Histomorphometrical Evaluation of Myocyte Types in the Lamb

S. VELOTTO, M.R. DI PRISCO, T. STASI, A. CRASTO

Dipartimento di Scienze Zootecniche e Ispezione degli Alimenti,
Facoltà di Agraria – Università degli Studi di Napoli "Federico II", Portici, Italy

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Abstract

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The aim of this study was to evaluate the effect of age and sex on histochemical and morphometric characteristics of muscle fibres (myocytes) in lambs born by single or twin birth. Forty lambs were fed mother's milk and slaughtered at 60 days; 40 weaned at 60 days and fed until 120 days with flakes (60%) and hay (40%) and then slaughtered. Muscle tissues were obtained from two muscles, namely *m. psoas major* and *caput longum m. tricipitis brachii* of all lambs. The muscles were stained for myosin ATPase, and succinic dehydrogenase. For each fibre type, area, perimeter, maximum and minimum diameter were measured and slow-twitch oxidative fibres, fast-twitch glycolytic fibres, fast-twitch oxidative-glycolytic fibres were histochemically differentiated. An image-analysing system was used. At 60 days, females had fibres larger than males while the opposite was observed at 120 days. Moreover, at 60 days, the lambs born by single birth had fibres larger than those born by twin birth, while the opposite was observed at 120 days. The fast-twitch glycolytic fibres had the largest size, followed by the slow-twitch oxidative and the fast-twitch oxidative glycolytic fibres. The dimensions of fibre types in *caput longum tricipitis brachii* muscle were larger than in the *psoas major* muscle ($P < 0.001$). This study provides important data on basic characteristics of lamb muscles that, as intrinsic factors, may influence some aspects of meat production and quality.

Postnatal development, single birth, twin birth, myosin ATPase, succinic dehydrogenase, meat quality

The Gentile of Puglia, also known by Apulian Merino, Merino di Puglia, Improved Apulian, Italian Merino, Merina Gentile, is a fine-wooled breed from southern Italy. Development of this breed began in the 15th century but the first improvement started from the 18th century. The breed was obtained from Spanish Merino crossed with the local breeds. Saxony and Rambouillet breed was introduced during the 19th century. In the second half of the century the number of head has dramatically decreased. Presently, there are about 15 000 sheep mainly in the Basilicata, Molise, and Puglia Regions. The breed is traditionally reared in the hilly pastures from late spring to early fall and in the fold or in the lowland pasture in the remaining seasons. In the last years researchers have focused on the potential of this breed with the purpose to produce meat. Meat quality is affected by age, sex, breed, diet, and animal husbandry practices as well as by factors that affect growth and differentiation of skeletal muscle fibre types. The muscle fibres represent the basic unit for the muscle activity. They contain enzymes which transform chemical into mechanical energy, and specific proteins that form myofibrils. During the neonatal period, the muscle fibres acquire metabolic and functional characteristics related to the locomotor or postural function of the muscle. The fibres are usually classified into three main groups with different names. The terminology used in this study for the three main types of muscle fibres is as follows: type FG (i.e. rapidly contracting fibres with glycolytic metabolism), type FOG (i.e. rapidly contracting fibres with glycolytic-oxidative metabolism), and SO (i.e. slowly contracting fibres with oxidative metabolism) corresponding to IIB IIA and I, respectively (Suzuki and Tamate 1988). These features are not stable in the sense that each

Address for correspondence:
Dipartimento di Scienze Zootecniche e Ispezione degli Alimenti,
Facoltà di Agraria – Università degli Studi di Napoli „Federico II".
Via Università, 133 – 80055 - Portici (NA), Italy.

Phone: +390812539269-264
Fax: +390817762886
e-mail: velotto@unina.it.
http://www.vfu.cz/acta-vet/actavet.htm

fibre may transform into another one due an adaptive response to a certain stimulus. The proportions of the three types of fibres change with the muscle, and the predominance of one type over the others depends on the metabolism as well as environmental stresses. The aim of this study is to evaluate the effect of age, sex, and birth type on the histochemical and morphometric characteristics of muscle fibres based on myosin ATPase, and succinic dehydrogenase methods. The information obtained by the distribution of different fibres types, their number and diameter and/or area, represent an element of paramount importance for the determination of some basis characteristics (morphological and functional) of the muscle that, as intrinsic factors, have great influence on some aspects of the meat production and quality.

Materials and Methods

Eighty clinically healthy lambs (40 males and 40 females) coming from a farm located in Benevento (Italy) were slaughtered at 60 and 120 days. They were born as single or twins and were all fed mother's milk until 60 days. Group 1 (40 animals) was slaughtered at 60 days, and Group 2 was weaned at 60 days and fed until 120 days with flakes (60%), and hay (40%).

Muscle Histochemistry

The following muscles were used in the study*: m. psoas major (PM), caput longum m. tricipitis brachii* (*ClTb*). From every muscle samples were collected and immediately frozen in liquid nitrogen (-196 °C), and successively stored at -80 °C until histochemical analyses were conducted. Transverse serial sections (8μm) were cut in a cryostat at -20 °C and transferred to glass cover slips. The sections were stained histochemically for myosin ATPase (m-ATPase shows the muscular contraction) and succinic dehydrogenase (SDH shows the fibres metabolism) simultaneously on the same muscular fibres (Padykula and Herman 1955; Nachlas et al. 1957; Barany 1967; Edstrom and Kugelberg 1968; Guth and Samaha 1970; Solomon and Dunn 1988; Velotto et al. 2002). The method used for the combined histochemical staining (acid m-ATPase + SDH) consisted of different phases. Acid pre-incubation was performed at room temperature for 20 min and was always followed by two 1-min rinses in $CaCl_2$ in tris hidroxymethyl aminomethane buffer rinses solution. Nitro blue tetrazolium (NBT) incubation was performed for the detection of SDH activity at 37 °C for 20 min followed by two rinses in distilled water. For the myofibrillar (acid) ATPase portions, the procedure was performed at 37 °C at pH 9.4 for 50 min along with one 30-s rinse in $CaCl_2$ solution and incubation for 3 min in CoCl solution. Finally, ammonium sulphide staining of the acid ATPase procedure was performed. Cover slips were placed over the stained tissue sections and fixed in place using glycerol jelly. Additional serial sections were also histochemically stained for detection of basic m-ATPase and SDH activities. The basic m-ATPase method was used for the first control procedure and consisted of different phases. Sodium-cacodylate and sucrose solutions were used for incubation for 5 min followed by two 1-min rinses in CaCl2 in tris hydroxymethyl aminomethane buffer rinse solution. Sigma 221 and $CaCl_2$ solutions were used for 10 min at range pH 10.3-10.5 followed by two 1-min rinses in $CaCl_2$ and tris hydroxymethyl aminnomethane buffer (Merck & Co USA) rinse solution. For the myofibrillar (acid) ATPase portions, the procedure was performed at 37 °C at a pH 9.4 for 50 min along with one 30-s rinse in $CaCl_2$ solution and incubation for 3 min in CoCl solution. Finally, ammonium sulphide staining of the acid ATPase procedure was performed. Cover slips were placed over the stained tissue sections and fixed in place using glycerol jelly. The SDH method was used for the second control procedure and was consisted of different phases. Incubation in NBT at 37 °C was performed for 40 min followed by two rinses in distilled water. Finally, formaldehyde solution was used for 10 min. Cover slips were placed over the stained tissue sections, and fixed in place using glycerol jelly.

Fibre size

Fibre size was determined from the same section used to determine myofibre number. The area, the perimeter, and the maximum and minimum diameter were measured using an image-analysing system (Zeiss, Kontron, KS 300). For each muscle not less than 200 fibres belonging to eight random fields were measured. The average fibre size (area, perimeter, diameter maximum and minimum) was calculated.

Statistical Analysis

Data were processed by analysing the variances and means were estimated by following the general linear model (Proc GLM; SAS, 1992) in which the factors considered are fixed, and the effect of the other factors is expressed as deviation from the general average (μ). The model used was:

$y_{ijklm} = \mu + Sex_i + Bt_j + Mu_k + Ft_l + (sex*Ft)_{il} + (Bt*Ft)_{jl} + (Mu*Ft)_{kl} + \varepsilon_{ijklm}$

y_{ijklm} = value of relative observation to the l^{mo} fibre type of the k^{mo} muscle, of the m^{mo} subject of i^{mo} sex, born of j^{mo} birth type.
Sex_i = fixed effect of the i^{mo} sex (I = 1,2).
Bt_j = fixed effect of the J^{mo} birth type (j = 1,2).
Mu_k = fixed effect of the k^{mo} muscle (k = 1, 2).
Ft_l = fixed effect of the l^{mo} fibre type (l = 1, 2, 3).

$(Sex*Ft)_{il}$ = fixed effect of the i^{mo} sex with the l^{mo} fibre type.
$(Bt*Ft)_{jl}$ = fixed effect of the j^{mo} birth type with the l^{mo} fibre type.
$(Mu*Ft)_{kl}$ = fixed effect of the k^{mo} muscle with the l^{mo} fibre type.
ε_{ijklm} = residual error.

Significance between the mean values was evaluated using the Student 's *t*-test.

Results

Fibre types

Based on combined m-ATPase reactions, after acid (pH range 4.35 - 4.4) and alkaline (pH range 10.3 - 10.5) preincubations in *PM* and *ClTb* muscles of the lambs slaughtered at 60 and 120 days three fibre types were identified. Type SO (slow-twitch oxidative) fibres were acid-stable and alkaline-unstable with low m-ATPase activity and high oxidative activity, type FG (fast-twitch glycolytic) fibres were acid-unstable and alkaline-stable with high m-ATPase activity and low oxidative activity; type FOG (fast-twitch oxidative-glycolytic) fibres were acid-unstable and alkaline-stable with moderate m-ATPase activity and intermediate oxidative activity (Plates V, VI, VII, VIII, Figs 1, 2, 3, 4, 5, 6, 7 and 8).

Percentage of the fibre types

The relative distribution of the three fibre types in the studied muscles (Fig. 9) revealed a high percentage of FOG fibres in the *PM* muscle (44% at 60 days and 47% at 120 days). The FOG fibres were characterized by a more versatile oxide-glycolytic metabolism, according to the necessity to obtain energy for the contraction. *ClTb* muscle at 60 days showed approximately the same distribution of FOG and SO fibres (37% and 35%, respectively); while at 120 days a considerable increase in SO fibre numbers was noticed (+ 9%) accompanied by a reduction of FG fibre (- 9%).

Sizes of the fibre types

The results of the analysis of variance show significant interactions between muscle × fibre type, birth type × fibre type, sex × fibre type (Table 1). In the group of lambs (60 days)

Table 1. F value relative to the experimental factors considered at 60 days and at 120 days

F(1)	Variable			
	Area	Perimeter	Diameter	
			Minimum	Maximum
Age, 60 days				
Sex	133.54***	71.72***	155.02***	25.10***
Birth type, Bt	28.85***	39.28***	9.88***	54.25***
Muscle, Mu	904.27***	1069.05***	631.67***	1066.4***
Fibre type, Ft	353.3***	347.57***	264.53***	319.31***
Sex*Ft	18.94***	14.92***	11.08***	11.90***
Bt*Ft	10.08***	8.16***	7.00***	6.79***
Mu*Ft	53.11***	37.03***	30.72***	32.48***
Age, 120 days				
Sex	151.50***	149.47***	65.90***	165.8***
Birth type, Bt	50.33***	35.62***	40.44***	28.55***
Muscle, Mu	783.49***	977.7***	572.12***	1003.63***
Fibre type, Ft	76.78***	74.14***	82.03***	58.32***
Sex*Ft	5.63*	6.01***	4.06*	6.5**
Bt*Ft	15.73***	14.9***	14.1***	11.68***
Mu*Ft	26.51***	25.24***	25.12***	20.04***

(1) * = $P < 0.05$; ** = $P < 0.01$; *** $P < 0.001$

Table 2. Mean value and variation coefficient (v.c., %) of morphometric characteristics of fibre types in the males and females in relation to age

Fibre Types	Area/μm²		Perimeter/μm		Diameter/μm			
					minimum		maximum	
	mean	v. c.,%	mean	v. c.,%	mean	v. c.,%	mean	v. c.,%
Age, 60 days								
Males								
FG	910.61	41	119.26	23	28.06	25	47.08	25
FOG	631.16	40	101.57	20	23.53	24	40.91	24
SO	720.8	45	106.24	22	25.26	25	40.17	25
Females								
FG	1000.47	55	122.64	25	30.63	29	47.77	28
FOG	700.64	52	102.9	24	25.59	27	40.86	24
SO	897.57	54	115.22	27	28.24	30	42.92	27
Age, 120 days								
Males								
FG	1140.5	58	131.6	30	32.45	30	51	36
FOG	838.89	57	115.46	30	27	29	43.7	32
SO	1025.33	56	125.43	29	31.75	29	48.15	33
Females								
FG	930.2	52	115.46	25	28.25	25	44.37	28
FOG	700.4	52	103.35	25	25.35	26	38.61	27
SO	930.69	54	116.32	25	26.96	28	43.75	29

Table 3. Mean value and variation coefficient (v.c., %) of morphometric characteristics of fibre types for birth type in relation to age.

Fibre Types	Area/μm²		Perimeter/μm		Diameter/μm			
					minimum		maximum	
	mean	v. c.,%	mean	v. c.,%	mean	v. c.,%	mean	v. c.,%
Age, 60 days								
Single birth								
FG	1150.01	35	139.68	20	32.45	22	54.38	24
FOG	740.94	30	110.68	15	26.09	20	45.43	20
SO	900.72	33	115.98	22	28.1	21	47.2	20
Double birth								
FG	900.3	45	117.4	22	29.36	18	47.35	24
FOG	640.45	40	100.99	20	25.27	20	41.54	21
SO	800.2	50	107.84	22	27.76	31	45.14	26
Age, 120 days								
Single birth								
FG	980.96	45	120.45	20	31.74	23	47.29	22
FOG	600.43	38	95.58	20	23.41	23	38.2	20
SO	790.93	36	110.58	35	27.95	20	40.42	20
Double birth								
FG	1074.25	55	125.3	28	31.62	26	50.46	30
FOG	809.15	49	110.25	26	26.61	25	44.3	28
SO	945.36	55	116.6	25	29.71	28	43.21	29

fed only mother's milk, the females had fibres larger than the males, while the opposite was observed in the other group (120 days). The difference between females and males (about fibre area at 60 days), was 9% for FG (1000.47 vs. 910.61, $P < 0.001$), 10% for FOG (700.64

vs. 631.16, $P < 0.01$) and 20% SO (897.57 vs. 720.8, $P < 0.001$). At 120 days the males had fibres larger than females, the differences between males and females were 18% for FG (1140.5 vs. 930.2, $P < 0.001$), 16% for FOG (838.89 vs. 700.64, $P < 0.001$), and 9% for SO (1025.33 vs. 930.69, $P < 0.001$) (see Table 2). With respect to the birth type, the lambs born by single birth and slaughtered at 60 days had fibres greater than those born by twin birth. The difference between single and twin birth (about fibre area) was 22% for FG (1150.01 vs. 900.3, $P < 0.001$), 14% for FOG (740.94 vs. 640.45, $P < 0.001$) and 11% for SO (900.72 vs. 800.2, $P < 0.001$) (see Table 3). The lambs born by twin birth and slaughtered at 120 had larger fibres than those born by single birth. The differences between twin and single birth in fibre area were 26% for FOG (600.43 vs. 809.15, $P < 0.001$), and 16% for SO (790.43 vs. 945.36, $P < 0.001$). The other morphometric parameters show a similar trend. In agreement with Velotto et al. (2003) in the *ClTb* muscle the dimensions of FG, FOG and SO fibres were larger than in the *PM* at 60 and 120 days (Table 4). The dimensions of FG fibres in muscles examined were larger than SO and FOG (Table 4).

Table 4. Mean value and variation coefficient (v.c., %) of morphometric characteristics of fibre types in considered muscle

Fibre Types [1]	Area/μm²		Perimeter/μm		Diameter/μm			
					minimum		maximum	
	mean	v. c.,%	mean	v. c.,%	mean	v. c.,%	mean	v. c.,%
Age, 60 days								
ClTb								
FG	1365.86	42	142.12	20	35	20	55.36	23
FOG	860.93	43	116.69	20	27.36	23	44.7	24
SO	960.65	44	122.32	21	28.99	24	46.31	22
PM								
FG	725.53	50	107.5	20	27.43	22	42.33	22
FOG	525.45	45	92.1	20	21.64	25	36.6	22
SO	735.42	50	106.2	22	25.92	30	39.31	24
Age, 120 days								
ClTb								
FG	1537.45	46	153.18	24	36.37	25	59.51	26
FOG	1194.18	46	135.1	24	31.32	24	52.55	26
SO	1335.06	50	140.73	25	33.59	26	55.64	26
PM								
FG	824.16	50	112.47	25	28.61	25	43.04	27
FOG	628.38	58	98.86	25	25.06	29	37.32	28
SO	978.34	56	120.65	28	30.77	31	46.28	30

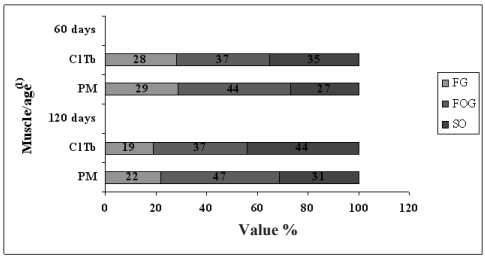
[1] *ClTb=Caput longum m. tricipitis brachii PM=m. psoas major*

Discussion

This study demonstrated that age, sex, and birth type influence the histochemical and morphometric characteristics of muscles in lambs.

Percentage of fibre types

The percentage of muscle fibre types in the skeletal muscle change according to the muscle function. Our study highlights that *PM* muscles show a high percentage of FOG. The massive presence of these fibres is explained by the fact that it is an in and out rotator of the femur, and this action is very effective when the thigh is in semi-flexion. When this muscle takes a fixed point on the lumbar region it flexes the thigh on the pelvis, while when it takes a fixed point on



(1) ClTb= *Caput longum triciptis brachii* PM= *Psoas major*

Fig. 9. Percentage distribution of fibre types in muscles under astudy

the femur it flexes the lumbar region. In *ClTb* muscle at 120 days it is possible to notice an increase of SO fibres and a reduction of FG fibres. This effect may be due to the conversion of FG fibres into SO fibres and could be addressed to trophic factors, or more reasonably to the pattern or frequency of impulses in the motoneurons. Repetitive stimulations of motor nerves in experimental animals enable conversion of fast fibres into the slow ones, as well as an increase in capillary density. However, these conditions are extreme, and conversion of fast to slow fibres is not found with exercise (Salmons and Henriksson 1981).

The high percentage of SO fibres and the low percentage of FG fibres can be explained by the function performed by this muscle which, together with the other heads of *m. triceps brachii* is an extensor of the forearm. When the limb is lifted, the triceps uses a first class lever and opens the angle of the elbow, instead, when the limb is supported, the action is the same, but it uses a second class lever. The lateral and long head of triceps muscle oppose the flexors and extend the elbow joint of raised limb in the swing phase, during the landing phase they also function to support the other extensors of the elbow (Menzel 1999). In his study Menzel (1999) removed muscle tissue from the extensors and flexors of the elbow joint of six male sheep (180 days old). He noticed that the medial head of triceps muscle had a very high percentage of SO-fibres (90%) and FG fibres were absent in this muscle. The dorsal part of the long head of triceps muscle contained only 13% SO fibres, but had the highest percentage of FG fibres (49%), which is representative of fast-muscle. Suzuki and Tamate (1988) studied the composition of the hip and thigh musculature of sheep by myofibre types and examined the differences in their distribution. They noticed that type IIA myofibres, showed little difference in distribution in the hip and thigh muscles. Type IIB myofibres, were distributed more in the cranial, caudolateral, and caudomedial portions than in the middle portions of the thigh. The distribution of type IIB myofibres is suited to powerful flexion and extension of the thigh and leg. In the hip and thigh musculature, it appears that type I myofibres are effectively distributed to maintain a standing posture without diminishing the propulsive force of the hind-limb.

Sizes of fibre types

Our results demonstrate that in the lambs slaughtered at 60 days the dimensions of fibres in the females are larger than in the males. The opposite was observed for the lambs slaughtered at 120 days. Ford and Klindt (1989) noticed that male farm animals grew to a larger mature size and had more muscle, especially in the neck and forequarter, than females. This increase may be attributed to the protein anabolic effects of testicular hormones (Buttery and Sinnett-Smith 1984; Jost and Magre 1984). Because of their nature muscle mass is larger and intact males grow more rapidly and utilize feed more efficiently. Moreover, their carcass contains less fat and more edible product (Unruh 1986). The data obtained by Arnold et al. (1997) showed that testosterone increased the combined weight of three muscles (*semitendinosus*, *splenius*, and *triceps brachii).* Additionally, this hormone may determine an increase in the muscular protein synthesis that causes an increase in dimension of the muscles fibres, an increase in percentage of the FG fibres along with a reduction in percentage SO fibres. McCoard et al. (2001) observed that twin neonate lambs sacrificed at 20 days had lower body weights and muscle weights compared to single-born lambs. Lower muscle weights in twins were associated with smaller myofibre cross-

sectional areas and lower total nuclei numbers and myogenic precursor cell numbers × muscle in selected hind-limb muscles. These results indicate that myofibre hypertrophy in late gestation and early postnatal life is related to myogenic precursor cell number which may have important implications for growth potential of the growth-restricted fetus. In agreement with the previous study our results highlight that at 60 days the lambs born by twin birth have fibres smaller than the lambs born by single birth.

Fibre area in the lambs increased ($P < 0.01$) with age in both oxidative and glycolytic fibres (Whipple and Koohmaraie 1992). The *longissimus* muscle had the smallest ($P < 0.05$) muscle fibres and the *supraspinatus* muscle tended to have the largest ($P = 0.06$) muscle fibres at 26 wk of age. White and McGavin (1978) reported that fibre diameter increased in ovine *quadriceps* muscles with age for both fibre types. Our study demonstrates that the *caput longum tricipitis brachii* muscle has shown a significant development in SO, FOG and FG fibres types higher than in the *psoas major* muscle at 60 and 120 days. Regardless of age of animal in agreement with our study Suzuki and Cassens (1983) observed that also in *serratus ventralis thoracis* muscle type FG myofibres were greater than type SO and FOG myofibres were always lowest.

Interest in the possible association between eating quality and fibre type characteristics has arisen from the observations that both variables vary between muscles (Klont et al. 1998; Sinclair et al. 2001).

Based on histochemical staining methods it has been shown that fibre type proportions and sizes vary both within and between muscles, but in general, glycolytic fibres attain a greater size than oxidative fibres (Maltin et al. 2003). Several studies of beef, lamb and pork have shown a positive relationship between proportions of SO fibres and either tenderness or juiciness (Ockerman et al. 1984; Maltin et al. 2001). Moreover, the larger the muscle fiber cross sectional area is, the less tender meat is. Our study shows the evolution of muscle fibre characteristics in the period 60–120 days of life. Significant interaction among the considered factors highlighted with variance analysis discussed in previous section demonstrates that age sex and birth type influence the dimensions of the fibres and consequently the meat quality.

Vliv věku, pohlaví a typu porodu na histochemické a morfometrické znaky dvou svalů jehňat

Cílem studie bylo porovnat vliv věku, pohlaví a typu porodu na histochemické a morfometrické znaky svalových fibril. Svalová tkáň byla získána z *m. psoas major* a *caput longum m. tricipitis brachii* osmdesáti jehňat (40 jehňat bylo krmeno mateřským mlékem a poraženo v 60 dnech; zbylých 40 bylo v 60 dnech odstaveno a do 120. dne byla jehňata krmena vločkami (60 %) a senem (40 %) a po té byla poražena). Získaná svalová tkáň byla barevně rozlišena pomocí myosin ATPázy a sukcinátdehydrogenázy na jednotlivé svalové fibrily. Sledovaní jedinci se narodili jednotlivě nebo jako dvojčata. Pro každý typ fibrily byl měřen její objem, obvod a maximální a minimální průměr pomocí systému obrazové analýzy. Pomalá červená vlákna, rychlá červená vlákna a rychlá bílá vlákna byla histochemicky odlišná. Samice měly v 60 dnech větší fibrily než samci, zatímco 120. den byl pozorován přesný opak. Navíc jehňata narozená jednotlivě, měla v 60 dnech větší fibrily než ta, která se narodila jako dvojčata, zatímco 120. den byl pozorován opět přesný opak. Největší objem měla rychlá červená vlákna, následovala vlákna pomalá červená a pak rychlá bílá vlákna. Vlákna všech typů fibril byla větší v c*aput longum m. tricipitis brachii*, než v *m. psoas major* ($P < 0.001$). Informace získané v této studii představuje důležitou bázi pro popis některých základních znaků svalu, jako podstatných faktorů, které mají vliv na aspekty kvality produkce jehněčího masa.

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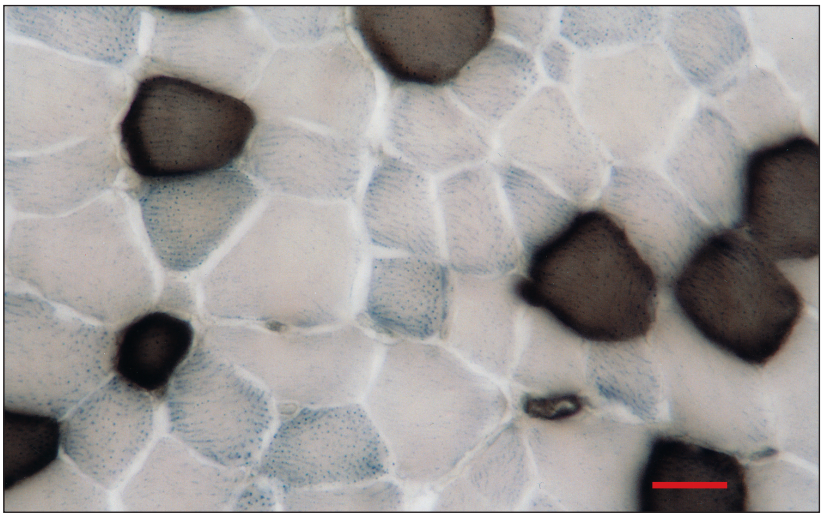

Fig. 1. *Caput longum m. tricipitis brachii* (60 days, males). A light micrograph showing histochemical staining of three types of fibres: slow oxidative, SO (darkest area); fast oxidative glycolytic, FOG (intermediate area) and fast glycolytic FG (lightest area). Muscle transverse section (× 400) treated for m-ATPase + SDH combined reaction after preincubation at pH range 4.35 – 4.40, bar 25 μm

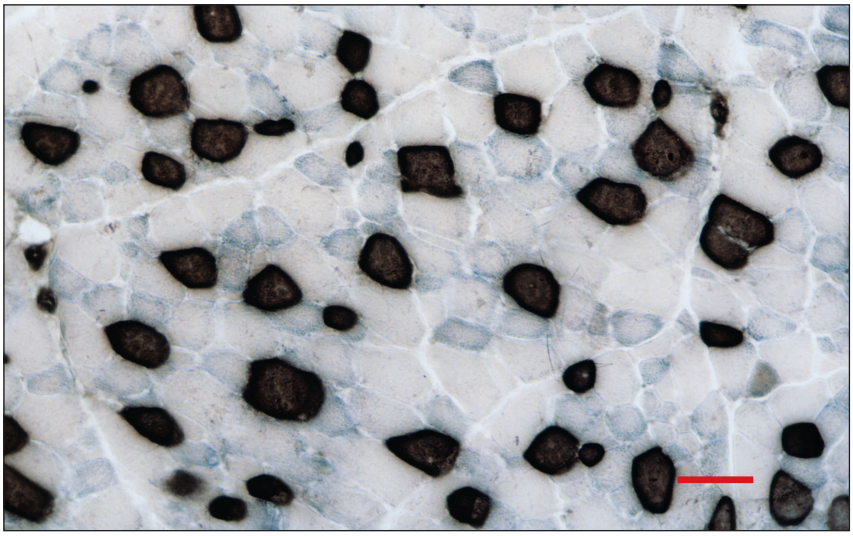

Fig. 2. *Caput longum m. tricipitis brachii* (60 days, females). A light micrograph showing histochemical staining of three types of fibres: slow oxidative, SO (darkest area); fast oxidative glycolytic, FOG (intermediate area) and fast glycolytic FG (lightest area). Muscle transverse section (× 160) treated for m-ATPase + SDH combined reaction after preincubation at pH range 4.35 – 4.40, bar 62.5 μm

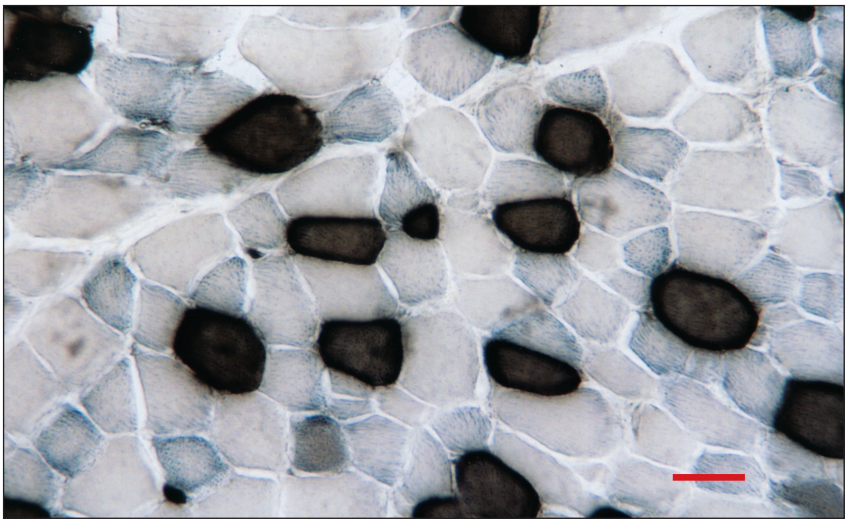

Fig. 3. *M. psoas major* (60 days, males). A light micrograph showing histochemical staining of three types of fibres: slow oxidative, SO (darkest area); fast oxidative glycolytic, FOG (intermediate area) and fast glycolytic FG (lightest area). Muscle transverse section (× 250) treated for m-ATPase + SDH combined reaction after preincubation at pH range 4.35 – 4.40, bar 40 µm

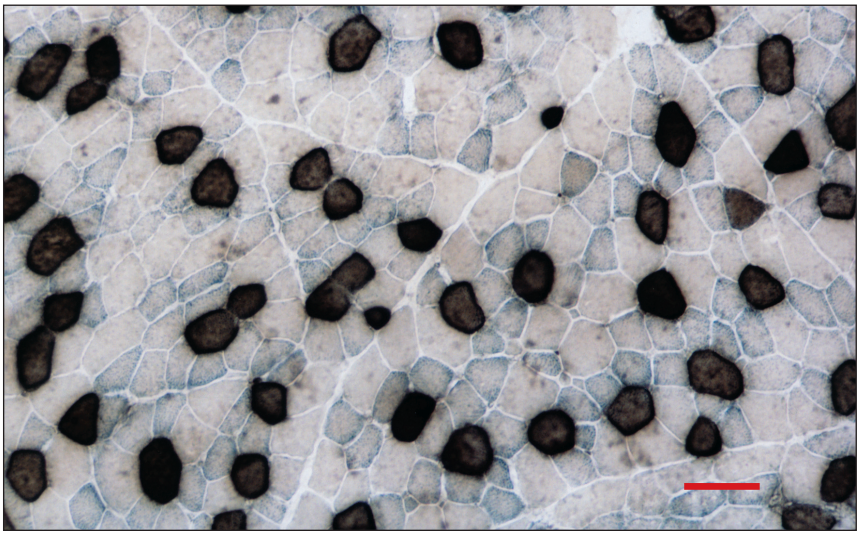

Fig. 4. *M. psoas major* (60 days, females). A light micrograph showing histochemical staining of three types of fibres: slow oxidative, SO (darkest area); fast oxidative glycolytic, FOG (intermediate area) and fast glycolytic FG (lightest area). Muscle transverse section (× 160) treated for m-ATPase + SDH combined reaction after preincubation at pH range 4.35 – 4.40, bar 62.5 µm

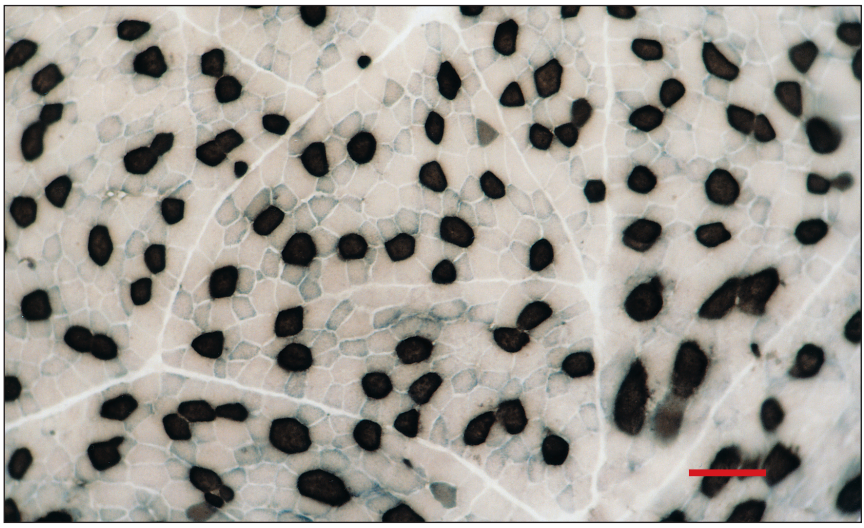

Fig. 5. *Caput longum m. tricipitis brachii* (120 days, males). A light micrograph showing histochemical staining of three types of fibres: slow oxidative, SO (darkest area); fast oxidative glycolytic, FOG (intermediate area) and fast glycolytic FG (lightest area). Muscle transverse section (× 100) treated for m-ATPase + SDH combined reaction after preincubation at pH range 4.35 – 4.40, bar 100 μm

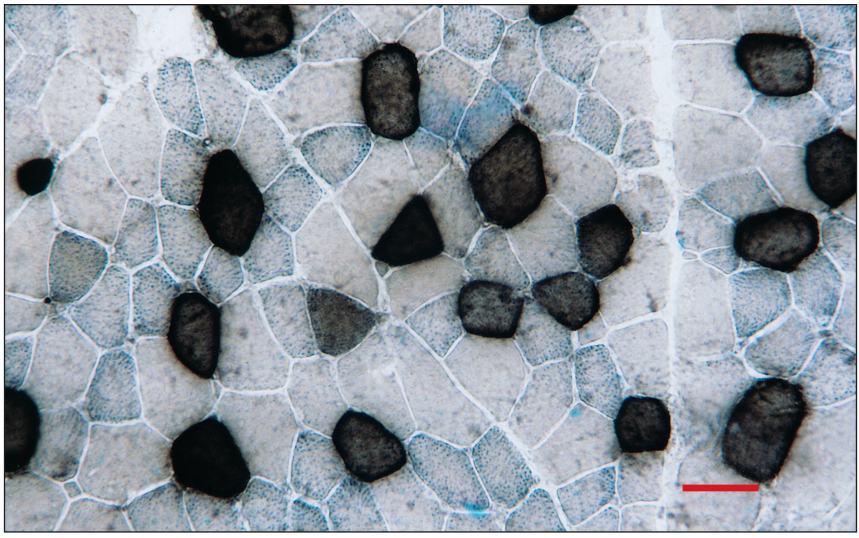

Fig. 6. *Caput longum m. tricipitis brachii* (120 days, females). A light micrograph showing histochemical staining of three types of fibres: slow oxidative, SO (darkest area); fast oxidative glycolytic, FOG (intermediate area) and fast glycolytic FG (lightest area). Muscle transverse section (× 250) treated for m-ATPase + SDH combined reaction after preincubation at pH range 4.35 – 4.40, bar 40 μm

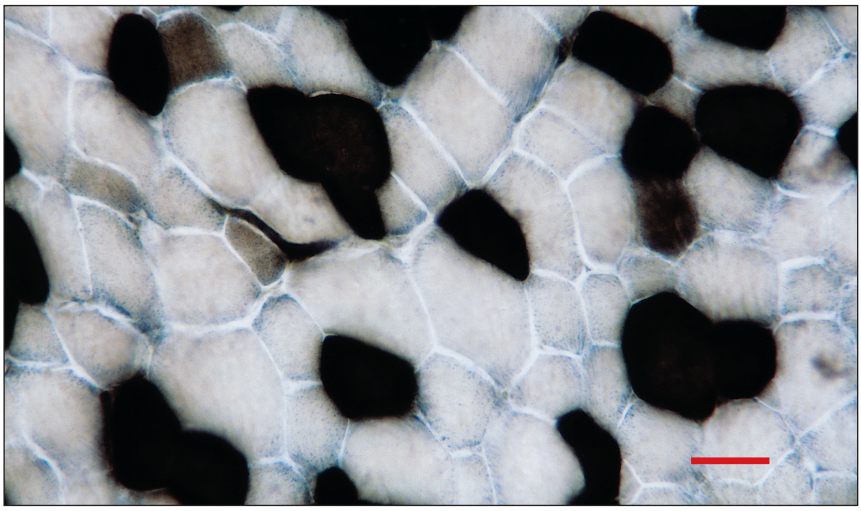

Fig. 7, *M. psoas major* (120 days, males). A light micrograph showing histochemical staining of three types of fibres: slow oxidative, SO (darkest area); fast oxidative glycolytic, FOG (intermediate area) and fast glycolytic FG (lightest area). Muscle transverse section (× 250) treated for m-ATPase + SDH combined reaction after preincubation at pH range 4.35 – 4.40, bar 40 µm

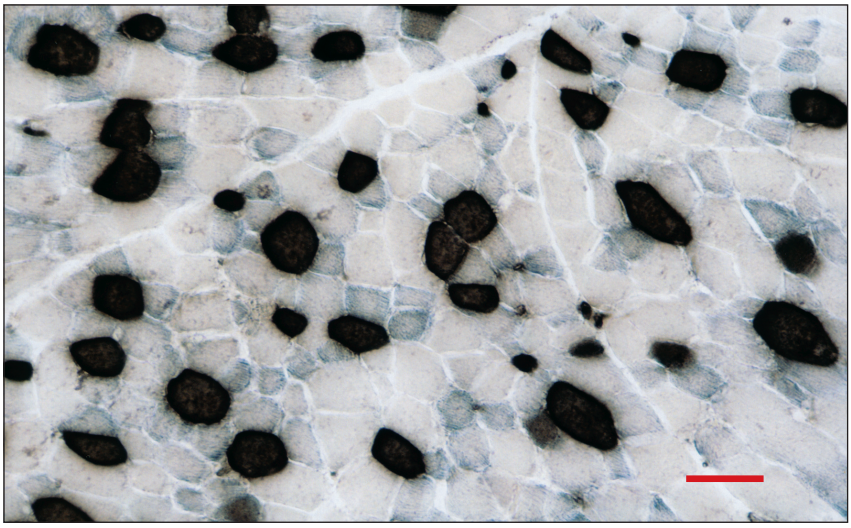

Fig. 8. *M*. p*soas major* (120 days, females). A light micrograph showing histochemical staining of three types of fibres: slow oxidative, SO (darkest area); fast oxidative glycolytic, FOG (intermediate area) and fast glycolytic FG (lightest area). Muscle transverse section (× 160) treated for m-ATPase + SDH combined reaction after preincubation at pH range 4.35 – 4.40, bar 62.5 µm