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Carcass, Meat Quality and Histochemical Traits of *m. longissimus lumborum* from Złotnicka Spotted Pigs and Commercial Pigs*

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Carcass, meat quality and muscle fibre traits of *m. longissimus lumborum* were studied in 61 Złotnicka Spotted (ZS) pigs and 35 commercial crossbred (Polish Large White × Polish Landrace) pigs. The animals received organic feed *ad libitum* and were slaughtered at 210 days of age. The current findings indicate that both slaughter weight and cold carcass weight were significantly lower, and backfat thickness higher for ZS pigs compared to commercial pigs, while the commercial pigs exhibited longer carcasses and greater area, height and width of the loin eye. No significant differences were found in IMF content between the analysed groups. Moreover, the percentage of type I fibres was higher and their diameter was larger in *m. longissimus lumborum* of ZS compared to commercial pigs, whereas the opposite occurred for type IIB fibre percentage and diameter, and that concurred with markedly higher pigment content and higher pH_u, redness (a*), plasticity values, and significantly lower drip loss, WHC – loose water, and colour lightness (L*) values in ZS pigs than those found in commercial pigs. Also, sensory assessment of raw meat confirmed that *m. longissimus lumborum* of ZS pigs was characterized by more favourable colour and less exudative meat.

Keywords: Złotnicka Spotted pigs, commercial pigs, carcass quality, meat quality, muscle fibres.

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Long-term selection and breeding programmes aimed at improving muscle content in slaughter pigs have resulted in the achievement of considerable progress in different breeds, but also a negative correlation between carcass meatiness traits and pork quality. One possible way to improve pork quality is to put greater emphasis on native breeds of pigs, including the Złotnicka Spotted. This breed offers many economically important advantages, such as high resistance to pathogenic agents, low feed requirements and very good quality of meat (BUCZYŃSKI *et al.* 2003; FLOROWSKI *et al.* 2006). Results of earlier studies provide conclusive evidence that Złotnicka Spotted pigs can be used in breeding programmes aimed at producing valuable slaughter material meeting high technological demands (FLOROWSKI *et al.* 2006).

An important component of meat quality assessment is identification of muscle microstructure. It is widely accepted that one of the main factors determining physico-chemical traits is fibre type composition. RYU and KIM (2006) suggested that fibre type composition is associated with *post mortem* changes in the conversion of muscle to meat, and subsequently meat quality. Hence, in addition to its cognitive significance, the linking of muscle microstructure with physico-chemical properties of the muscle makes it possible to understand the

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basis of meatiness and regulation of meat characteristics. Therefore, the purpose of the present study was to compare selected parameters of meat production, quality and microstructure of *m. longissimus lumborum* in Złotnicka Spotted pigs with commercial pigs.

Material and Methods

The subjects included 96 fatteners representing two different groups: Złotnicka Spotted (ZS) (n=61) and commercial crossbreds Polish Large White × Polish Landrace (PLW × PL) (n=35). The study was carried out at the Rolmięs organic farm in Łabiszyn, Poland. All animals were reared in an organic farming system. Fattening started at a body weight of about 30 kg in group pens on deep litter. During this time, pigs received organic feed containing 12.6 MJ ME and 156 g crude protein. The feed consisted of a 40% triticale-lupin mixture, 20% rye, 10% barley-pea mixture, 10-15% rapeseed and oat grain, and 5% vitamin-mineral supplement. Animals were weighed and slaughtered at 210 days of age at a commercial abattoir (Rolmięs in Łabiszyn) according to routine procedure.

The day after slaughter, carcass quality was evaluated according to the Pig Testing Station method (RÓŻYCKI 1996). The following traits were measured: cold carcass weight, cold carcass yield (percentage of cold carcass in body weight), carcass length (from the anterior edge of the symphysis pubis to the recess of the first rib), backfat thickness (BFT) at five locations: over the shoulder (at the thickest point) (BFT1), on the back (behind the last thoracic vertebra and the first lumbar vertebra) (BFT2) and at three locations over the loin (cross-section of the gluteal muscle): over the rostral edge of the gluteal muscle (BFT3), in the middle of the gluteal muscle (BFT4), and over the caudal edge of the gluteal muscle (BFT5). The arithmetic mean was calculated from the five measurements of backfat thickness (BFT1-BFT5). In addition, the contour of the cross-sectional area of the *longissimus lumborum* muscle was traced behind the last thoracic vertebra and on the rostral surface, the height and width of this area was measured, and then the cross-sectional area (loin eye area) was determined using the LUCIA computer image analysis system.

The samples for meat quality assessment were taken from the right carcass side from the *longissimus lumborum* muscle at the level of the 1st-4th lumbar vertebrae.

The pH was measured at 45 min (pH_{45}) *post mortem* between the 3rd and 4th lumbar vertebra, and the acidity of meat, i.e. ultimate pH (pH_u) was determined 48 h *post mortem* in water extracts of meat (obtained by adding water to meat at a 1 : 1 ratio) using a pH meter (Matthäus, Germany) 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 muscle temperature.

Meat colour (CIE 1976) was measured at 24 h postmortem with a Minolta colorimeter (Chroma Meter CR-310, Minolta Camera C., Osaka, Japan) after exposing the surface to the air for 30 min at 4°C. The L* (lightness), a* (redness) and b* (yellowness) values were recorded from the average of three random readings across each muscle surface. Sensory evaluation of colour, exudation and consistency was performed by a panel of 5 judges using a 5-point scale described by CLAUSEN and THOMSEN (1956), where 3 is the optimum score. Drip loss (%) was determined as the weight loss of a meat sample (80 g), placed on a plastic bag, after a storage time of 48 h (24-72 h *post mortem*) at 4°C (HONIKEL 1987). Water holding capacity (WHC) was determined according to the Grau-Hamm method (GRAU & HAMM 1952), in which a weighed 300 mg sample of ground meat was placed on Whatman No. 1 filter paper under constant pressure of 2 kg for 5 min, and the expressible juice area was measured using the LUCIA computer image analysis system. The area of the pressed meat sample was used as a measurement of meat plasticity (GRAJEWSKA *et al.* 1998).

Tenderness of meat was determined by an instrumental method using an Instron 3342 tensile tester fitted with a Warner-Bratzler attachment. Meat samples were taken after 24 h carcass cooling at 4°C, and frozen until analysis. After thawing, they were warmed in a water bath until an internal temperature of 70°C was reached. The sample size was about 120 g and the heat treatment was conducted in a 0.85% sodium chloride solution. Next, they were wrapped in plastic film and stored in a cooler overnight before coring. At least six 1.25 cm diameter cores from each sample were removed parallel to the longitudinal orientation of the muscle fibers. The cores were sheared perpendicular to the muscle fibres at blade speed 50 mm/nim.

The intramuscular fat content (IMF) of the *m. longissimus lumborum* was determined on lyophilized muscle samples using an ANKOM XT10 semi-automated extraction system (AOCS 2004). Muscle pigment content was determined according to the method of HORNSEY (1956).

Within 45 min *post mortem,* muscle samples for histochemical and immunohistochemical analysis were taken from the right side of the carcass from the *m. longissimus lumborum* at the level of the 1-2 lumbar vertebra and deep within the muscle. Muscle samples were cut into 1 cm^3 pieces (parallel to the muscle fibres) and frozen in isopentane that was cooled using liquid nitrogen and stored at -80°C until subsequent analyses. Samples were mounted on a cryostat chuck with a few drops of tissue-freezing medium (Tissue-Tek; Sakura Finetek Europe, Zoeterwoude, The Netherlands). Transverse sections (10-μm thick) were cut at -20°C in a cryostat (Slee MEV, Germany). To determine the muscle histochemical composition, we used a modified combined method of NADH-tetrazolium reductase activity and immunohistochemical determination of the slow myosin heavy chain on the same section (WOJTYSIAK & KACZOR 2011). First, sections were air-dried for 1 h and incubated for 1 h at 37°C with medium for the determination of NADH-tetrazolium reductase (DUBOVITZ & BROOKE 1973). Next, on the same section, immunohistochemical staining with monoclonal antibodies against the skeletal slow myosin heavy chain was performed for 1 h at RT (clone WB-MHCs Leica, Germany, dilution 1:80). The reaction was visualized by NovoLink™ Polymer Detection System (Leica, Germany) according to the manufacturer's instruction. Finally, all sections were dehydrated in a graded series of ethyl alcohol, cleared in xylene and mounted in DPX mounting medium (Fluka, Buchs, Switzerland). A minimum of 300 fibres were counted in each section using a NIKON E600 light microscope. The percentage and diameter of muscle fibre types were quantified with an image analysis system using the Multi Scan v. 14.02 computer program.

The experimental data were analysed by a one-way analysis of variance with Statgraphics 5.0 (STSC Inc., Rockville, MD) software. The data were presented as LSM±SE.

Results and Discussion

The values and the statistical analysis of the carcass traits of the pigs studied are shown in Table 1.

Analysis of the obtained results showed that both slaughter weight (P<0.01) and cold carcass weight (P<0.001) were significantly lower for ZS pigs than for commercial pigs, although slaughter age was similar for the pig groups (210 days). Importantly, cold carcass yield (%) was only slightly lower compared to the slaughter weight of ZS pigs (P<0.05). In addition, ZS pigs had shorter carcasses (P<0.01) as well as a significantly smaller area, height and width of the loin eye (P<0.001). Backfat thickness measured at five locations and average backfat thickness were both higher (P<0.001) in ZS pigs when compared with commercial pigs. These findings are typical of native breed pigs. For example, Iberian pigs (SERRA *et al.* 1998) and Lantang pigs, native to China (DAI *et al.* 2009) had a significantly higher backfat thickness compared to Landrace pigs. Likewise, Korean native pigs exhibited a higher backfat thickness than European breeds (KIM *et al.* 2008). EDWARDS (2005) also reported that in traditional breeds the values of backfat thickness are in general much higher than those obtained from modern breeds, which are selected for leaner carcasses. Also in the case of loin eye parameters, similar to our study, DAI *et al.* (2009) reported significantly smaller loin eye at 60, 90 and 150 days of age in Lantang pigs compared to Landrace pigs.

The results for meat and sensory quality of the *m. longissimus lumborum* of the examined pig groups are represented in Table 2. The meat obtained from ZS pigs compared to that of commercial pigs was characterized by a higher pH_u value (P<0.05) and lower values for drip loss (P<0.05) and WHC

Table 1

Least squares means (LSM) and standard errors (SE) of carcass traits of ZS and commercial pigs

Traits	ZS	PWLxPL	Sig
Slaughter weight [kg]	107.12 ± 1.25	119.80 ± 2.29	**
Cold carcass weight [kg]	79.66 ± 1.01	91.66 ± 1.48	***
Carcass yield [%]	74.36 ± 0.84	76.51 ± 1.03	NS
Carcass length [cm]	83.76 ± 0.36	88.57 ± 0.52	**
Loin eye area [cm^2]	33.60 ± 0.75	51.15 ± 1.39	***
Loin eye height [cm]	4.62 ± 0.06	5.81 ± 0.13	***
Loin eye width [cm]	9.23 ± 0.09	11.06 ± 0.16	***
BFT1 [mm]	41.26 ± 1.01	9.51 ± 1.13	***
BFT2 [mm]	24.97 ± 0.72	20.83 ± 0.79	***
BFT3 [mm]	28.90 ± 0.94	22.13 ± 1.03	***
BFT4 [mm]	22.72 ± 0.67	15.60 ± 0.74	***
BFT5 [mm]	29.67 ± 0.88	21.11 ± 0.96	***
Average BFT1-5 [mm]	29.49 ± 0.72	21.83 ± 0.75	***

NS – non significant; ** P<0.01; *** P<0.001

Table 2

Least squares means (LSM) and standard errors (SE) of meat quality and sensory characteristics of *m. longissimus lumborum* of ZS and commercial pigs

Traits	ZS	PWLxPL	Sig.
Meat quality traits			
pH_{45}	6.33 ± 0.04	6.26 ± 0.06	Ns
pH_u	5.52 ± 0.01	5.45 ± 0.02	*
Colour L*	49.30 ± 0.32	52.37 ± 0.42	*
a*	17.34 ± 0.11	16.28 ± 0.15	*
b*	3.74 ± 0.22	4.49 ± 0.28	Ns
Drip loss [%]	2.55 ± 0.19	4.31 ± 0.31	*
WHC, % loose water	16.51 ± 0.41	20.32 ± 0.56	*
Plasticity [cm^2]	2.69 ± 0.05	2.42 ± 0.06	*
Warner-Bratzler shear force [N/ cm^2]	43.61 ± 1.66	43.57 ± 1.47	Ns
Pigments [μg hematin/ 1g]	43.45 ± 0.92	36.22 ± 0.89	*
IMF [%]	1.87 ± 0.09	1.70 ± 0.12	NS
Visual appraisal			
Colour, score	3.08 ± 0.04	2.66 ± 0.06	***
Exudation, score	2.92 ± 0.02	2.76 ± 0.03	**
Consistency, score	2.94 ± 0.02	2.91 ± 0.04	NS

NS – non significant; * P<0.05; ** P<0.01; *** P<0.001

(P<0.05). Moreover, as could be expected, the higher values of the expressible juice ratio (P<0.05) and greater plasticity (P<0.05) were characteristic of meat from ZS pigs. These results are consistent with the data of FLOROWSKI *et al.* (2006), who reported significantly higher pH_{48} values and lower drip loss and WHC values for *m. longissimus thoracis* of ZS pigs compared to Puławska and Polish Landrace pigs. Also earlier studies showed lower drip loss in Korean native black pigs (PARK *et al.* 2007) and higher pH_u values in Iberian pigs (SERRA *et al.* 1998) for *m. longissimus lumborum* compared to the commercial breeds. Moreover, in the current study the variables of meat colour showed differences between examined pigs: the *m. longissimus lumborum* from commercial pigs was lighter (higher L*) (P<0.05) than the muscles of ZS pigs. Regarding redness (a* value), ZS pigs had higher a* (P<0.05) than the commercial pigs. The colour measurements (L*, and a* indices) in *m. longissimus lumborum* of ZS pigs revealed values that correspond with the dark and red meat of native pig breeds (SERRA *et al.* 1998; FORTINA *et al.* 2005; ESTEVEZ *et al.* 2006). The observations are also confirmed by sensory tests, which showed that the meat from ZS pigs was characterized by more beneficial colour (P<0.001) and lower exudation (P<0.01). Moreover, darker, redder colour of ZS meat compared to commercial meat is probably associated with a higher haeme pigment content (P<0.05) observed in our study. Pigment content also increases with the age of the animal (GIL *et al.* 2008) and in our study the average age was the same for both examined groups of pigs (210 days). On the other hand, the higher pigment content in *m. longissimus lumborum* of ZS pigs is probably due to the fact that the content of pigments is influenced by muscle physiology, and our study demonstrated that muscle metabolism is more oxidative in ZS pigs than in commercial pigs, as discussed below. All the other meat quality traits: pH_{45}, yellowness (b*), and shear force, were not significantly affected (P>0.05) by the pig groups. Similarly, PARK *et al.* (2007) did not find significant differences in shear force between Korean native black pigs and Landrace pigs. On the other hand, concurrent to our findings, FLOROWSKI *et al.* (2006) reported significantly lower shear force values in *m. longissimus thoracis* of ZS pigs compared to Puławska and Polish Landrace pigs. It should be remembered that meat tenderness is determined by many factors such as the composition of muscle fibres, intramuscular fat and collagen content, sarcomere length, water holding capacity and the calpain system in addition to animal breed.

Intramuscular fat (IMF) is one of the main factors affecting the quality of pork, in particular its tenderness, juiciness and palatability (ESSEN-GUSTAVSSON *et al.* 1994). It is generally accepted that traditional breeds produce a higher IMF content (SERRA *et al.* 1998; ROSENVOLD & ANDERSEN 2003; FLOROWSKI *et al.* 2006; PARK *et al.* 2007; DAI *et al.* 2009). In our experiment we found no significant differences in IMF content

(P>0.05) between the pig groups studied. There was only a tendency towards a greater proportion of IMF in *m. longissimus lumborum* of ZS pigs compared to the commercial pigs. Different results were obtained by FLOROWSKI *et al.* (2006), who found a significantly higher IMF content of *m. longissimus thoracis* in ZS pigs compared to Puławska and Polish Landrace pigs. Such a low IMF content found in the present study in *m. longissimus lumborum* of ZS pigs is in line with data obtained by BOCIAN *et al.* (2009). One of the main factors determining meat quality is the muscle fibre composition. Many reports have examined the relationship between muscle fibre type characteristics and meat quality (LARZUL *et al.* 1997; RYU & KIM 2005). In the current study, in agreement with the meat quality traits analysed, we found significant differences in both the composition and size of muscle fibres between the pig groups studied (Table 3, Fig. 1). Accordingly, *m. longissimus lumborum* of ZS pigs had a greater percentage of type I fibres (P<0.05) and a smaller percentage of type IIB fibres (P<0.05) than the muscles of commercial pigs. Moreover, the diameter of type I fibres was larger and type IIB fibres smaller in ZS pigs than in commercial pigs, which may explain greater loin eye area found in commercial pigs in our study. However, it should be remembered that the amount of meat is determined not only by muscle fibre size but, above all, by muscle fibre quantity. The increase in the diameter of muscle fibres, especially white fibres (IIB) with a glycolytic metabolism, is connected with the increase in carcass meat content and lower fat content. On the other hand, it contributes to poorer meat quality, as reflected in a decreased number of capillaries and the associated muscle oxygen deficiency and increased production of lactic acid during glycolysis (RUUSUNEN & PUOLANNE 2004). This may ultimately be indicative of poorer meat quality in commercial pigs. The effect of breed on muscle fibre composition and size was also reported in earlier studies (LARZUL *et al.* 1997; SERRA *et al.* 1998; BOGUCKA & KAPELAŃSKI 2005; GIL *et al.* 2008; ORZECHOWSKA *et al.* 2008). SERRA *et al.* (1998) found similar relationships in Iberian pigs compared to Landrace pigs, similarly to those reported here. Likewise, RUUSUNEN & PUOLANNE (1997) reported that *longissimus* muscles of Hampshire pigs are more oxidative compared to those of others pigs, and showed that variation in muscle fibre composition in pigs within the breeds is larger than

Table 3

Least squares means (LSM) and standard errors (SE) of muscle fibre percentages (%) and diameter (Ø) measured in *m. longissimus lumborum* of ZS and commercial pigs

Traits	ZS	PLWxPL	Sig.
%IIB	72.36 ± 1.07	75.72 ± 1.63	*
%IIA	13.81 ± 0.77	12.33 ± 0.52	Ns
%I	13.83 ±0.75	11.95 ±0.59	*
ØIIB	60.06 ± 1.54	68.39 ± 0.97	*
ØIIA	42.31 ±0.99	45.02 ± 0.80	Ns
ØI	51.87 ± 1.36	47.96 ± 1.01	*

NS – non significant; * P<0.05

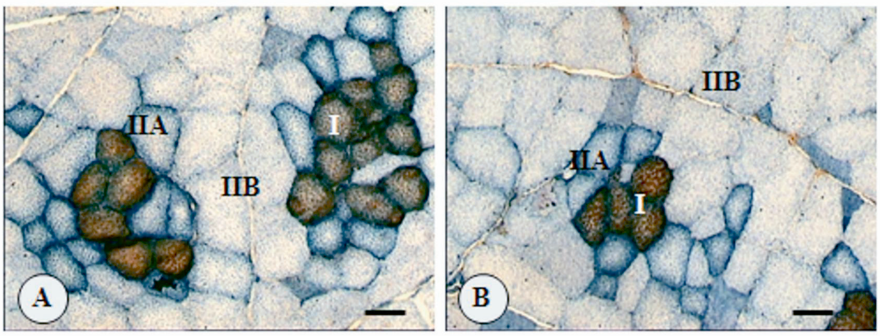

Fig. 1. Exemplary cross-sections of *m. longissimus lumborum* of Złotnicka Spotted (A) and commercial crossbreds Polish Large White × Polish Landrace (B) pigs: NADH-TH and immunohistochemical MHCslow staining: I – red fibres; IIA – intermediate fibres; IIB – white fibres. Bar 50 μm.

the average variation between the breeds. Meanwhile, DAI *et al.* (2009) found no differences in muscle fibre composition and size between Lantang and Landrace pigs in any of the analysed periods, i.e. 60, 90 and 150 days of age. In Korean native black pigs, PARK *et al.* (2007) noted significantly higher levels of MyHC I compared to the Landrace breed. The MyHC I (slow) isoform is a useful marker for type I fibres (PICARD *et al.* 1994). Also KIM *et al.* (2008) noted that the mRNA levels of oxidative and intermediate fibres were elevated in the Korean native pigs, whereas the glycolytic fibres were more highly expressed in the Landrace and Yorkshire pigs. KARLSSON *et al.* (1993) and WIELER *et al*. (1995) noted that breeds selected for faster growth and leanness have a higher percentage of fast fibres and stronger glycolytic metabolism than native, unselected, breeds or wild boars. All in all, our results indicate that the *m. longissimus lumborum* of ZS pigs is much more oxidative than in commercial pigs. In addition, our data also suggest a relationship between muscle fibre composition and meat quality, which is in accordance with previous reports (SERRA *et al.* 1998; RYU & KIM 2005). Hence, the greater proportion of type I oxidative fibres in the structure of *m. longissimus lumborum*, observed in our study in ZS pigs, is probably related to more beneficial meat quality traits, such as lower drip loss and WHC – loose water, darker colour (lower L* value), and higher ultimate pH. HENCKEL *et al.* (1997) suggested that haem pigments had a positive correlation with the percentage of type I muscle fibre and a negative correlation with that of type IIB fibre. These suppositions were corroborated by CHANG *et al.* (2003) and GIL *et al.* (2003), who reported that MyHC I (slow) isoform content is positively correlated with ultimate muscle pH. Likewise KANG *et al.* (2011), who analysed the relationships between quality parameters of *m. longissimus dorsi* in Berkshire pigs, showed a significant positive correlation between the pH_{24} and the content of MyHC I slow isoforms and a negative correlation with fast isoforms. Similar results have been reported in the *longissimus* muscle of three different genetic lines (GIL *et al.* 2003) and in the *psoas* muscle of Berkshire pigs (CHANG *et al.* 2003).

In summary, although the carcasses of ZS pigs had poorer quality parameters (lower slaughter weight, lower cold carcass weight, shorter carcasses, lower loin cross area), the pigs of this breed are characterized by greater fatness (higher backfat thickness), better meat quality as shown by higher pH_u, redness (a*), plasticity, and higher pigment content, significantly lower drip loss, WHC, and lightness (L*) values, as well as more beneficial colour and greater exudation of meat, determined by sensory analysis in *m. longissimus lumborum*, compared to the commercial pigs. In addition, the results obtained for microstructure of *m. longissimus lumborum*, namely the greater percentage of type I fibres of greater diameter coupled with the lower percentage of type IIB fibres of smaller diameter, found in ZS pigs compared to the commercial pigs, might be one of the reasons for the differences in carcass and meat quality.

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