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Pathological Changes in the Microstructure of *longissimus lumborum* Muscle from Five Breeds of Pigs*

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The aim of the study was to determine the extent of histopathological changes in *m. longissimus lumborum* of PL, PLW, Duroc, Pietrain, and Puławska pigs (N=30 per breed) aged 210 days. Changes in fibre size (atrophy, hypertrophy – giant fibres), changes in fibre shape (angular fibres), degenerative lesions (necrosis with phagocytosis) and connective tissue hypertrophy were evaluated. The percentage of individual pathological changes in *m. longissimus lumborum* of the analysed pig breeds was relatively low. Significantly more normal fibres were found in the muscles of Puławska compared to Pietrain pigs. Muscle fibre atrophy was the most frequent and extensive histopathological change. The muscles of Puławska pigs had significantly fewer atrophic, giant and angular fibres, significantly less necrosis with phagocytosis, and less animals with connective tissue hypertrophy compared to the other pig breeds. On the other hand, Pietrain pigs were characterized by a greater number of animals with giant fibres and a significantly higher proportion of giant fibres compared to the other breeds. Also the diameter of giant fibres was the largest in Pietrain, intermediate in PL and PLW, and the smallest in Duroc and Puławska pigs. Moreover, current findings indicate that giant fibres may arise from each muscle fibre type (I, IIA and IIB). It is concluded that selection of pigs for increased leanness contributes to the incidence of histopathological changes, which may decrease pork quality.

Key words: Histopathological changes, *m. longissimus lumborum*, breed, pig.

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Long-term selection and breeding efforts have contributed to the development of many pig breeds and lines with increased rate of growth and improved slaughter indicators. The intensive selection of pigs for improved leanness considerably increased carcass lean content and decreased carcass fat while having an adverse effect on the microstructure of muscle tissue, which caused overall meat quality to deteriorate (BORZUTA 2000). It is generally accepted that muscle structure is related to final meat quality (FIEDLER *et al.* 1999; KŁOSOWSKA & FIEDLER 2003). Therefore, changes in pork as a result of selection are well reflected in microstructural data that encompass histopathological analysis of muscle tissue and shed light on the determinants of leanness and the causes of meat defects. High-lean animals were found to exhibit meat quality aberrations such as PSE meat, which are paralleled by pathological changes in muscle tissue that include changes in both the size and structure of muscle fibres (SOŚNICKI 1987; KŁOSOWSKA *et al.* 1995). Connective tissue hypertrophy is also common. Earlier research has shown that the incidence of pathological changes in pig muscles depends largely on the histochemical profile of the muscles as well as the age and body weight of pigs (RAHELIC & PAUC 1980; SOLOMON & WEST 1985; KŁOSOWSKA *et al.* 1995; WOJTYSIAK & MIGDAŁ 2006). Abundant evidence shows that the type and extent of histopathological changes may have a genetic background (WIMMERS *et al.* 2006) and may also depend on animal breed (KŁOSOWSKA *et al.* 1995; WALASIK *et al.* 2000; BOGUCKA *et al.* 2006; SCHUBERT-SCHOPPMEYER *et al.* 2008; SOBCZAK *et al.* 2009). Therefore, the aim of this study was to determine the extent of histopathological changes in *m. longissimus lumborum* of pigs representing different breeds: the highly selected Pietrain breed, meat-type breeds such as Polish Landrace,

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Polish Large White and Duroc, and the Polish native breed Puławska.

Material and Methods

A total of 150 pigs from five breeds: Polish Landrace (PL), Polish Large White (PLW), Duroc, Pietrain, and Puławska (N=30 per breed) were used in this study. Animals were kept from 60 to 210 days of age at the Agricultural Production Cooperative in Kędzierzyn-Koźle, Poland, and fed a complete diet. All animals were reared under the same environmental and production regime. Pigs of each breed were slaughtered at 210 days of age in a commercial slaughterhouse belonging to the Agricultural Production Cooperative in Kędzierzyn-Koźle. Feed was withdrawn 12 h before slaughter but water was freely available in lairage. The pigs were stunned with CO_2 and processed according to normal slaughterhouse procedures (exsanguinated, scalded, dehaired and eviscerated). Muscle samples were taken 45 min *postmortem* from the right carcass side from the *longissimus lumborum* muscle (LL) at the level of the 2nd lumbar vertebra and frozen in isopentane that was cooled using liquid nitrogen and stored at -80^{o}C until histological analysis. Serial transverse sections of 10 μm were cut at -20^{o}C in a cryostat (Slee MEV, Germany) and stained by haematoxylin and eosin. Further sections were exposed to a histochemical reaction for NADH-tetrazolium reductase (NADH-TR), which enables classification of type I, IIA and IIB muscle fibre types (DUBOWITZ & SEWERY 2007). The histopathological changes were assessed by analysing 10 photos of muscle samples of 1000 μm^2 area each using a NIKON E600 (Japan) light microscope. The following histopathological changes were examined according to DUBOWITZ and SEWERY (2007): changes in fibre size (atrophy, hypertrophy – giant fibres) and shape (angular fibres), degeneration (necrosis with phagocytosis) and connective tissue hypertrophy (fibrosis).

The percentage of histopathological changes and diameter of giant fibres and their classification as I, IIA and IIB types were determined by image analysis (Multi Scan v. 14.02). A minimum of 300 muscle fibres per sample were counted except connective tissue, which was estimated subjectively according to the following scale: “0” – no change, “+” – minor change, and “++” – major change.

Associations of the pig breeds with histopathological parameters were calculated using the General Linear Model (GLM) procedure of the Statistical Analysis System program (SAS v. 8.02, 2001). The data were presented as LSM±SE.

Results and Discussion

The proportion of animals with histopathological changes of varying extent in the *m. longissimus lumborum* of the analysed animals is shown in Table 1 and 2. In all pig breeds studied, there were histopathological changes in *m. longissimus lumborum* such as changes in fibre size (fibre atrophy, hypertrophy – giant fibres) and shape (triangular, trapezoid and elongated fibres – angular fibres), degenerative changes of fibres (necrosis with phagocytosis), and connective tissue hypertrophy (fibrosis).

Muscle fibre atrophy was the most frequent histopathological change observed in all pig breeds analysed (Fig. 1A). It was observed in all Pietrain, Duroc and PL animals, and in most PLW fatteners (93.3%). In the Puławska breed, this

Table 1

Percentage of pigs showing histopathological changes in *longissimus lumborum* muscle

Traits	PLW	PL	Puławska	Duroc	Pietrain
Giant fibres (%)	53.3	66.7	6.7	63.3	100
Giant fibres of type (%)					
I	0	3.3	0	6.7	16.7
IIA	6.7	16.7	0	23.3	43.3
IIB	50	56.7	6.7	46.7	86.7
Angular fibres (%)	56.6	53.3	30	66.6	63.3
Atrophic fibres (%)	93.3	100	73.3	100	100
Necrosis with phagocytosis (%)	20	16.7	6.7	23.3	26.7
Fibrosis (%)					
“0“	53.3	56.7	66.7	50	40
“+”	26.7	23.3	20	26.7	23.3
“++”	20	20	13.3	23.3	36.7

Table 2

Least squares means and standard errors (SE) for incidence of histopathological changes in *longissimus lumborum* muscle

Traits	PLW	PL	Puławska	Duroc	Pietrain
Normal fibres (%)	95.07[ab] (2.05)	95.20[ab] (3.19)	97.68[a] (1.94)	94.64[ab] (2.76)	93.55[b] (1.89)
Giant fibres (%)	0.78[a] (0.23)	0.97[a] (0.16)	0.09[b] (0.02)	0.89[a] (0.21)	1.74[c] (0.57)
Giant fibre diameter (μm)	158.2[a] (6.21)	153.9[a] (7.54)	135.7[b] (6.92)	132.8[b] (5.46)	184.9[c] (8.31)
Angular fibres (%)	0.81[a] (0.17)	0.73[a] (0.22)	0.37[b] (0.08)	0.94[a] (0.32)	0.89[a] (0.16)
Atrophic fibres (%)	3.06[a] (0.12)	2.86[a] (0.15)	1.74[b] (0.09)	3.21[a] (0.18)	3.47[a] (0.24)
Necrosis with phagocytosis (%)	0.28[a] (0.05)	0.24[a] (0.05)	0.12[b] (0.04)	0.32[a] (0.06)	0.35[a] (0.08)

a, b, c – LSMs marked with different superscripts differ significantly at P<0.05.

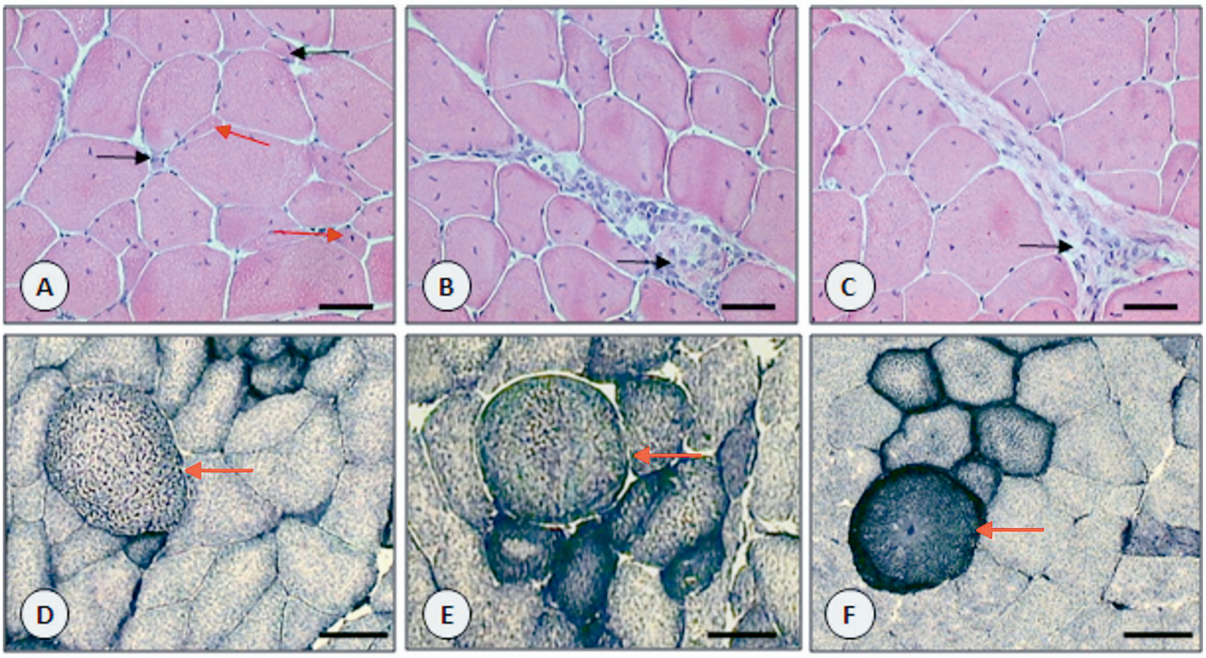

Fig. 1. Exemplary cross-section of *longissimus lumborum* muscle: fibre atrophy (black arrow), angular fibres (red arrow) – A; fibre necrosis with phagocytosis (arrow) – B; connective tissue hypertrophy (arrow) – C; giant fibres (arrow) of type IIB – D, IIA – E and I – F stained for NADH-TR activity, bar = 50 μm.

change occurred in the smallest number of animals with significantly lower extent. The analysis of muscle fibre shape (Fig. 1A) revealed that the smallest number of animals with angular fibres and a significantly lower percentage of angular fibres were found in Puławska pigs compared to other breeds. The observation that muscle fibre atrophy was the most frequent pathological change observed in pig muscles agrees with earlier research by WALASIK *et al.* (2000), BOGUCKA *et al.* (2006) and WOJTYSIAK and MIGDAŁ (2006). Atrophy is a gradual reduction in muscle fibre volume, leading to a loss of both myofibrils and sarcoplasm (PACIELLO & PAPPARELLA 2009; THUILLIEZ *et al.* 2009). Fibres undergoing atrophy may show different types of degeneration (a change in cross-sectional shape from circular to polygonal – angular fibres), but oftentimes atrophic fibres exhibit normal structure. According to HAUSMANOWA-PETRUSEWICZ (1993) and THUILLIEZ *et al.* (2009), muscle fibre denervation is one of the most frequent causes of atrophic and angular fibre emergence. Neurogenic changes result from damage to the peripheral motor neuron, which causes the atrophy of all muscle fibres innervated by that neuron.

Degenerative changes that cause the breakdown of muscle fibres (necrosis with phagocytosis) are presented in Fig. 1B. According to HAUSMANOWA-PETRUSEWICZ (1993), necrosis with phagocytosis is indicated by the appearance of histiocytes, lymphocytes and macrophages within muscle fibre. KŁOSOWSKA *et al.* (1998), WALASIK *et al.* (2000) and PACIELLO and PAPPARELLA (2009) showed this process to be often accompanied by connective tissue hypertrophy (Fig. 1C). This supposition is confirmed by the present study which demonstrated that Puławska pigs, characterized by the lowest percentage and smallest number of animals affected by necrosis with phagocytosis in *m. longissimus lumborum*, also have a lower percentage of animals with connective tissue hypertrophy compared to the other pig breeds analysed. In an analysis of the extent of histopathological changes in *m. semimembranosus* of hybrid pigs derived from specialized production programs (the Dutch Stamboek and the Polish Torhyb), BOGUCKA *et al.* (2006) showed that the latter had a greater proportion of fibres undergoing necrosis with phagocytosis which was paralleled by connective tissue hypertrophy.

In agreement with previous reports (RAHELIC & PAUC 1980; FIEDLER *et al.* 1999; FAZARINC *et al.* 2002; SCHUBERT-SCHOPPMEYER *et al.* 2008; SOBCZAK *et al.* 2009), the current study also showed the presence of giant fibres in *m. longissimus lumborum* (Fig. 1D-F). They were mostly oval, sometimes round in shape. A thicker layer of endomysium occurred around these fibres, which detached them from the rest of fibres. Giant fibres can be found grouped or isolated and are predominantly located at the periphery of bundles. However, they can also be found within the bundles (KULIŠEK *et al.* 2009). The aetiology of this fibre anomaly is the subject of inconsistency and is not yet fully clarified. Most researchers consider giant fibres to arise from hypercontraction of individual fibres (SINK *et al.* 1986; SOŚNICKI 1987). On the other hand, WEGNER and ENDER (1980) and WILSON *et al.* (1990) characterized them as fibres in a degenerative, pre-necrotic stage. However, all reports on giant fibres have in common that these fibres can be detected only *postmortem* and not *in vivo* (SOLOMON & EASTIDGE 1987; FIEDLER *et al.* 1999; SCHUBERT-SCHOPPMEYER *et al.* 2008). RAHELIC and PAUC (1980) indicated that the number of giant fibres increases in "white" muscles, which consist predominantly of type IIB fibres with a glycolytic metabolism. Evidence to the contrary was provided by SOLOMON and WEST (1985) who observed a higher number of giant fibres in "red" porcine muscles comprising a greater proportion of I and IIA fibres. Recent research shows that preslaughter environmental conditions may contribute to the giant fibre syndrome (BEE *et al.* 2006; SHEN *et al.* 2006). On the other hand, WIMMERS *et al.* (2006) suggest that the occurrence and frequency of giant fibres is in part genetically determined. The presence of giant fibres in pig muscles is also important for pork quality. Many studies showed that giant fibre frequency and size are significantly correlated to acidity and texture parameters of the meat (FAZARINC *et al.* 2002; SCHUBERT-SCHOPPMEYER *et al.* 2008; SOBCZAK *et al.* 2009). In the current study, differences in giant fibre frequency and size were significantly related to breed. Thus, giant fibres occurred in 100% of highly selected Pietrain pigs, followed by PL, Duroc and PLW, and finally the native Puławska breed (only 6.7%). The proportion of giant fibres within muscle sections followed the same order, with the highest percentage found in Pietrain and the lowest in Puławska pigs. In Duroc, PLW and PL pigs the percentage of giant fibres was intermediate. This is in accordance with KŁOSOWSKA (1984) and SOBCZAK *et al.* (2009) who reported the highest number of giant fibres to appear in the muscles of Pietrain pigs. SCHUBERT-SCHOPPMEYER *et al.* (2008) analysed the frequency of giant fibres in *m. longissimus* of four pig breeds and showed that Pietrain pigs had a significantly greater proportion of giant fibres compared to Large White, Landrace and Leicoma pigs. In the present study, such a high proportion of giant fibres in *m. longissimus lumborum* of Pietrain pigs is probably associated with the fact that pigs of this breed have the highest stress susceptibility (KŁOSOWSKA *et al.* 1995; BRYM *et al.* 2002), and ESSEN-GUSTAVSSON *et al.* (1994) claimed that the presence of giant fibres in muscles of high meatiness is connected with the HAL gene and stress susceptibility. Likewise, KŁOSOWSKA and FIEDLER (2003) suggest that the quantity of giant fibres is related to a genetic predisposition of animals to generate PSE meat. Meanwhile, FAZARINC *et al.* (2002) reported that the giant fibre syndrome is not necessarily associated with the mutation of the RYR1 gene, but the incidence of affected fibres is higher in pigs homozygous for the RYR1 gene mutation (RYR1 nn). These results corroborate the findings of FIEDLER *et al.* (1999), who also reported a significantly higher frequency of giant fibres in RYR1 dimutant pigs. Moreover, FAZARINC *et al.* (2002) and SCHEFFLER and GERRARD (2007) suggest that the occurrence of giant fibres depends mostly upon the rate and intensity of early *postmortem* glycolysis, which results in acidity of muscle tissue. On the other hand, such a small number of animals with giant fibres and the lowest frequency of these fibres in *m. longissimus lumborum* of Puławska pigs, found in the current study, confirm that native breeds of pigs are characterized by a lower proportion of histopathological changes compared to specialized breeds of high-lean pigs (SCHUBERT-SCHOPPMEYER *et al.* 2008). However, an earlier study has also indicated that giant fibres were not detected in all breeds of pigs. VELOTTO *et al.* (2007) reported that these fibres did not occur in Black Sicilian pigs.

In addition, the present histochemical study shows that giant fibres were exclusively type IIB in Puławska pigs. Similar observations were made by FAZARINC *et al.* (2002) for a local Slovenian breed (Krško polje) and by SCHUBERT-SCHOPPMEYER *et al.* (2008) for Large White pigs. The present study revealed type IIA giant fibres in the *m. longissimus lumborum* of PLW pigs, whereas Duroc, PL and Pietrain fatteners also exhibited type I giant fibres. Moreover, in agreement with earlier studies (SCHMITT & DUMONT 1981; SCHUBERT-SCHOPPMEYER *et al.* 2008) the results of the current study confirm that giant fibres can arise from each muscle fibre type (I, IIA and IIB), and within the breeds examined giant fibres arose mostly from type IIB fibres, which form the highest percentage of all three fibre types found in *m. longissimus lumborum* (RAHELIČ & PAUC 1980; FIEDLER *et al.* 1999; FAZARINC *et al.* 2002). Regarding the size of giant fibres, the largest were observed in the muscles of the Pietrain breed, intermediate in PLW and PL breeds, and significantly the smallest diameter was characteristic of the muscles of Puławska and Duroc pigs. Similarly, SOBCZAK *et al.* (2009) showed that giant fibres were significantly larger in Pietrain pigs and in crossbreed pigs with Pietrain genes compared to Duroc pigs. SCHUBERT-SCHOPPMEYER *et al.* (2008) found that Pietrain pigs tended to exhibit the largest giant fibres in *m. longissimus* compared to Large White, Landrace and Leicoma pigs. The size of giant fibres depends largely on the size of normal fibres. FAZARINC *et al.* (2002) noted that giant fibres are three times as large as normal fibres. For this reason, the smallest giant fibres found in Puławska and Duroc pigs are probably associated with smaller (thinner) normal fibres that form *m. longissimus lumborum* (FALKOWSKI & MILEWSKA 1999; SOBCZAK *et al.* 2009).

In conclusion, the percentage of individual pathological changes in the *m. longissimus lumborum* of the analysed pig breeds was relatively low. Significantly more normal fibres were found in the native Puławska breed compared to the highly selected Pietrain breed. In the other pigs, i.e. PL, PLW and Duroc breeds, the proportion of normal fibres was intermediate and not significantly different from that in the Puławska and Pietrain breeds.

It is also concluded that selection for increased leanness contributes to the incidence of pathological changes in *longissimus lumborum* muscle tissue and in particular increases the frequency of giant fibres, which may decrease pork quality.

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