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Histochemical profile of two parts of *longissimus dorsi* muscle in relation to sex of fatteners

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In six castrated males and six gilts, crossbreds [female (femalePolish Landrace × Polish Large White male) × Line 990 male)], different parts of *longissimus dorsi* (LD) muscle were examined histochemically in relation to sex of fatteners. Tissues for histochemical analysis were obtained from *m. longissimus thoracis* (LT) on the level of 6th thoracic vertebra and from *m. longissimus lumborum* (LL) on the level of 5th lumbar vertebra. Muscle fibres were cathegorized according to their enzymatic activity (types I, IIA, and IIB). Muscle fibre per cent and diameter were measured and correlation coeficients estimated between fibre traits. The results showed no significant differences in fibre types share between LT and LL. On the other hand, independently of sex of animals, larger diameter characterized muscle fibre types of LL compared to LT. Moreover, sex had no effect on fibre type percentages, but affected the fibre type diameter of all examined LD fibre types. Phenotypic correlations between fibre type percentages were negative, in contrast to positive correlations between fibre type diameters.

KEY WORDS: muscle fibres / histochemistry / *m. longissimus* / pigs

Mammalian skeletal muscles contain fibres with a broad spectrum of physiological and biochemical properties, namely slow-twitch oxidative, fast-twitch oxidative glycolytic and fast-twitch glycolytic fibres [Peter *et al*. 1972]. Brooke and Kaiser [1970] using histochemical methods described these fibre types as type I (red), IIA (intermediate) and IIB (white), and this classification can be applied to all mammalian muscles. Different muscles contain different proportion and composition of fibre types and these determine the function of the muscle. *Longissimus dorsi* (LD) is the most frequently used reference muscle in meat quality studies in pigs. This unipennate muscle is located on the dorso-lateral plane of the vertebral column throughout the thoracic (*m. longissimus thoracis*) and lumbar (*m. longissimus lumborum*) region of LD. Some fibre characteristics are mostly determined genetically, while others are affected mainly by external factors such as animal's age and sex [Larzul *et al.* 1997], its physical activity, nutrition or intensive selection [Brocks *et al.* 1998, Kłosowska and Fiedler 2003]. Few studies suggest that part of variation in fibre type characteristics and metabolic potentials within muscle may explain part of the variation in some meat quality characteristics. There are well known correlations between the distribution of fibre types and meat quality, or the effect of fibre type per cent and fibre diameter on meat tenderness, juiciness and colour [Cameron *et al.* 1998, Oksbjerg *et al.* 2000]. The proportions of fibre types vary between animals and between muscles, depending on their function. Additionally, several histochemical studies of LD in cattle [Morita *et al.* 2000], pig [Iwamoto *et al.* 1989] and rabbit [Vigeneron *et al.* 1976] showed regional differences to exist in fibre type composition.

Therefore the aim of this study was to determine muscle fibres composition in different parts of *m. longissimus* in relation to sex of pigs.

Material and methods

The histochemical examination was performed on six castrated males and six gilts, crossbreeds [female (female Polish Landrace × Polish Large White male) × Line 990 male)]. Animals were from the same litter and were slaughtered at 105-kg body weight. Muscle samples about 2 cm thick were taken within 20 min post-slaughter, cooled in liquid nitrogen and stored at -80°C until histochemical analyses were performed. Samples (two from each animal) were taken from the right carcass-side from *m. longissimus thoracis* (LT) on the level of 6th thoracic vertebra and from the *m. longissimus lumborum* (LL) on the level of 5th lumbar vertebra, deep within the muscle. Transverse sections 10 μm thick were cut at -20°C in a cryostat (Slee MEV, Germany). The activity of dehydrogenase $NADH_2$ (diaphorase) was detected using specific histochemical tests [Dubovitz *et al.* 1973] for distinguishing muscle fibre types: I – red fibres of very high enzymatic activity, IIA – intermediate or medium activity fibres, and IIB – white fibres of low enzymatic activity. The incubation medium contained nicotinamide adenine dinucleotide ($NADH_2$) and nitro blue tetrazolium (NBT). The per cents and diameter of muscle fibre types were quantified with an image analysis system (Multi Scan Base 98). A minimum of 300 fibres were examined from each cross-section. Moreover, correlation coefficients (r) between fibre traits were estimated. The data were evaluated with the analysis of variance and presented as means and their standard errors (SE).

Results and discussion

Microstructure of LT and LL parts of LD muscle in castrated males and gilts is illustrated in Photos 1 and 2. In all sections a typical pattern of labelling was observed, with the type I fibres being surrounded by type IIA and these then being surrounded by type IIB fibres in a fibre cluster pattern. No change in this localization pattern or the intensity of the staining was seen, independently of sex of animals, in both LD parts examined.

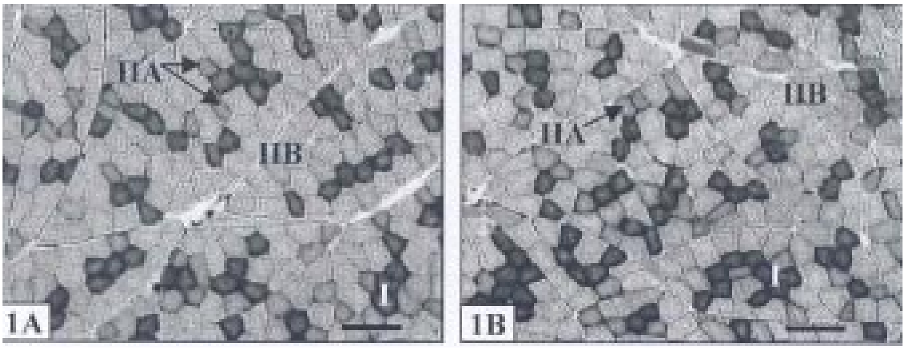

Photo 1. Cross section of *m. longissimus thoracis* in castrated males – 1A and gilts – 1B. Diaphorase activity reaction: I – red fibres, IIA – intermediate fibres, IIB – white fibres. Scale bar 200 µm.

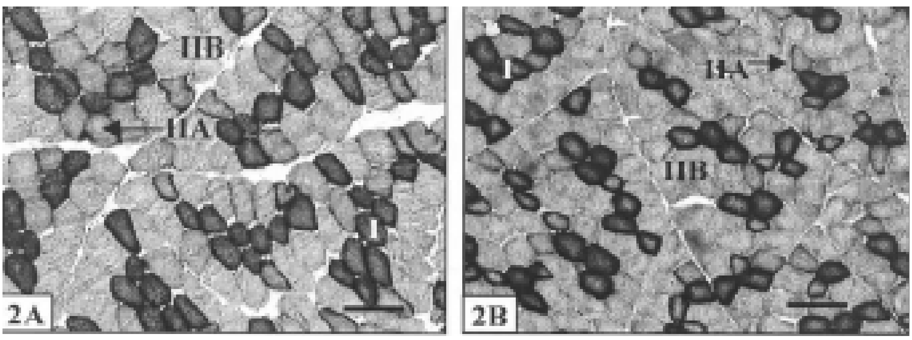

Photo 2. Cross section of *m. longissimus lumborum* in castrated males – 2A and gilts – 2B. Diaphorase activity reaction: I – red fibres, IIA – intermediate fibres, IIB – white fibres. Scale bar 200 µm.

The percentage and diameter of fibre types are presented in Table 1. Between the two LD parts examined no significant differences in the per cent of fibre types were found. Likewise sex had no effect on fibre type per cent. However, in castrated males as well as in gilts, a tendency of declining number of IIB, and increasing number of type I fibres was observed in LL as compared to LT muscle. The fact that sex has no

Table 1. Percentage and diameter of red (I), intermadiate (IIA) and white (IIB) muscle fibres of *m. longissimus thoracis* and *m. longissimus lumborum* in gilts and castrated males (means± SE)

Fibre type	Castrated males				Gilts			
	m. longissimus thoracis		*m. longissimus lumborum*		*m. longissimus thoracis*		*m. longissimus lumborum*	
	mean	SE	mean	SE	mean	SE	mean	SE
	Per cent of muscle fibres							
I	18.66	1.56	22.18	3.52	19.84	1.52	21.81	2.65
IIA	11.97	1.26	14.29	3.43	15.66	1.24	15.83	1.83
IIB	69.37	2.19	63.53	5.59	64.50	2.22	62.36	3.91
	Diameter of muscle fibres (µm)							
I	52.14^{ax}	1.94	78.11^{bx}	3.15	52.77^{ax}	1.77	62.91^{by}	2.06
IIA	59.31^{ax}	2.14	93.45^{bx}	3.29	61.64^{ax}	2.01	69.25^{by}	1.59
IIB	72.64^{ax}	1.81	100.67^{bx}	3.09	68.72^{ax}	1.65	78.99^{by}	2.22

^{ab}Within sexes means for LT and LL bearing different superscripts differ significantly at $P \leq 0.05$.

^{xy}Within muscles means for sexes bearing different superscripts differ significantly at $P \leq 0.05$.

effect on fibre type composition confirms the earlier results of Larzul *et al.* [1997], and Wojtysiak *et al.* [2003]. On the other hand, in the present study, independently of sex of animals, larger diameter of muscle fibres of LL than in LT can probably be interpreted on the basis of different behavioral and biological functions of these muscles. Moreover, males had larger diameter of all examined muscle fibre types than females, but only in LL muscle. Differences in fibre types diameter in LL appear similar to those reported earlier by Wojtysiak *et al.* [2003]. Similarly, Kłosowska and Kłosowski [1975] observed the greater muscle fibre type diameter in castrated males than in gilts. On the other hand, these results still remain open to criticism, as some previous studies did not report any significant inter-sex differences in fibre type diameter [Sośnicki 1987], whereas others observed the opposite [Larzul *et al.* 1997]. Additionally, Larzul *et al.* [1997] suggested that larger fibre cross-sectional area in gilts could be biologically meaningful in that females exhibit a greater loin eye area and higher lean per cent of carcass. It is also possible, that variation in the fibre size between castrated males and gilts was affected by various examined parts of LD muscle. This is confirmed by the present study, where in the LT, in contrast to LM, any significant differences were not found. On the other hand, some fibre characteristics are determined genetically [Brocks *et al.* 1998, Kłosowska and Fiedler 2003]. Moreover, other studies have demonstrated that muscle fibre composition and size is specific for different pig breeds or lines [Karlsson *et al.* 1993, Larzul *et al.* 1997]. Ruusunen and Puolanne [1997] found that the muscles of Hampshire are more oxidative than those of Landrace or Yorkshire pigs. Thus, the presence (or lack) of differences in fibre type size can be interpreted not only as sex, but as breed effect.

Additionally, variation in fibre type diameter between LT and LL as well as inter-sex differences reported here may help explaining some of the variation in their mechanical properties [Morrison *et al.* 1998]. Moreover, it has been reported that fibre type composition can also affect meat eating quality – tenderness, juiciness, drip loss, intramuscular fat and colour [Warriss *et al.* 1990, Larzul *et al.* 1997, Oksbjerg *et al.* 2000]. Cameron *at al.* [1998] suggests that diameter of white fibres, contrary to that of red fibres, positively correlates with tenderness and negatively with juiciness of porcine meat. According to Oksbjerg *et al.* [2000] an increase in the diameter of intermediate fibres can be related to higher daily gain. Moreover, meat colour is a major factor limiting the quality and acceptability of meat and meat products. The proper colour of meat can be conditioned by the ferrous oxymioglobin (oxyMb) – Philips *et al.* [2001] – what is directly related to the per cent and diameter of the muscle fibre types [Warriss *et al.* 1990]. Therefore, differences in diameter of the muscle fibre between castrated males and gilts, and between LT and LL muscle observed in the present study, can also affect the meat colour and eating quality.

Table 2. Phenotypic correlation coefficients between muscle fibre characteristics: I – red fibres, IIA – intermediate fibres, IIB – white fibres

Fibre type	Per cent		Diameter		
	IIA	IIB	I	IIA	IIB
I (%)	-0.092*	-0.788**	ns	ns	ns
IIA (%)		-0.730**	ns	ns	ns
IIB (%)			ns	ns	ns
Diameter I				0.912***	0.872***
Diameter IIA					0.956***

*P≤0.05; **P≤0.01; ***P≤0.001.

Phenotypic correlations between fibre type percentages were found significant and negative (Tab. 2), similarly to the results by Larzul *et al.* [1997] who found significant negative correlations between all fibre types. Additionally, correlations between fibre type diameters were all positive and highly significant, corroborating the data of Larzul *et al*. [1997] and Dietl *et al.* [2000]. On the other hand, correlations between fibre per cent and fibre diameter were not found significant. In contrast, the earlier studies by Larzul *et al.* [1997] and Dietl *et al.* [2000] showed low correlations between fibre percentages and fibre cross-sectional areas.

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Profil histochemiczny *m. longissimus thoracis* i *longissimus lumborum* tuczników obu płci

S t r e s z c z e n i e

Na 6 wieprzkach i 6 loszkach mieszańcach [samice (samica pbz × samce wbp) × samce 990] przeprowadzono badania histochemiczne polegające na określeniu kompozycji włókien mięśniowych w różnych częściach *m. longissimus*, w zależności od płci tuczników. Fragmenty mięśni do analizy histochemicznej pobierano z *m. longissimus thoracis* (LT) na wysokości szóstego kręgu piersiowego oraz z *m. longissimus lumborum* (LL) na wysokości piątego kręgu lędźwiowego. Typy włókien mięśniowych określano na podstawie ich aktywności enzymatycznej (I, IIA, IIB). Badano udział (%) i średnicę włókien mięśniowych, a także obliczono współczynnik korelacji dla zależności między poszczególnymi cechami włókien mięśniowych. Wykazano brak istotnych różnic w kompozycji włókien mięśniowych między LT a LL. Dodatkowo, niezależnie od płci badanych zwierząt, większą średnicą charakteryzowały się włókna mięśniowe LL niż LT. Stwierdzono także, że płeć nie ma wpływu na udział (%) poszczególnych typów włókien mięśniowych, natomiast w LL wpływa istotnie na średnicę wszystkich analizowanych typów włókien. Współczynniki korelacji między udziałem (%) włókien mięśniowych okazały się ujemne, podczas gdy zależności dodatnie oszacowano między średnicami włókien mięśniowych.