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Histochemical muscle fibre characteristics of German Heath lamb meat

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Abstract

Histochemical characteristics of muscle were evaluated in 12 German Heath lambs (6 males and 6 females). The lambs were slaughtered at the age of 150 days and samples of musculus longissimus lumborum et thoracis and quadriceps femoris were collected and measurements of fibre cross sectional area (μm^2), diameter (μm^2), and perimeter (μm^2) were performed. Subsequently the fibre type distribution (%) was calculated. The dataset was evaluated using SAS 9.3. Significantly higher ($P < 0.05$) cross sectional area, diameter, and perimeter attributes were observed in females compared to males. Significant differences ($P < 0.05$ to 0.01) were observed in fibre type distribution between musculus longissimus lumborum et thoracis and quadriceps femoris muscle. The presented study describes evaluation of morphological formation of skeletal muscle tissue performed on a sheep breed of German origin. The results may be useful for meat scientists or meat industry due to close connection of these characteristics to meat quality.

Cross sectional area, fibre type distribution, musculus longissimus lumborum et thoracis, quadriceps femoris muscle

The German Heath is an original coarse-wool German sheep breed. The total number of German Heath reached 300 ewes in 2013, according to the performance recording system in the Czech Republic (Bucek et al. 2014). This sheep breed is characterized by a specific taste of meat that resembles venison and is low in fat. Vertebrate skeletal muscles are made up of bundles of muscle fibres that are peculiar to their morphological characters, contractile and metabolic properties. The muscle fibre is a major determinant factor of muscle mass because it occupies 75–90% of the total muscle volume (Lee et al. 2010). Myofibres can be defined by cross-sectional area, length attributes or total number of muscle fibres (Lefaucheur 2010). Brooke and Kaiser (1970) described three different skeletal muscle types (I, IIA and IIB fibres) based on differences in myosin ATPase and NADH tetrazolium reductase activity (Suzuki and Tamate 1988) after pH preincubations. The muscle fibre types are influenced by feed ration (De Marzo et al. 2012), body part (Daniel et al. 2007), sex or genotype (Wegner et al. 2000) as previously reported in sheep or other different livestock. Moreover the muscle fibre characteristics have practical importance to meat scientists, breeders, and the meat industry to provide a better understanding of the involvement of muscle fibres with regard to the determination of muscle growth and final meat quality traits such as tenderness, water holding capacity, juiciness or fat content (Hawkins et al. 1985; Čandek-Potokar et al. 1999; Rehfeld et al. 2000; Wegner et al. 2000). As noted by Petracci et al. (2013) higher cross-sectional fibre area is coupled with a significantly higher ($P \leq 0.001$) incidence of chicken meat abnormalities.

There is practically no information about lamb meat characteristics of German Heath sheep. The population of German Heath sheep has a minority distribution also from the global viewpoint and there are not many studies focused on this rustic sheep breed.

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Therefore the aim of this study was the detailed analysis of German Heath lamb meat with regard to determining the effects of sex and body part on meat properties.

Materials and Methods

Flock management

The study was performed on a selected sheep farm in the south border of the Usti nad Labem region. The farm was situated at the altitude of 275 m above the sea level, with the average annual rainfall of 700 to 800 mm per year and average annual temperature of 8.0 °C. The animals were kept extensively year-round outdoor using natural shelters only. The feed ration during the grazing season (April to September) consisted of grassland pasture and hay *(ad libitum)* only. There was no flushing applied before the mating season. In the non-grazing season, the ewes' feed ration consisted entirely of hay (*ad libitum)*. The lamb's feed ration consisted of ewe's milk, grassland pasture, and hay. There was no concentrate supply either for mothers or their lambs. All the animals had permanent *ad libitum* access to drinking water and mineral licks.

Experimental animals and sampling

A total of 12 German Heath lambs (males, n = 6; females, n = 6) were purposely selected from a basic flock of 230 ewes. All the lambs – born and reared singles – came from different mothers at the productive age of 2 to 4 years. The lambs were born from April 1 till April 13 and their birth weight was 2.0 ± 0.3 kg. They were naturally reared together with the flock. At the age of 150 days the lambs were slaughtered in compliance with the EU laws. Procedures were conducted according to the guidelines of the Council Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. After slaughter the carcass weight (CW) was evaluated. At the same time the samples (5 × 5 × 15 mm) of musculus longissimus lumborum et thoracis (MLLT; loin eye area) and quadriceps femoris muscle (QFM; tight area) were collected for subsequent histochemical analyses.

Histochemical analysis

Samples were frozen in 2-methylbutane cooled by liquid nitrogen (–156 °C) and then stored at –80 °C until analysis. Cross-sections (12 µm) were cut with a cryostat Leica CM1850 (Leica Microsystems Nussloch GmbH, Nussloch, Germany) at –20 °C. Subsequently, staining for myofibrillar ATPase was performed after preincubation in alkaline buffer according to methodology by Brooke and Kaiser (1970). The types of muscle fibres were classified as type I (slow-twitch oxidative, slow oxidative, beta red, or red fibres), type IIA (fast-twitch oxidative, fast oxidative glycolytic, alpha red, or intermediate fibres), or as type IIB (fast-twitch glycolytic, fast glycolytic, alpha white, or white fibres) according to the nomenclature of previous authors (Plate I, Fig. 1). Characteristics of muscle fibres (cross sectional area – CSA, diameter, perimeter) were determined using NIS Elements AR software (Version 3.2, Nikon, Tokyo, Japan, 2006). Subsequently the fibre type distribution (FTD; %) was calculated.

Statistical analysis

Statistical analysis was carried out using the statistical programme SAS version 9.3 (SAS/STAT® 9.3., SAS Institute Inc., NC, USA, 2011). The correlation coefficients were expressed by using CORR procedure. Detailed evaluation was performed by GLM procedure (ANOVA). The fixed effects in model equation were the sex of lambs (2 classes – males and females) and sampling area (2 classes – MLLT muscle fibre and QFM muscle fibre). Regression on the carcass weight was taken into account. Interaction of sex and sampling area was also counted but finally it was excluded from the trial due to its non-significance in the model equation. Tukey-Kramer method was applied for comparison and evaluation the significance of differences between least squares means. Significance levels $P < 0.05$, $P < 0.01$, and $P < 0.001$ were used to evaluate the differences between groups.

Results

The correlation coefficients among selected meat attributes are presented in Table 1. The carcass weight was correlated with CSA attributes (r = 0.509 to r = 0.582) or FTD attributes (r = -0.462 to r = 0.360). Significant correlations (r = 0.802 to r = 0.989; $P < 0.001$) were observed among CSA of respective muscle types one another. Significantly positive correlations (r = 0.427 to r = 0.558; $P < 0.05$ to 0.001) were observed among type I muscle fibres in CSA and FTD attributes while negatively correlations among CSA and FTD attributes in both IIA and IIB types were observed (r = -0.263; $P > 0.05$ to r = -0.506; $P < 0.001$). Negative correlations (r = -0.075; $P > 0.05$ to r = -0.850; $P > 0.001$) were also observed in FTD of all muscle fibre types, mutually.

The basic statistical characteristics of dataset are presented in Table 2. The highest values of CSA (mean = 1133.38 µm^2; SD = 374.62 µm^2), diameter (mean = 36.49 µm;

Table 1. Correlation analysis of selected muscle fibre characteristics.

		CSA – total	CSA – I	CSA – IIA	CSA – IIB	FTD – I	FTD – IIA	FTD – IIB
CW	r	0.539	0.582	0.509	0.520	0.360	0.097	-0.462
	P	0.008	0.004	0.012	0.011	0.091	0.661	0.026
CSA – total	r		0.849	0.973	0.989	0.558	-0.506	-0.326
	P		< 0.001	< 0.001	< 0.001	0.006	0.014	0.127
CSA – I	r			0.828	0.802	0.427	-0.366	-0.263
	P			< 0.001	< 0.001	0.042	0.086	0.225
CSA – IIA	r				0.956	0.512	-0.489	-0.286
	P				< 0.001	0.012	0.018	0.187
CSA – IIB	r					0.477	-0.449	-0.270
	P					0.021	0.032	0.213
FTD – I	r						-0.641	-0.850
	P						0.027	< 0.001
FTD – IIA	r							-0.075
	P							0.733

CW - carcass weight; CSA – total - cross sectional area of all muscle fibres (μm^2); CSA – I - cross sectional area of type I muscle fibre (μm^2); CSA IIA - cross sectional area of type IIA muscle fibre (μm^2); CSA – IIB - cross sectional area of type IIB muscle fibre (μm^2); FTD – I - fibre type distribution of type I muscle fibre (%); FTD – IIA - fibre type distribution of type IIA muscle fibre (%); FTD – IIB - fibre type distribution of type IIB muscle fibre (%).

Table 2. Basic statistical characteristics of dataset.

Variable	n	Means	SD	CV	Min	Max
CSA – total	24	894.22	395.16	44.19	362.71	1868.02
CSA – I	24	1133.38	374.62	33.05	431.39	2028.50
CSA – IIA	24	716.44	295.00	41.18	331.29	1469.32
CSA – IIB	24	883.56	404.55	45.79	342.79	1940.75
Diameter – I	24	36.49	6.32	17.32	21.67	49.93
Diameter – IIA	24	28.75	5.92	20.60	19.56	42.55
Diameter – IIB	24	31.13	6.72	21.59	19.71	46.15
Perimeter – I	24	128.76	22.58	17.54	78.61	179.04
Perimeter – IIA	24	110.56	40.20	36.36	68.08	236.46
Perimeter – IIB	24	112.81	41.73	36.99	11.55	243.34
FTD – I	24	18.35	13.29	72.43	3.24	57.32
FTD – IIA	24	20.03	7.02	35.04	3.88	31.98
FTD – IIB	24	61.62	11.82	19.19	28.05	76.44

CSA – total = total cross sectional area regardless of muscle fibre type (μm^2); CSA – I = cross sectional area of type I muscle fibres (μm^2); CSA – IIA = cross sectional area of type IIA muscle fibres (μm^2); CSA – IIB = cross sectional area of type IIB muscle fibres (μm^2); Diameter – I = diameter of type I muscle fibre (μm); Diameter – IIA = diameter of type IIA muscle fibre (μm); Diameter – IIB = diameter of type IIB muscle fibre (μm); Perimeter – I = perimeter of type I muscle fibre (μm); Perimeter – IIA = perimeter of type IIA muscle fibre (μm); Perimeter – IIB = perimeter of type IIB muscle fibre (μm); FTD – I = fibre type distribution of type I muscle fibre (%); FTD – IIA = fibre type distribution of type IIA muscle fibre (%); FTD – IIB = fibre type distribution of type IIB muscle fibre (%); n = number of observation; Min = the lowest value of the muscle fibre histochemical characteristic; Max = the highest value of the muscle fibre histochemical characteristic; Means = average mean of the muscle fibre histochemical characteristic; SD = standard deviation; CV = coefficient of variance.

Table 3. Effect of sex of lambs and sample collection area on histochemical muscle fibre characteristics.

		CSA (µm²)				Diameter (µm²)			Perimeter (µm²)			FTD (%)		
		total	I	IIA	IIB	I	IIA	IIB	I	IIA	IIB	I	IIA	IIB
Sex	Males	717.72^{a}	978.12^{a}	578.38^{a}	680.82^{a}	33.56^{a}	25.74^{a}	27.62^{a}	118.39^{a}	91.28	92.28^{a}	18.99	21.27	59.74
	Females	1049.04^{b}	1264.31^{b}	839.89^{b}	1065.61^{b}	38.93^{b}	31.41^{b}	34.25^{b}	136.88^{b}	127.68	130.37^{b}	17.00	19.23	63.77
	RMSE	94.54	87.69	72.20	97.42	1.39	1.44	1.52	4.85	11.58	10.35	2.34	1.95	2.71
Muscle	MLLT	785.04	1035.63	647.90	805.11	34.05	27.20	29.37	121.46	102.10	103.78	8.12^{A}	24.02^{a}	67.86^{A}
	QFM	981.72	1206.80	770.36	941.33	37.94	29.95	32.50	134.35	116.85	118.87	27.48^{B}	16.48^{b}	55.65^{B}
	RMSE	89.63	83.14	68.46	92.37	1.32	1.36	1.44	4.60	10.98	9.81	2.22	1.85	2.57
Significance														
Sex		0.034	0.047	0.029	0.019	0.021	0.019	0.010	0.026	0.055	0.027	0.586	0.504	0.344
Muscle		0.148	0.173	0.233	0.323	0.093	0.183	0.152	0.069	0.367	0.303	< 0.0001	0.012	0.004
CW		0.080	0.041	0.125	0.122	0.042	0.173	0.090	0.023	0.809	0.174	0.014	0.425	0.009

RMSE = root mean square error; CSA – cross sectional area; FTD – fibre type distribution; I - slow-twitch oxidative; IIA - fast-twitch oxidative-glycolytic; IIB - fast-twitch glycolytic; MLLT - musculus longissimus lumborum et thoracis (loin eye area); QFM - quadriceps femoris muscle (tight area); CW - carcass weight; a-b or A-B. C-D different letters in rows means significant differences $P < 0.05$ or $P < 0.01$.

SD = 6.32 µm) and perimeter (mean = 128.76 µm; SD = 22.58 µm) attributes were observed in type I muscle fibres. The highest FTD attribute was marked in type IIB muscle fibres (mean = 61.62%; SD = 11.82%). Detailed evaluation of histochemical muscle fibre characteristics performed by ANOVA test is presented in Table 3. Differences in the sex of lambs were observed in CSA, diameter and perimeter attributes. All these attributes were higher in females compared to males ($P < 0.05$). Significantly lower FTD of type I (-1.99%; $P > 0.05$) and type IIA (-2.04%; $P > 0.05$) were observed in females while non-significantly higher FTD of IIB type muscle was observed in females as well. Non-significantly higher values of CSA, diameter and perimeter attributes were marked in QFM compared to MLLT muscle. Significantly higher values of FTD-I resp. FTD-IIA (+19.36%; $P < 0.01$, resp. +7.54%; $P < 0.05$) were detected in QFM while the higher FTD-IIB muscle fibre type (+12.19%; $P < 0.01$) was marked in MLLT muscle.

Discussion

The feed ration influenced the histochemical characteristics of Delle Langhe lambs as previously published by De Marzo et al. (2012). All the lambs evaluated in the study originated from the same flock and were kept under same flock management and breeding conditions; effects of flock, grazing pasture quality or natural conditions were thus eliminated.

The study of Čandek-Potokar et al. (1999) evaluated relationships of CSA and FTD attributes of the longissimus dorsi muscle fibre. According to their results, FTD both in I and IIA muscle fibres were negatively correlated to FTD in IIB fibres (r = -0.70 and -0.66, respectively). These results largely corresponded to ours. Oppositely significant negative correlation between FTD in I and IIA observed in our results indicated that fewer type I fibres were compensated for by an increase of IIA type fibres. The non-significant relationships between CSA and FTD attributes detected in the meat of four different crossbred pigs presented by Borosky et al. (2010) were also observed in our study.

Peinando et al. (2004) described in detail the histochemical indicators of skeletal muscle fibres in Segurena lambs. The value for the diameter of MLLT muscle ranged between 10.45 and 25.20 µm in type I fibre; between 5.62 and 13.30 µm in IIA and between 6.59 and 21.65 µm in IIB type fibres. They noted that type I muscle fibre represented 8.57 to 10.61%; IIA muscle fibre 30.23 to 48.53% and IIB muscle fibre 41.49 to 60.84% of distribution of particular fibre types in lambs at the age from one day to 90 days. These results are in partial accordance with ours as detected in German Heath lambs where different figures but similar proportions of fibre type distribution were found. Differences in partial values could be explained mainly by different genotype, age of lambs, feed ration or environment. On the other hand similar proportion of fibre type distribution compared to previous studies confirmed similar physiological development in different breeds of sheep.

As also published by Wojtysiak et al. (2010) the sex of Polish Longwool lambs significantly influenced the diameter of muscle fibres: males had larger diameter of type IIB and IIA fibres than females. Velotto et al. (2010, 2005) confirmed the effect of sex on the muscle fibre characteristics of Laticuada or Italian Merino lambs. According to their results males had higher ($P < 0.001$) CSA, perimeter and diameter attributes of muscle fibres compared to females. On the other hand, no effect of the sex of lambs on all the types of FTD muscle fibres in MLLT was noticed in the study published by Wojtysiak et al. (2010). Sex differences on muscle fibre characteristics were affirmed also in other species (e.g. pigs or cattle) by Ozawa et al. (2000), Klont et al. (1998) or Johnston et al. (1981). Our results also confirmed significant differences between males and females in the CSA, perimeter and diameter attributes in the German Heath sheep breed, but in a reversed order. Higher dimension of muscle fibre characteristics in females could be connected with their muscle conformation. As an example, non-significantly higher MLLT depth at the age of 100 days was observed in Texel sheep females compared to males, despite the higher live weight of males (Štolc et al. 2011).

Differences among muscle fibres were confirmed by Daniel et al. (2007) who evaluated muscle fibre characteristics of different body parts in (Swaledale × Leicester Blue Face) × Charollais lambs slaughtered at the age of 24 weeks. They found that the IIB muscle fibres were in a majority and had the largest diameters ($P < 0.01$) in MLLT as well as in the semitendinosus muscle. Differences among muscle fibres between two body parts (m. psoas major – PM – and caput longum m. tricipitis brachii – ClTb) in lambs were detected by Velotto et al. (2005, 2003). As they presented the CSA, perimeter and diameter dimensions of I, IIA and IIB muscle fibres were higher in ClTb muscle compared to PM at the age of 60 and 120 days. The differences between MLLT and QFM in the German Heath lambs were marked also in our study. On the contrary, Velotto et al. (2005, 2003) noted that significant differences ($P < 0.05$ to 0.01) were obvious in fibre type distribution attribute only. It is possible to assume that in rustic breeds the muscle fibres of two valuable carcass parts have a similar dimension regardless of the body part but a different distribution of individual muscle fibres.

The quality and sensorial characteristics of meat are influenced mainly by the dimension of muscle fibres or by the distribution of IIB in muscle fibres as explained by Čandek-Potokar et al. (1999).

From the practical point of view, these muscle fibre attributes are closely connected to qualitative and sensory properties of meat such as fattiness (Hawkins et al. 1985), water holding capacity, tenderness (Rehfeld et al. 2000) or meat juiciness (Čandek-Potokar et al. 1999).

This study presents results obtained in an indigenous German sheep breed ranked among the national genetic resources in Germany. Practically no data about the meat characteristics of this breed were published before. Therefore our results, partially in conformity with the findings of other authors provide new information.

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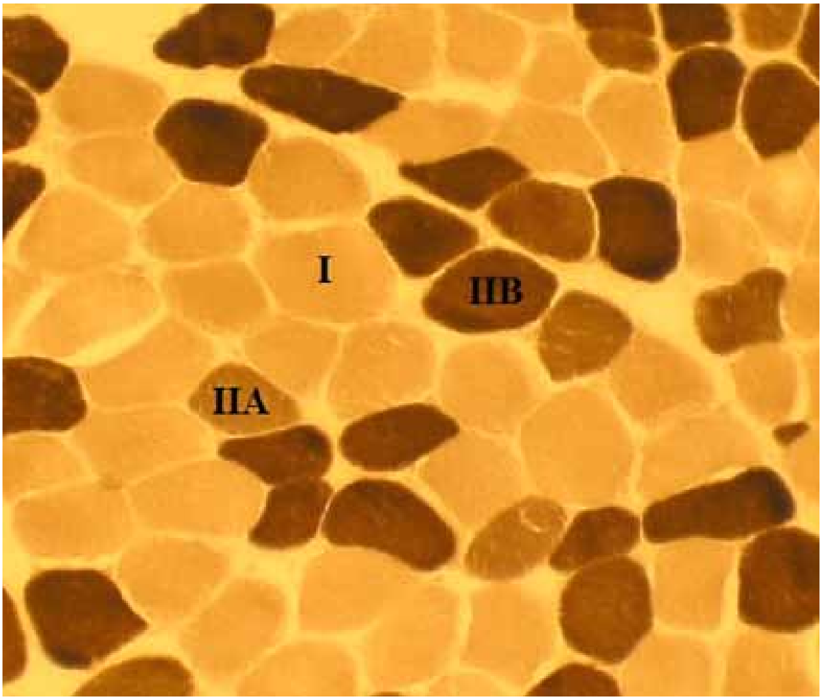

Fig. 1. Muscle tissue fibres after preincubation in alkaline buffer

I - slow-twitch oxidative muscle fibre; IIA - fast-twitch oxidative muscle fibre; IIB - fast-twitch glycolytic muscle fibre (Brooke and Kaiser 1970)