs directly after birth on the basis of ATP-ase activity. The findings of Alabaya et al. (1995) were similar, which suggested the use of the ATP-ase method for objective distinction of individual muscle fibre types. Our results show that only red muscle fibres are distinguishable in pigs from birth until 18 days. In the study of Tristan et al. (2009) it was concluded that pig weight at birth is associated with muscle cellularity of pig, which may influence the postnatal muscle growth and final size of muscle fibres and meat quality. According to the results of Kaman and co-workers (1995a), one month after birth the muscles are divided into those with a predominance of anaerobic fibres (MLD, MTB, MG and MM), and those which are metabolically inclined to myopathy. According to Beerman et al. (1978), pig muscles consist only of red muscle fibres at the beginning. These fibres are converted, and white fibres are created later. According to our monitoring, ß-Red muscle fibres are present in small groups which are surrounded by intermediate (α-Red) and then by white muscle fibres (α-White). This observation was noted from the 48th day of post-natal growth, which is similar to the results of Lefaucheur et al. (1995).

According to Sandoval et al. (1995), the number of α-White muscle fibres increased with the pig's growth. These data correspond to those of Alabay et al. (1996) and Ruusunen and Puolanne (2004). The study from Elias et al. (2007) shown that Red muscle fibre abundance was only slighty higher than intermediate muscle fibre abundance. Concerning the average muscle fibre diameter, the highest values in white and the lowest values in red muscle fibres were fnoted. According to Orzechowska et al. (2008), an increase in the daily live weight gain was related to increased size of White fibres and shear force was negatively related to Red muscle fibre size. Velotto et al. (2007) observed no differences between males and females in percentage of the fibre types, but there were significant differences between sews in size of all the three fibre types. In our study the predominance of α-White muscle fibres was shown from the age of 48 days. We also detected that at birth the average thickness of all the muscle fibres of pigs was 19.50 μm and at the age of 1055 days 151.70 μm. Among all the muscles observed, cross striated skeletal muscles dominated over interstitial tissues.

Sencic et al. (1994) analyzed the skeletal muscles in Slovak White and Swedish Landrace pigs at 105 kg. They determined that there are differences in the percentage representation of muscle tissue between the two breeds. Our results also depict differences in the percentage representation of skeletal muscles within one breed in different body parts. Fofana and Manukhina (1998) conducted a study in which they monitored the post-natal growth of skeletal muscles of Slovak White and Duroc pigs. Their results shows that from birth until 30 days of age muscle tissue increased intensively, which is in accordance with our results. They assert that the growth period of muscle fibres definitely ends at 105 days of age, which is contrary to our results. The authors suggest the end of the pig's growth in terms of the onset

of a balance with the eventual increase or conversion of fat tissue at the expense of muscle tissue.

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