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MICROSTRUCTURE OF *LONGISSIMUS LUMBORUM* MUSCLE AND MEAT QUALITY OF NATIVE POLISH PIG BREEDS: ZŁOTNICKA SPOTTED AND PUŁAWSKA*

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Abstract

In 50 fattening pigs representing two Polish native breeds: 24 Złotnicka Spotted, 10 Puławska and 16 F_1 ♀ (Polish Large White × Polish Landrace) × F_1 ♂ (Duroc × Pietrain) crosses microstructure of muscle, carcass and meat quality were studied. Puławska pigs had the thickest backfat, but the loin eye area was smaller only in comparison to crossbreds. Compared to the Puławska breed, the meat of Złotnicka Spotted pigs was darker, which was associated with a greater percentage of type I fibres and a smaller percentage of type IIB fibres. Puławska pigs distinguished themselves from the other groups under study by the greatest density of fibres per mm². Smaller diameter of type IIA and IIB fibres and higher total number of fibres were found in Puławska breed pigs compared to Złotnicka Spotted, despite the absence of differences in the loin eye area. Smaller thickness of the fibres favourably affects meat quality, and might be considered an indicator of a delicate structure of meat.

Keys words: pigs, meat quality, *longissimus lumborum* muscle, muscle fibres

As consumers look for good quality pork with excellent taste in the Polish market, the interest in the native pig breeds Złotnicka Spotted and Puławska has increased. These old breeds were created from local primitive pigs and have undergone little genetic improvement. They are well adapted to the environment, tolerant of unfavourable housing conditions, and undemanding in feed. The meat and meat products made from these pigs possess unique quality and taste, and are highly nutritious. This meat is excellent for making long matured cured meats and can be used to produce quality regional products (Blicharski, 2001; Bocian et al., 2012; Florowski et al., 2006 a, b; 2007; 2008; Native breeds of pigs – state of breeding and results of evaluation, 2012; Kapelański et al., 2013). The latest research indicates that the meat from native breeds of pigs is more interesting in terms of quality traits than the meat from improved breeds and their crosses from intensive breeding, which makes it

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a viable alternative for many consumers. These traits include more desirable colour, better water holding capacity, and unique taste of the dishes made from this meat (Bocian et al., 2012; Florowski et al., 2006 a; Fortina et al., 2005; Renaudeau and Mourot, 2007; Wojtysiak and Połtowicz, 2014).

Muscle histology, in particular the enzyme profile and the kinetics of muscle contraction, as well as the total number of fibres per muscle are important determinants of meat quality (Bogucka and Kapelański, 2005; Henckel et al., 1997; Kłosowska and Fiedler, 2003; Lefaucheur, 2010). Skeletal muscles are heterogenous structures because they are composed of different muscle fibre types. These fibres, referred to as type I (slow-twitch, oxidative), IIA (fast-twitch, oxidative glycolytic) and IIB (fast-twitch, glycolytic) (Brooke and Kaiser, 1970), have distinct metabolic, morphological and functional characteristics (Karlsson et al., 1999). Final muscle weight is determined by the total number of fibres, their thickness, and the percentage of different types. These different characteristics of muscle microstructure in pigs affect the quality of their meat (Fiedler et al., 1999; Rehfeldt and Kuhn, 2006).

The present study compares the microstructure of *longissimus lumborum* muscle in two native Polish breeds of pigs: Złotnicka Spotted and Puławska, and in their highly-productive crossbreds as related to some carcass and meat quality traits.

Material and methods

Animals

The experiment used 50 fattening pigs (approximately 180–190 days old) representing two Polish conservation breeds: Złotnicka Spotted (24 head) and Puławska (10 head) and their F_1 ♀ (Polish Large White × Polish Landrace) × F_1 ♂ (Duroc × × Pietrain) crosses (16 head). The sex ratio of gilts to barrows was similar in the groups. Piglets were reared and fattened in two conventional pig houses under similar environmental conditions. Piglets were obtained, raised by sows, weaned and fattened under identical housing, feeding and nursing conditions. All animals were fed a diet which had the same nutritive value and contained the same proportion of farm-produced fodders. Nutritive value of the feed was 12.6 MJ ME and 156 g crude protein (composition of complete diet: soybean meal – 12%, peas – 5%, barley – 60%, wheat/triticale – 20%, premix – 3% (lysine content 1%)). Animals were slaughtered and muscle samples collected for analysis in accordance with meat industry standards and regulations.

Carcass quality and meat quality measurements

All the right half-carcasses were evaluated in detail using a method described by Różycki and Tyra (2010). Backfat thickness was measured at five positions: over the shoulder, on the back (behind the last thoracic vertebra and the first lumbar vertebra) and at three locations over the loin (cross-section of the gluteal muscle): over the rostral edge of the gluteal muscle, in the middle of the gluteal muscle and over the caudal edge of the gluteal muscle. Cross-section of *longissimus lumborum* muscle was traced and its area was determined by planimetry under laboratory conditions.

Forty-five minutes after slaughter, the pH_{45} of *longissimus lumborum* was measured with a portable pH meter (Matthäus, Germany) with a glass electrode standardized for pH 4.0 and 7.0 according to Polish Standard PN-77/a-82058. During dissection, samples were collected from the lumbar section for meat quality analyses. Meat colour parameters were determined with a Minolta CR 310 using the LAB colour model (L* – lightness, a* – redness, b* – yellowness) (Itten, 1997). Water holding capacity of meat was determined according to Grau and Hamm (1952), in which a weighed 300 mg sample of ground meat was placed on Whatman No. 1 filter paper subjected to 2 kg pressure for 5 minutes. Then, expressible juice area was measured with a compensating planimeter. The area of expressible juice was used to calculate (after deducting the compressed meat area) the free water content of meat, assuming that 1 cm^2 of the juice area corresponds to 10 mg of free water. Forty-eight hours postmortem, ultimate pH (pH_u) was measured again in water extracts of meat, which were obtained by adding water at a 1:1 ratio.

Histochemical analysis

Forty-five minutes postmortem, samples of *longissimus lumborum* muscle were taken between the 4th and 5th lumbar vertebrae for histological examination. Immediately after collection, the samples were frozen in liquid nitrogen and transported in cryovials to a laboratory. Frozen muscle samples were mounted on a cryostat sample disc with a few drops of water. Mounted tissue was cut into sections (10 µm thick) at around –25°C using a cryostat (Thermo Shandon, United Kingdom). Microstructure of porcine *longissimus lumborum* muscle was evaluated on histological slides using an enzymatic reaction (Ziegan, 1979). This method allows staining the same section with two enzymes: tetrazolium reductase (incubation of the preparations in incubation fluid: NADH, NBT, 0.1M phosphate buffer pH 7.4 at 37ºC for 1 h) and myofibrillar ATPase (preincubation of the preparations in acid solution pH 4.0 for 3 min, followed by incubation in incubation fluid: ATP, $CaCl_2$, sodium barbiturate pH 9.6 at 37ºC for 30 min). The reaction is based on the reduction of colourless tetrazolium salts to coloured crystals of formazan, which enables a conclusive determination of the metabolic and structural type of fibres: type I (slow-twitch oxidative fibres, which stain dark brown or black), type IIA (fast-twitch oxidative fibres, which stain blue) and type IIB (fast-twitch glycolytic fibres, which stain light yellow). A Carl Zeiss microscope (Jena, Germany) equipped with a Toup View™ camera was used to record microscopic images on a computer disk. MultiScan v. 18.03 microscope imaging software (Computer Scanning Systems II Ltd, Warsaw, Poland) was used to calculate the percentage of different types of muscle fibres and their diameter. Muscle fibre diameter was measured according to a method of smallest diameters described by Brook (1970). Muscle fibre density expressed as a number of fibres per mm^2 was calculated from the mean number of fibres per mm^2. Total number of fibres (TNF) was calculated by multiplying fibre density per mm^2 by loin eye area.

Statistical analysis

The experimental data were analysed by a one-way analysis of variance. The results of the study (means ± standard error of the mean (SEM), significant differences using Tukey's HSD test for uneven sample sizes) were statistically analysed using STATISTICA AXAP ver. 10.0 MR1 software.

Results

Carcass and meat quality traits

The results of slaughter performance are presented in Table 1. Preslaughter weight ranged from 99 kg in Puławska pigs to 102 kg in Złotnicka Spotted pigs. No statistically significant differences were found for this parameter among the studied groups. Puławska pigs had thickest backfat (P<0.01). The largest loin eye area was found in crossbreds and this group differed significantly from the Puławska breed (P<0.05). Table 2 shows the results of meat quality. pH value measured 45 min post-mortem (pH_{45}) was similar in meat for all the groups. Differences in pH were only found after 48 hours (pH_u). The meat of four-breed crosses (PLW × PL × (Duroc × × Pietrain)) had a significantly lower pH_u compared to the meat of conservation breeds (P<0.01). Water holding capacity (WHC) was at a similar level in all the groups and ranged from 18.32% in Puławska to 18.91% in crossbred pigs. The analysis revealed significant differences in L* and a* colour coordinates. The lowest colour lightness was characteristic of meat from Złotnicka Spotted pigs with a significant difference in relation to the Puławska breed and the crossbreds (P<0.01). In addition, the meat of Złotnicka Spotted pigs showed that a* colour value was significantly greater than in the other groups. For b* colour coordinate no significant differences were observed among the groups.

Table 1. Carcass traits of pigs

Trait	Złotnicka Spotted	Puławska	Crossbreds (PLW × PL) × × (Duroc × Pietrain)
Slaughter weight (kg)	102±2	99±4	101±2
BFT (mm)	23.72 B±1.19	31.24 A±1.07	24.40 B±0.70
Loin eye area (cm²)	39.47 ab±2.20	36.90 b±2.24	44.81 a±1.66

BFT – average backfat thickness measured at five points.
a, b – values in rows with different letters differ significantly (P<0.05).
A, B – values in rows with different letters differ significantly (P<0.01).

Table 2. Meat quality traits of pigs

Trait	Złotnicka Spotted	Puławska	Crossbreds (PLW × PL) × × (Duroc × Pietrain)
pH_{45}	6.57±0.08	6.44±0.08	6.36±0.06
pH_u	5.69 A±0.06	5.73 A±0.05	5.45 B±0.01
WHC (%)	18.56±0.75	18.32±0.59	18.91±0.51
Colour L*	49.30 B±0.45	51.99 A±0.90	53.84 A±0.60
a*	17.22 A±0.31	15.88 B±0.33	14.81 B±0.31
b*	2.53±0.29	2.87±0.31	2.29±0.26

pH_u – 48 h after slaughter; water holding capacity (WHC) – % loose water.
A, B – values in rows with different letters differ significantly (P<0.01).

Microstructure of *longissimus lumborum* muscle

In the present study, it was observed that pig breed caused significant differences in some microstructural characteristics of the analysed muscle (Table 3). Muscles of Puławska pigs had the lowest proportion of type I fibres and the highest proportion of type IIB fibres (P<0.01). For type IIA fibres, no differences were observed among the analysed pig groups and the value of this trait was uniform from 24.87% in the Puławska breed to 26.73% in crossbreds. Diameter of slow-twitch fibres (type I) was similar. The thinner fast-twitch fibres (type IIA and IIB) were noticed in the muscle of Puławska pigs and this group differed significantly from Złotnicka Spotted pigs (P<0.01). Because the diameters of type IIA and IIB fibres in the muscle of Puławska pigs were smaller, this breed had the greatest fibre density per mm^2 (P<0.01). In addition, in Puławska pigs the density of type IIB fibres was significantly higher than in the other pig groups under study (P<0.01). The total number of fibres (TNF) was significantly higher in Puławska than in Złotnicka Spotted pigs (P<0.05). This is because the smaller diameters of type IIA and IIB fibres in comparison to Złotnicka Spotted breed and greater fibre density per mm^2 were observed in Puławska pigs.

Table 3. *Musculus longissimus lumborum* microstructure in pigs

Trait		Złotnicka Spotted	Puławska	Crossbreds (PLW × PL) × × (Duroc × Pietrain)
Percentage of muscle fibres (%)	I	24.32 A±1.53	15.41 B±1.53	22.86 A±1.24
	IIA	25.80±1.59	24.87±2.37	26.73±1.95
	IIB	49.88 B±1.63	59.72 A±3.27	50.41 B±2.35
Fibre diameter (µm)	I	55±2	56±2	56±2
	IIA	51 A±2	43 B±1	46 AB±1
	IIB	69 A±2	57 B±2	63 AB±2
Muscle fibre density (fibre number/mm^2)	Sum	151 B±8	206 A±15	153 B±7
	I	37±3	32±4	35±2
	IIA	40±3	53±8	40±3
	IIB	75 B±4	122 A±8	78 B±6
Total number of muscle fibres (×1000) (TNF)		596 b±41	760 a±41	686 ab±36

I – slow twitch oxidative fibres; IIA – fast twitch oxidative fibres, IIB – fast twitch glycolytic fibres.
a, b – values in rows with different letters differ significantly (P<0.05).
A, B – values in rows with different letters differ significantly (P<0.01).

Discussion

Carcass and meat quality traits

In the present study, the body weight of pigs was uniform, but the native breeds reached that weight around 3 weeks later due to their slower growth rate. Similarly, in a study of productive traits and meat quality of local Creole pigs and Large White pigs, Renaudeau and Mourot (2007) demonstrated that Creole pigs reached the same slaughter weight 34 days later than Large White pigs. Backfat thickness is the main indicator of carcass fatness, and loin eye area is an indicator of carcass muscular development. In the present study, thickest backfat was found in Puławska pigs. The thickest backfat in this breed is consistent with Wojtysiak and Połtowicz (2014), who showed backfat to be thicker in Puławska than in Polish Large White (PLW) pigs. However, the value of this trait was lower than in our study (23.64 vs. 31.24 mm). In addition, the above authors reported loin eye area in the Puławska breed to be greater than in our study (43.28 vs. 36.90 cm^2). In turn, Florowski et al. (2007, 2008) obtained similar backfat thickness and greater loin eye area compared to the values presented in our study. Mean backfat thickness in Złotnicka Spotted and crossbred pigs was at a similar level. For the Złotnicka Spotted breed, we obtained smaller backfat thickness and greater loin eye area compared to an earlier study by Bocian et al. (2012). Many authors have reported differences in slaughter traits between native breeds and other (genetically different) types of pigs. Gispert et al. (2007) showed loin eye area to be smallest and backfat thickness to be greatest in Meishan pigs compared to the commercial breeds such as Landrace, Large White, Duroc and Pietrain. Likewise, Bocian et al. (2012) in Złotnicka Spotted pigs, Kim et al. (2008) in Korean native pigs and Serra et al. (1998) in Iberian pigs obtained poorer slaughter parameters. It should be noted that in our study, which compared two native Polish breeds of pigs, the Złotnicka Spotted breed showed more favourable results than the Puławska breed for both backfat thickness and loin eye area.

Breed of pigs is one of the main factors affecting pork quality attributes (Lee et al., 2012). Many countries are increasing the emphasis on breeding native breeds of pigs as one of the ways to improve meat quality. These breeds produce good quality meat, as reported by da Costa et al. (2015) for Bísaro pigs, by Kim et al. (2008) for Korean native pigs, by Maiorano et al. (2007) for Casertana pigs, by Park et al. (2007) for Korean native black pigs, and by Serra et al. (1998) for Iberian pigs. The present study accounted for three basic determinants of pork quality: pH, colour, and water holding capacity. Differences between the native breeds Złotnicka Spotted and Puławska and the highly productive crossbred pigs were only observed for pH_u and meat colour coordinates (L* and a*). The pH meat value reflected changes after slaughter. This is an indicator of maturity of meat, durability and technological usefulness. Dynamics of changes in pH is the basic criterion for evaluation of deviations from the normal process of *postmortem* changes. Meat pH measured 48 h postmortem was higher in the native pigs, although in the all experimental groups of pigs pH indicated the occurrence of normal meat (RFN – reddish-pink, firm, non-exudative). In addition, pH_{48} decreased significantly, around 0.7–0.9 units, which is evidence that postmortem glycolytic changes in the muscles were normal. The higher pH_u values in the Złotnicka Spotted and Puławska breeds are in agreement with the findings of other authors (Bocian et al., 2012; Wojtysiak and Połtowicz, 2014). On the contrary, Florowski et al. (2006 a) found no statistically significant differences in pH_{48} among Złotnicka Spotted, Puławska and Polish Landrace breeds. Similar values to those reported for Polish native breeds as regards pH_1 and pH_u were also obtained by Maiorano et al. (2007), who investigated meat quality traits in local Italian Casertana pigs. Higher pH_u values were reported by Juárez et al. (2009) in different varieties of Iberian pigs and in IB × Duroc crosses. WHC is an important factor of meat quality because it shows the suitability of raw meat for further processing. In our study, WHC of meat from the studied pig groups was similar. Wojtysiak and Połtowicz (2014) found water holding capacity to be significantly lower (P<0.01) in Puławska than in Polish Large White breed, which is indicative of better water binding by muscle proteins and may suggest smaller water loss during technological processing of the meat. This is supported by Florowski et al. (2006 a), who noted much lower drip loss and cooking loss in *longissimus thoracis* muscle from Złotnicka and Puławska compared to Polish Landrace pigs. Analysis of meat colour coordinates showed significantly lower colour lightness (L*) and significantly higher redness (a*) in Złotnicka Spotted pigs compared to the other groups. The meat colour measurements (L* and a* values) correspond with the findings of other authors, who obtained darker meat from native breeds of pigs (Bocian et al., 2012; Estevez et al., 2006; Fortina et al., 2005; Serra et al., 1998; Wojtysiak and Połtowicz, 2014).

Microstructure of *longissimus lumborum* muscle

One of the more important factors influencing the quality of pork meat is the proportion of different muscle fibre types. Many authors point to the relationship between pork quality and the presence of muscle fibres that differ in contraction rate, diameter, and metabolism (Bogucka and Kapelański, 2004, 2005; Kłosowska et al., 1998, 2002; Lefaucheur, 2010; Larzul et al., 1997; Ryu and Kim, 2005; Serra et al., 1998). The muscles of Puławska pigs were characterized by the smallest proportion of type I fibres (16.71%) and the greatest proportion of type IIB fibres (59.73%), although these values compare favourably with the findings of Wojtysiak and Połtowicz (2014), who obtained 11.56% of type I fibres and 72.12% of type IIB fibres in Puławska pigs. However, these authors found that Puławska pigs had significantly more type I fibres compared to Polish Large White. Similarly, Serra et al. (1998) showed a greater proportion of red fibres in Iberian compared to Landrace pigs. In turn, Dai et al. (2009) found no statistically significant differences in fibre size and composition between Lantag and Landrace pigs. In our study, the highest proportion of type I fibres was characteristic of *longissimus lumborum* muscle from Złotnicka Spotted pigs, and, surprisingly, from four-breed crosses. The lowest proportion of type I fibres in the Puławska breed is quite surprising because a slightly greater proportion of red fibres could reasonably be expected in native breeds of pigs originated from local primitive breeds. This is confirmed by an earlier study of Bogucka et al. (2008), who compared muscle microstructure in wild boars, pigs, and wild boar pig hybrids. These authors demonstrated that the greater proportion of oxidative fibres was characteristic of wild boar muscles, whereas crossing of wild boars with Duroc and Polish Landrace pigs decreased the percentage of oxidative fibres and increased the proportion of glycolytic fibres compared to wild boars. Likewise, Elminowska-Wenda (2006), who analysed the structure of *longissimus lumborum* muscle in wild boar pigs, showed a large (about 30%) proportion of intermediate fibres. The Złotnicka Spotted breed was found to contain many more type I fibres compared to earlier studies by Bogucka and Kapelański (2005) and Bocian et al. (2012) (23.39% vs. 13.83%). However, in the study by Bogucka and Kapelański (2005) it is worth noting a high proportion of intermediate fibres in the Złotnicka Spotted breed compared to PL, Pietrain and P × (PLW × PL) pigs, finally this breed had the least glycolytic fibres anyway. As regards the enzymatic profile of muscle fibres in our study, four-breed (PLW × PL) × (Duroc × Pietrain) crosses turned out more favourably by achieving a high proportion of type I fibres and a low proportion of type IIB fibres. Furthermore, comparison of our results with the findings of other authors (Bocian et al., 2012; Kim et al., 2013; Lee et al., 2012; Wojtysiak and Kaczor, 2011; Wojtysiak and Połtowicz, 2014) in all the analysed pig groups showed a low proportion of type IIB fibres. Muscle fibre density in *longissimus lumborum* muscle, just like density of type IIB fibres, was found to be significantly higher in the Puławska breed compared to the other groups of pigs under study. Muscle fibre density is a trait directly related to muscle fibre diameter. The diameter of slow-twitch fibres (type I) reached similar values. However, the thickness of type IIA and IIB fibres was smaller in the muscle of Puławska pigs than Złotnicka Spotted pigs. Wojtysiak and Połtowicz (2014), comparing the thickness of the muscle fibers among Puławska and PLW breed received significantly larger cross-sectional area of type I fibers and smaller of type IIA fibres in Puławska pigs. Also Bogucka et al. (2008) observed the diameter of all fibre types to be smallest in wild boars compared to domestic pigs and wild boar/domestic pig hybrids. The surprisingly large muscle fibre diameters in the Złotnicka Spotted breed, and consequently their lower density per mm^2 make the structure of their muscles similar to the muscles of commercially produced pigs. Much smaller fibre diameters in the muscles of Złotnicka Spotted pigs were observed in earlier studies by Bogucka and Kapelański (2005) and Bocian et al. (2012). Differences in the thickness of different muscle fibre types are noticeable on microscopic images of the analysed muscle (Figures 1, 2, 3). Many authors point to TNF as an important factor in muscle growth and a determinant of meat quality (Kim et al., 2013; Lee et al., 2012; Lefaucheur, 2010; Ryu and Kim, 2005). On the other hand, Larzul et al. (1997) found myofibre cross-sectional area to be positively correlated to loin eye area and carcass meat percentage. Total number of fibres was found to be significantly greater in Puławska compared to Złotnicka Spotted pigs, which is possibly indicative of the fibrillarity of meat from Puławska pigs. Wojtysiak and Połtowicz (2014) showed larger loin eye area and greater TNF in PLW compared to Puławska pigs. The same authors also observed a relationship between TNF and intramuscular fat content, where higher IMF content was accompanied by smaller TNF value in Puławska compared to the PLW breed. This was also suggested by Kim et al. (2013) for Korean Native × Landrace pigs. Both Nuernberg et al. (2005) in Pietrain × German Landrace pigs and Wojtysiak and Połtowicz (2014) in Puławska and PLW pigs showed TNF to be higher than in our study.

Microscopic images

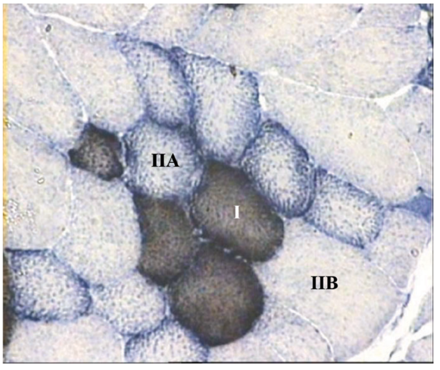

Figure 1. Cross section of *longissimus lumborum* muscle in Złotnicka Spotted pig. Mag. 125x

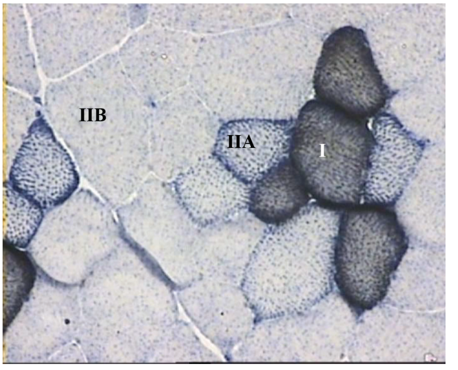

Figure 2. Cross section of *longissimus lumborum* muscle in Puławska pig. Mag. 125x

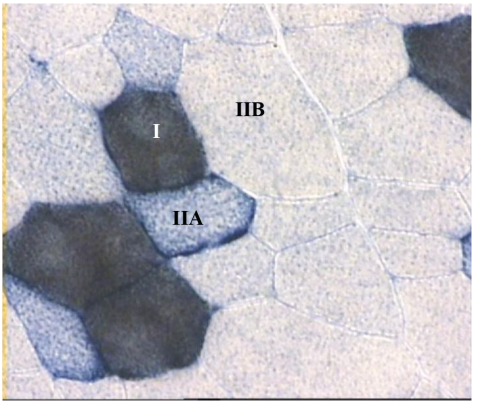

Figure 3. Cross section of *longissimus lumborum* muscle in crossbred pig. Mag. 125x

Conclusions

Puławska pigs had the thickest backfat, but the loin eye area was smaller only in comparison to crossbreds. The meat of Złotnicka Spotted pigs was darker than that of Puławska breed. Colour of Złotnicka Spotted meat was associated with a greater percentage of type I fibres and a smaller percentage of type IIB fibres. The lightness of meat colour in crossbreds was similar to Puławska breed, despite a larger percentage of type I fibres. It could be due to the different structure of meat, which does not allow penetration of light into the deeper layers of muscle. Consequently, the reflection of light from the surface of the muscle fibres is large, and thus the lightness of meat colour is high. Puławska pigs distinguished themselves from the other study groups by the greatest density of fibres per mm^2. Smaller diameter of type IIA and IIB fibres and higher total number of fibres were found in Puławska breed pigs compared to

Złotnicka Spotted, despite the absence of differences in the loin eye area. Smaller thickness of the fibres favourably affects meat quality, and might be considered an indicator of a delicate structure of meat.

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