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Relationships between muscle fibre characteristics and physico-chemical properties of *longissimus lumborum* muscle and growth rate in pig fatteners of three breeds*

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In 40 Polish Landrace, 38 Polish Large White and 14 Pietrain fatteners the *longissimus lumborum* (LL) muscle fibres were characterized and LL quality indicators determined. The correlation was estimated between the LL fibres traits and daily live weight gain, muscle pH and texture parametres. Muscle fibres were identified as I, IIA and IIB types based upon their dehydrogenase (NADH) activity. The daily live weight gain of fatteners and their muscle pH_{45}, pH_{24}, Warner-Bratzler shear force and texture properties were determined.

Breed was found related neither to meat quality traits, nor fibre type per cent and fibre relative area, but it affected the size of fibres. The LL from Pietrain fatteners had larger diameter of fibres within each fibre type compared to fatteners of remaining two breeds ($P \leq 0.01$). The phenotypic correlations between histological and physico-chemical traits were generally low. The content (%) and relative area of type IIB fibres, unlike those of type I, positively correlated with daily live weight gain ($P \leq 0.01$). Moreover, increase in the daily live weight gain was related to increased size of type IIB fibres. Shear force was negatively related to type IIA muscle fibre size. A similar tendency was found between pH_{24} and diameter of type IIA fibres.

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Meat quality can be evaluated objectively by measuring its physico-chemical traits such as pH, water-holding capacity, colour, and instrumental texture. The most important attribute of porcine meat which affects consumer's satisfaction is tenderness. There is now sufficient evidence that many important characteristics of pig meat quality are affected by the proportion and size of skeletal muscle fibre types [Henckel *et al*. 1997, Larzul *et al*. 1997, Brocks *et al*. 1998, Ryu and Kim 2005]. In general, mammalian skeletal muscles contain a heterogeneous population of fibres differing in contraction ability and metabolism, among which the slow-twitch oxidative, fast-twitch oxidative glycolytic and fast-twitch glycolytic fibres are distinguished [Peter *et al*. 1972]. On the other hand, using histochemical methods these fibre types are categorized as I, IIA and IIB [Brooke and Kaiser 1970], or βR (red), and αR and αW (white), respectively [Ashmore *et al*. 1972]. There are many factors that contribute to muscle fibres composition such as animal species and breed, genotype and sex, selection intensity and post-slaughter processing [Ruusunen and Puolanne 1997, Brocks *et al*. 1998, Ryu and Kim 2005, Wojtysiak and Migdał 2006]. Moreover, muscle fibre characteristics, and especially muscle fibre size, are influenced by age/body weight and growth rate of animals [Wojtysiak *et al*. 2005, Ruusunen and Puolanne 2004]. It is well known that muscle fibres number and size as well as percentage of individual fibre types may be associated with *post-mortem* changes in the conversion of muscle to meat and subsequently meat quality. Some recent studies found correlations between muscle fibre characteristics and meat quality traits in pigs [Larzul *et al*. 1997, Ryu and Kim 2005].

It is believed that there is a relationship between the properties of muscle fibres and physico-chemical traits of meat. In light of this the current study aimed at (i) comparing the fibre type distribution and physico-chemical properties of meat in fatteners of three breeds and (ii) to estimate the correlation between the muscle fibre characteristics and daily gain, muscle pH and texture parametres of meat.

Material and methods

Ninety-two fatteners of Polish Landrace (PL, n=40), Polish Large White (PLW, n=38) and Pietrain (P, n=14) were used, fed *at libitum* and slaughtered at a commercial abattoir at the live weight of 100 kg. Growth rate (daily live weight gain) was determined for the period 25-100 kg live weight.

Shortly (15-30 min) after slaughter the carcassess were divided into sides and samples of the right *longissimus lumborum* (LL) muscle for histological analyses were taken at the level of the 5^{th} lumbar vertebra and deep within the muscle. Samples were immediately formed into 1 cm^3 cubes (cutting parallel to the muscle fibres), frozen in isopentane cooled with liquid nitrogen, and stored at -80ºC. Frozen samples were mounted on a cryostat chuck with a few drops of tissue-freezing medium (TISSUE-TEC. SAKURA FINETEK Europe, Zoeterwoude, The Netherlands). In order to classify the muscle fibres into type I, type IIA and type IIB, 10 µm slides were made at -25ºC in a cryostat (SLEE MEV, Germany) and stained for dehydrogenase (NADH) activity according to Dubovitz *et al*. [1973]. The percentage and diameter of muscle fibres of individual types were quantified with an image analysis system using a computer programme MULTI SCAN v.14.02. Additionally, the relative area (RA) occupied by fibres of each type was derived from the corresponding numerical percentages and mean diameter. A minimum of 300 fibres were considered from each slide.

The pH was measured in the LL muscle between the 3-rd and 4-th lumbar vertebra 45 min (pH_{45}) and 24 h (pH_{24}) *post-mortem* using a MATTHÄUS, pH-meter with a glass electrode standardized for pH 4.01 and 7.0 according to Polish Standard PN-77/a-82058. The pH-meter automatically corrected pH values, taking into account the muscle temperature.

Meat samples for texture analysis were taken from right carcass side at the level of the 2-nd and 4-th lumbar vertebra after 24 h carcass cooling at 4°C. The chops for Warner-Bratzler (WB) shear force and texture profile analysis (TPA) were roasted at 180°C to reach an internal temperature of 78°C and then cooled to room temperature. Next, ten 14 mm in diameter cores were taken from each chop parallely to the muscle fibre orientation. Shear force was measured using a Texture Analyser TA-XT2 (STABLE-MICRO SYSTEMS, UK) with Warner-Bratlzer unit and with a triangular blade. The cores for texture profile (TPA) determination were doubly compressed by a cylinder (SMS P/25, base diameter 50 mm) to 70% of their height, at the rate of 2 mm/s and with a 3 s break between the storage of compression. In all cases measurement was carried out at 6±1°C. The following parametres were defined: hardness, springiness, cohesiveness, chewiness and resilience. The TPA parametres were determined using a Texture Analyser TA-XT2 (STABLE-MICRO SYSTEMS, UK).

For statistical evaluation of results the SAS Institute (Cary, NC, USA) software was used to perform analysis of variance (General Linear Models procedure) and to test for significance of differences among means by Tukey test. Body weight at slaughter was included as a covariant. Additionally, phenotypic correlations between muscle fibre traits and meat quality traits were estimated.

Results and discussion

The breed effect was investigated on muscle fibres properties, daily live weight gain, meat quality traits and texture parametres of LL muscle.

The microstructure of fatteners' muscle fibres of different types as related to breed is characterized in Table 1. No significant effects of breed were found on muscle fibre types percentage and RA. However, in the PL fatteners a tendency towards an increased number and RA of type I fibres, and a declining number and RA of type

Table 1. Means for histological muscle fibre type traits of *longissimus lumborum* in fatteners of three breeds

Trait	Polish Landrace	Polish Large White	Pietrain	SE
Composition (%)				
IIB type	71.49	73.79	72.62	0.511
IIA type	11.59	10.29	11.67	0.257
I type	16.92	15.92	15.71	0.348
Diameter (μm)				
IIB type	78.66[a]	78.97[a]	94.27[b]	0.785
IIA type	58.03[a]	59.81[a]	66.06[b]	0.601
I type	54.46[a]	54.37[a]	67.57[b]	0.579
Relative area (%)				
IIB type	77.91	79.73	78.88	0.465
IIA type	9.32	8.42	8.88	0.221
I type	12.77	11.85	12.24	0.315

[ab]Means within columns bearing different superscripts differ significantly at $P \leq 0.05$.

IIB fibres were observed compared to PLW and Pietrain fatteners. On the other hand, significant differences were shown between the examined groups of fatteners in the size of muscle fibres. In that case, the LL muscle of P fatteners showed significantly larger diameter of fibres of all examined types compared to the PLW and PL fatteners, between which no significant differences were observed. The fact that muscle fibre characteristics are mainly determined genetically corroborates the earlier results of Brocks *et al*. [1998] and Kłosowska and Fiedler [2003]. According to Stickland *et al*. [1975] the number of muscle fibres is determined genetically and thereafter only the length and the cross-sectional area of the muscle fibres increase. Other studies have demonstrated that muscle fibre-type composition (%) and size are specific for different breeds, lines and crossbreds of pigs [Ruusunen and Poulanne 1997, Wojtysiak and Migdał 2006]. Ruusunen and Puolanne [1997] found that the muscles of Hampshire are more oxidative than those of Landrace or Yorkshire pigs. These authors also showed that variation in muscle fibre composition in pigs within the breeds is larger than the average interbreed variation. Weiler *et al*. [1995] and Lafaucheur *et al*. [2004] both suggest that selection in modern pig breeds induced a shift in muscle metabolism towards a more glycolytic and less oxidative fibre types.

When the meat quality traits were compared, breed of fatteners showed no effect on mean daily gain, meat pH_{45} and pH_{24} and shear force (Tab. 2). Similarly, results of the TPA test showed no significant differences in hardness, springiness, cohesiveness, chewiness and resilience between the fatteners of examined breeds. However, trends towards higher shear force and hardness were observed in P compared to PL and PLW fatteners. Chang *et al*. [2003] have shown that differences in meat quality between the same muscles may be evident in specific breeds of any one species. They suggest that the shear force of *longissimus dorsi* and *psoas* muscles are significantly different within Duroc, but not within Berkshire, Tamworth or Large White pigs.

Table 2. Means for daily live weight gain, meat pH and texture profile parametres of *longissimus lumborum* in fatteners of three breeds

Trait	Polish Landrace	Polish Large White	Pietrain	SE
Live weight gain (g/day)	848	870	820	9.552
pH_{45}	6.13	6.22	6.20	0.047
pH_{24}	5.60	5.62	5.56	0.017
Shear force	5.08	5.15	5.25	0.126
Hardness	124.09	118.52	129.56	2.288
Springiness	0.62	0.59	0.59	0.010
Cohesiveness	0.48	0.49	0.48	0.006
Chewiness	37.30	34.67	36.92	1.024
Resilience	0.22	0.21	0.21	0.004

No significant differences between breed means.

The relationships between muscle fibre characteristics and daily gain, meat quality and texture parametres were generally limited and only a few significant correlations were found (Tab. 3). The daily gain was negatively correlated with percentage and RA of type I and positively with the IIB fibres percentage and RA. Moreover, positive and significant was found the correlation between daily gain and diameter of type IIB fibres. The fact that growth rate may be a source of variation in muscle fibre size corroborates the earlier results achieved by Ruusunen and Puolanne [2004] who found that size of all fibre types of *longissimus dorsi* muscle in pigs increased when animal growth rate increased, but, in contrast to our results, only the correlation between daily gain and size of type I fibres was significant. Larzul *et al*. [1997] also found a low but significant correlation between mean daily live weight gain and muscle fibre characteristics. Moreover, Staun [1963] reported that rapidly growing pigs have large *longissimus dorsi* muscle fibres. On the other hand, Dwyer *et al*. [1993] in *semitendinosus* and Henckel *et al*. [1997] in *longissimus dorsi* found that in pigs growing fast muscle fibres are more numerous but smaller than in pigs that grow slower.

In general, glycolysis and *rigor mortis* occur faster in white than in red muscles. IIA and IIB fibres mainly follow the glycolytic pathway and their metabolism contributes to a fast pH drop. Moreover, pig muscles with many oxidative fibres tolerate stressful conditions durng transport better, resulting in a slower fall in pH after slaughter [Ruusunen and Puolanne 1997]. The negative correlation between the size of type IIA fibres and pH_{24} observed in the current study is in accordance with the results of Ruy and Kim [2005]. On the other hand, no significant phenotypic correlations were identified between pH_{24} and percentage and RA of muscle fibre types (Tab. 3). In contrast, Ruy and Kim [2005] showed pH_{24} correlating negatively with percentage of IIB and positively with percentage of IIA fibres, whereas Larzul *et al*. [1997] did not observe any significant relationship between muscle fibre traits and pH_{24} of meat.

Table 3. Phenotypic correlation coefficients between histological muscle fibre traits of *longissimus lumborum* and daily live weight gain, meat quality and texture profile in fatteners of three breeds

Histological trait	Daily gain	pH_{45}	pH_{24}	Shear force	Hardness	Springiness	Cohesiveness	Chewiness	Resilience
Composition (%)									
IIB type	0.235*	0.129	0.065	-0.009	-0.104	-0.050	-0.055	-0.008	-0.023
IIA type	-0.143	-0.025	-0.162	0.017	0.053	0.002	0.072	0.054	0.104
I type	-0.241*	-0.173	0.025	0.034	0.115	0.073	-0.137	-0.028	-0.044
Diameter (μm)									
IIB type	0.095*	-0.066	-0.132	-0.027	-0.027	-0.144	0.076	-0.023	0.049
IIA type	0.129	-0.142	-0.235*	-0.182*	-0.089	-0.104	-0.012	-0.108	-0.084
I type	0.032	-0.067	-0.193	-0.081	-0.054	-0.115	0.028	-0.016	0.033
Relative area (%)									
IIB type	0.251*	0.088	0.065	0.026	-0.102	-0.081	0.118	0.034	0.038
IIA type	-0.127	0.042	-0.113	-0.021	0.022	0.075	-0.042	-0.046	-0.026
I type	-0.279*	-0.163	-0.021	-0.029	0.140	0.064	-0.145	-0.017	-0.038

$P \leq 0.05$.

Moreover, the results obtained in this study, in which significant correlations between muscle fibre traits and pH_{45} were not found, are in contrast with the earlier report by Larzul *et al.* [1997], who found that pH at 30 min was negatively related to both fibre size and per cent of type IIB fibres.

Shear force, another important quality parametre of meat, was negatively related to IIA fibre size (Tab. 3). Moreover, non-significant were found the phenotypic correlations between fibre traits and parametres of texture. A similar tendency of relation between type IIA fibres and shear force was observed by Ruy and Kim [2005]. However, Cameron *et al*. [1998] showed that diameter of type I, unlike that of type IIB fibres, positively correlated with hardness and shear force. The fact that shear force is, as shown in this study, associated with muscle fibre characteristics, including fibre type size, confirms also the earlier results of Renand *et al*. [2001]. Opinions about the effect of muscle fibre traits on meat tenderness are not uniform. Totland *et al.* [1988] noted that the superficial regions in bovine *semitendinosus* muscle contained a high percentage of type IIB fibres and were tenderer than the deeper muscle layers, with a high percentage of type I fibres. Similarly, Karlsson *et al*. [1993] in pigs and O'Halloran *et al.* [1997] in cattle noted that the percentage of type IIB fibres was negatively correlated with toughness, while Renand *et al*. [2001] showed a positive correlation between percentage of type I fibres and meat toughness in bulls. On the other hand, Strydom *et al.* [2000] working on cattle showed a positive relationship between the percentage of type I fibres and sensory tenderness and negative between the tenderness and percentage of type IIB fibres.

Summarizing, the muscle fibre percentage and RA were not found related to fatteners' breed which, however, affected the size of muscle fibres. Moreover, neither daily gain and meat pH, nor shear force and textural parametres were also related to breed of fatteners. However, an increase in daily live weight gain was accompanied by increased percentage and RA of type IIB fibres, unlike the per cent and RA of type I fibres. On the other hand, size of type IIA fibres was negatively related to shear force and pH_{24}.

The results obtained can be used in breeding practice once genetic markers that determine an animal's histochemical profile have been developed. This would enable breeding work aimed at improving the slaughter yield of pigs while retaining good quality of meat and meat products.

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Zależności między właściwościami włókien mięśniowych a cechami fizykochemicznymi mięśnia *longissimus lumborum* i tempem wzrostu tuczników trzech ras

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

Celem pracy była identyfikacja włókien mięśniowych i cech jakościowych *m. longissimus lumborum* tuczników trzech ras oraz określenie zależności między cechami włókien mięśniowych a przyrostami dziennymi, pH i parametrami tekstury mięśnia. Badania przeprowadzono na 92 tucznikach trzech ras: polskiej białej zwisłouchej (n=40), wielkiej białej polskiej (n=38) i pietrain (n=14). Na podstawie aktywności dehydrogenazy (NADH) wyróżniono trzy typy włókien mięśniowych: I, IIA i IIB. Określono średnie przyrosty dobowe w przedziale od 25 do 100 kg masy ciała oraz parametry jakościowe mięsa: pH_{45}, pH_{24}, siłę cięcia i cechy tekstury. Rasa nie wpływała ani na cechy jakości mięsa, ani na proporcje (%) i RA (*relative area*) włókien mięśniowych poszczególnych typów, wiązała się natomiast istotnie z wielkością (średnicą) włókien. Mięsień *longissimus lumborum* tuczników rasy pietrain charakteryzował się większymi włóknami mięśniowymi wszystkich typów w porównaniu z takim samym mięśniem tuczników rasy polskiej białej zwisłouchej i wielkiej białej polskiej. Współczynniki korelacji między cechami histologicznymi a fizykochemicznymi były niskie. Udział (%) i RA włókien typu IIB, w przeciwieństwie do włókien typu I, korelował dodatnio z przyrostami dziennymi. Wykazano także dodatnie zależności między wielkością włókien mięśniowych typu IIB a przyrostami dziennymi. Z kolei siła cięcia korelowała ujemnie z wielkością włókien typu IIA. Podobne zależności odnotowano między średnicą włókien mięśniowych typu IIA a pH_{24}.