

# Meat quality and ultrastructure of muscle tissue from fatteners of Wild Boar, Pulawska and its crossbreed Pulawska × (Hamshire × Wild Boar)

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## Abstract

The investigation included three genetic groups, i.e. Pulawska, Wild boar and Pulawska × (Hampshire × Wild boar) crossbreds. Each group comprised 8 individuals (1:1 sex ratio). The animals were presented for slaughter at 90 kg body weight. The muscles of the analysed genetic groups have shown good quality, pH 6.07-6.54. Lower diameters were characteristic for both, wild boars and wild pigs, that averaged 33.74 µm and 35.4 µm for *m. longissimus dorsi* (MLD) and then 42.3 µm and 46.43 µm for *m. semimembranosus* (MS), respectively. It is likely to arise from their higher rate in the muscles regarding total red fiber content. The shortest sarcomeres were established in a group of wild boars (2.03 µm MLD and 2.31 µm MS) that may confirm the association with their meat tenderness. The most juicy and tender meat was produced by pigs of Pulawska breed, whereas the meat obtained from Pulawska pigs and crossbreds earned the highest score for flavour intensity and taste.

Markedly higher sarcomeres number was characteristic for the *semimembranosus* muscles. Contractile fibres in wild boars' muscles were separated by greater amount of the sarcoplasm as compared to other animals. The analysis of *longissimus dorsi* and *semimembranosus* muscle microstructure confirmed that the presented genetic groups showed proper structure of the muscle tissue and did not show any degenerative lesions. The muscles visualized the well-preserved sarcomeric structure, appropriate cell nuclei and mitochondria. Besides, the regular cross striation was visible giving evidence of the muscle decontraction.

**Keywords:** pig, meat, ultrastructure, muscle fibres, meat quality, Pulawska, Wild boar

## Zusammenfassung

### Fleischqualität und Ultrastruktur des Muskelgewebes von Mastschweinen von Wildschweinen, Pulawi und Kreuzungen Pulawi × (Hampshire × Wildschwein)

Untersucht wurden je acht Mastschweine (Geschlechterverhältnis 1:1) der drei Genotypen Wildschwein, Pulawi und Kreuzungen aus Pulawi × (Hampshire × Wildschwein). Die Muskeln der mit 90 kg geschlachteten Tiere zeigten eine gute Qualität mit pH Werten von 6,07 bis 6,54. Kleinere Faserdurchmesser waren sowohl für Wildschweine als auch die Kreuzungstiere charakteristisch. Sie betragen 33,74 µm und 35,41 µm beim *M. longissimus dorsi* (MLD) sowie 42,30 µm und 46,43 µm beim *M. semimembranosus* (MS),

was wahrscheinlich eine Folge des höheren Anteils an roten Fasern im Muskelgewebe ist. Die kleinsten Sarkomere wurden mit 2,03  $\mu\text{m}$  (MLD) und 2,31  $\mu\text{m}$  (MS) bei Wildschweinen beobachtet, was einen Zusammenhang mit der Zartheit ihres Fleisches bestätigt. Das saftigste Fleisch mit der größten Zartheit wurde bei Pulawitieren festgestellt. Die besten Noten für Geruchs- und Geschmacksintensität des Fleisches erzielten Pulawi- und Kreuzungstiere. Eine entschieden größere Anzahl von Sarkosomen war für MS charakteristisch. Die Myofilamente in den Wildschweinemuskeln wurden von einer größeren Sarkoplasmamenge als bei den anderen Gruppen getrennt. Die Mikrostruktur von MLD und MS zeigte, dass die untersuchten genetischen Gruppen eine einwandfreie Muskelgewebestruktur hatten und keine destruktiven Veränderungen aufwiesen. In den Muskeln fanden sich eine gut erhaltene Sarkomerstruktur sowie entsprechende Zellkerne und Mitochondrien, sichtbar waren ebenfalls regelmäßige Querstreifen welche die Lockerung des Muskels belegen.

**Schlüsselwörter:** Schwein, Fleisch, Ultrastruktur, Muskelfaser, Fleischqualität, Pulawi, Wildschwein

## Introduction

The ongoing intensive selective breeding practices aim to achieve genetic improvement through selection of the animals that will produce carcasses of increased lean meat content and a reduced intramuscular fat level. This breeding goal, however, has given rise to concern that the technological and eating quality of pork may be compromised. According to the studies of numerous authors (ENDER *et al.* 2003, FIEDLER *et al.* 2001, FIEDLER *et al.* 2004, GLODEK *et al.* 2004, KARLSON *et al.* 1999, LENGERKEN VON *et al.* 1994, SERRA *et al.* 1998), to gain more scientific insight into the histological structure of muscle tissue would be very helpful in this respect. The changes in muscle fibre size and percentage may affect not only the muscle motor characteristics but its overall quality and appetizing attractive appearance as well (BAROWICZ 2006). While, studying the microstructure of a pig muscle tissue at the pre-rigor stage, its heterogenous structure in relation to meat biochemical parameters may contribute to the early detection of desirable and undesirable meat quality traits (FIEDLER *et al.* 1993, REHFELD 2005, SOBINA and KONDRATOWICZ 1999). Therefore, muscle fibre properties are recognized as the extra criterion for meat quality evaluation. Recently, the consumers' perception of pork has appeared to relate to its nutritional value which seems to drive purchase intent (CERNADAS *et al.* 2005, DRANSFIELD 1999, DRANSFIELD 2001, FIEDLER *et al.* 1998, TYSZKIEWICZ 1995, ŻOCHOWSKA-KUJAWSKA *et al.* 2006). However, the survey studies indicate that consumer acceptability of pork and its products is significantly influenced by the sensory assessment of meat, among others, tenderness being the top rank. The studies on relationships between the fibre morphological traits and meat tenderness are very scanty and they focus primarily on cattle and sheep (ILLIAN *et al.* 2004, SWATLAND 2003). Mechanisms controlling pork quality development are often associated with altered *post mortem* muscle metabolism. Specifically, changes in the extent or rate of glycolysis can create unfavourable muscle pH. A high rate of pH decline and a low ultimate pH result in muscle protein denaturation and diminished quality parameters

(KLOSOWSKA 1984). Some more recent studies suggest relationship between fibre size or type and eating quality (tenderness, juiciness, colour) in pork (BOGUCKA and KAPELANSKI 2004, FIEDLER *et al.* 2004, ILIAN *et al.* 2004, REHFELD 2005, WOJTYSIAK and MIGDAL 2007). Crossing the pig makes it possible to obtain high-quality raw meat with attractive culinary and dietary properties.

The objective of the study was to determine the impact of a fatter genetic group on meat quality and ultrastructural properties of muscle tissue.

## Material and methods

The investigation included three genetic groups, i.e. Pulawska, Wild boar and Pulawska × (Hampshire × Wild boar) crossbreeds of the first litters. Each group comprised 8 individuals (1:1 sex ratio). The animals were presented for slaughter at 90 kg ( $\pm 3$  kg) body weight and slaughtered at the same period in the pork butchery in Lublin. The meat quality traits were assessed on the meat samples excised from the right half carcasses, *m. longissimus dorsi* (MLD) at 13th-14th thoracic vertebrae height and *m. semimembranosus* (MS) 45 min following slaughter. Meat acidity of carcasses was determined at 45 min (so-called pH1) and 24 h *post mortem* (pH2) using pH STAR CPU pH meter equipped with a glass-calomel electrode, while meat colour defined according to World Health Organization. The organoleptic and culinary characteristics of meat from various fatter classes were evaluated by a panel of 6 assessors.

The muscle samples for ultrastructural examinations were fixed in 4% glutaraldehyde buffered by 0.1M phosphate buffer of 7.4 pH for 12 h at room temperature. Then, the tissue material rinsed in cold phosphate buffer was fixed in 1% osmium tetroxide in the same 0.1 M buffer for 2 h. The subsequent processing step was the dehydration commenced by placing the sections in a series of ethanol solutions of graded concentration and followed by the experimental material baths in the solutions with increasing concentration of propylene oxide in alcohol. Afterwards, the specimens rinsed in a series of resin (Spurr) solutions of increasing concentration were embedded in polyethylene capsules containing resin and allowed to stay at 60°C for 12 h to polymerize. The obtained material was cut into half thin sections of 1  $\mu$ m thickness and 50 nm-thick ultrathin ones using an ultramicrotome Reichert Ultracut S. device. The half thin serial samples were stained by methylene blue with 1% azure II in 1% sodium tetrabromide, whereas the ultrathin ones with 8% uranyl acetate in 0,5% acetic acid for 45 min. Washed and dried serial series were poststained with lead citrate for 10 min. The ultrathin sections were examined and photographed with a TEM TESLA BS-500 transmission electron microscope in The Laboratory of Electron Microscopy, The Subdepartment of Histology and Embryology, Medical Faculty in Lublin. The measurements of contractile myofibril diameters and sarcomere length were performed using the computer image analysis (CIA).

The obtained results were analyzed statistically with one-factor analysis of variance assisted by STATISTICA ver. 5.0 computer software. The statistical model account: sex, age, body weight and breed.

## Results

The data summarized in Table 1 reveal that MLD reaction determined 45 min *post mortem* was slightly differentiated between the genetic groups (pH 6.07-6.33). Higher concentration of hydrogen ions was characteristic for the MS (pH from 6.32 up to 6.54) (Table 2).

Table 1  
Meat quality and morphological parameters of muscle fibres of the *m. longissimus dorsi*  
*Fleischqualität und morphologische Parameter der Muskelfasern von M. longissimus dorsi*

Trait	Pulawska		Wild boar		Crossbreds	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
pH1	6.07 <sup>a</sup>	0.25	6.33 <sup>b</sup>	0.28	6.21 <sup>b</sup>	0.30
pH2	5.41 <sup>A</sup>	0.20	5.80 <sup>B</sup>	0.18	5.75 <sup>B</sup>	0.22
Colour the WHO standards (points)	2.25	0.87	4.75	0.14	3.60	0.42
Flavour						
Intensity	4.60 <sup>a</sup>	0.50	4.30 <sup>b</sup>	0.05	4.60 <sup>a</sup>	0.60
Desirability	4.70 <sup>A</sup>	0.30	4.10 <sup>B</sup>	0.20	4.65 <sup>A</sup>	0.30
Taste						
Intensity	4.60 <sup>A</sup>	0.20	4.00 <sup>B</sup>	0.20	4.70 <sup>A</sup>	0.30
Desirability	4.60 <sup>aA</sup>	0.30	4.10 <sup>B</sup>	0.10	4.80 <sup>bA</sup>	0.15
Juiciness	4.70 <sup>A</sup>	0.25	3.60 <sup>aB</sup>	0.35	4.00 <sup>bB</sup>	0.40
Tenderness	4.60 <sup>A</sup>	0.40	3.80 <sup>B</sup>	0.10	4.50 <sup>A</sup>	0.30
Fibre diameter, $\mu\text{m}$	71.07 <sup>A</sup>	12.09	33.74 <sup>B</sup>	13.15	35.41 <sup>B</sup>	9.21
Sarcomere length, $\mu\text{m}$	2.81 <sup>A</sup>	0.25	2.43 <sup>B</sup>	0.21	2.82 <sup>B</sup>	0.27

Means values marked with differences letters are statistically significantly different capital letters at  $P \leq 0.01$ , small letters at  $P \leq 0.05$ .

Table 2  
Meat quality and morphological parameters of muscle fibres of the *m. semimembranosus*  
*Fleischqualität und morphologische Parameter der Muskelfasern von M. semimembranosus*

Trait	Pulawska		Wild boar		Crossbreds	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
pH1	6.32 <sup>a</sup>	0.23	6.54 <sup>b</sup>	0.15	6.47 <sup>b</sup>	0.20
pH2	5.50 <sup>A</sup>	0.28	5.85 <sup>B</sup>	0.12	5.70 <sup>B</sup>	0.23
Colour the WHO standards (points)	2.87	0.16	4.89	0,08	3.64	0.58
Flavour						
Intensity	4.70 <sup>A</sup>	0.30	4.10 <sup>B</sup>	0.10	4.60 <sup>A</sup>	0.30
Desirability	4.80 <sup>A</sup>	0.50	3.80 <sup>B</sup>	0.10	4.70 <sup>A</sup>	0.30
Taste						
Intensity	4.60 <sup>A</sup>	0.40	4.15 <sup>B</sup>	0.20	4.70 <sup>A</sup>	0.40
Desirability	4.70 <sup>a</sup>	0.80	4.00 <sup>bA</sup>	0.10	4.50 <sup>bA</sup>	0.30
Juiciness	4.80 <sup>aA</sup>	0.60	3.70 <sup>B</sup>	0.20	4.20 <sup>bA</sup>	0.40
Tenderness	4.75 <sup>aA</sup>	0.30	4.00 <sup>bB</sup>	0.20	4.40 <sup>b</sup>	0.50
Fibre diameter, $\mu\text{m}$	83.23 <sup>a</sup>	13.39	42.30 <sup>B</sup>	9.00	46.43 <sup>B</sup>	10.05
Sarcomere length, $\mu\text{m}$	2.75 <sup>a</sup>	0.09	2.51 <sup>b</sup>	0.34	2.83 <sup>a</sup>	0.25

Means values marked with differences letters are statistically significantly different capital letters at  $P \leq 0.01$ , small letters at  $P \leq 0.05$ .

From sensory profiling, the highest concentration of flavour and taste was recorded for meat from crossbreds and Pulawska breed pigs. Besides, meat from the latter breed animals had the highest scores for juiciness. Whereas meat produced by wild boars, as compared to other genetic groups, earned a lower meat tenderness and juiciness score.

There were indicated significant differences in muscle fibre thickness between the investigated genotypes. Shorter diameters were characteristic for both, wild boars and crossbred pigs which averaged 33.74  $\mu\text{m}$  and 35.41  $\mu\text{m}$  for MLD and 42.3  $\mu\text{m}$  and 46.43  $\mu\text{m}$  for MS muscle, respectively. The sarcomere length values showed significant differences between the analyzed groups. The shortest sarcomeres were noted in the wild boar group (2.03  $\mu\text{m}$  MLD and 2.31  $\mu\text{m}$  MS) that supports their relation with meat tenderness.

The microscopic image of muscles from Pulawska breed pig meat visualized the typical cross striation resulting from the organized arrangement of interdigitating actin thin and myosin thick filaments (Figure 1). The MLD muscle ultrastructure revealed abundance of thick bundles of contractile fibres. Slight amount of the sarcolemma detected between the fibres contained few glycogen granules. In the electronograms, scarce oval mitochondria were found located around the cell nuclei, while big, oval and regular-shaped cell nuclei with dense chromatin were distributed immediately below the nucleus capsule. The MS muscle showed the presence of considerable amount of sarcoplasm and relatively scanty bundles of contractile fibres. Besides, there were noticeable great numbers of big-sized, densely arranged mitochondria with marked mitochondrial cristae located between the contractile fibre bundles (Figure 2). In sarcoplasm, few glycogen granules were detected.

Loin contractile fibres (MLD) in the wild boar group exhibited a well preserved sarcomere structure. Besides, considerable amount of sarcoplasm between the contractile fibres with substantial quantity of glycogen particles was observed. There were also visualized fine, oval mitochondria of normal morphology, no degenerative lesions were recorded (Figure 3). The cell nuclei appeared as oval and elongated.

Analyzing the specimens obtained from wild boar ham muscle (MS), the normal ultrastructure of cross-striated muscles was observed. There were seen regularly arranged sarcomeres with clearly marked Z lines and isotropic bands and anisotropic ones (Figure 4). Contractile fibres separated by abundant sarcoplasm were visible as, just like in MLD case, very wide and delicate. The sarcoplasm contained numerous and large-sized sarcosomes, while in the mitochondrial matrix, cristae were quite distinct.

The sarcoplasm was shown to be rich with glycogen granules. The cell nuclei were big, oval with nuclear chromatin dense at the nucleus circumference area. In the crossbred pig group, in both MLD (Figure 5) and MS (Figure 6) muscle there were observed short contractile fibres with well marked sarcomeres and abundant sarcoplasm lying between them. The sarcoplasm contained numerous mitochondria of various shapes and well observable cristae. The cell nuclei appeared as elongated with dense chromatin material located below the nuclear capsule. In the MLD, great amount of glycogen particles was noted.

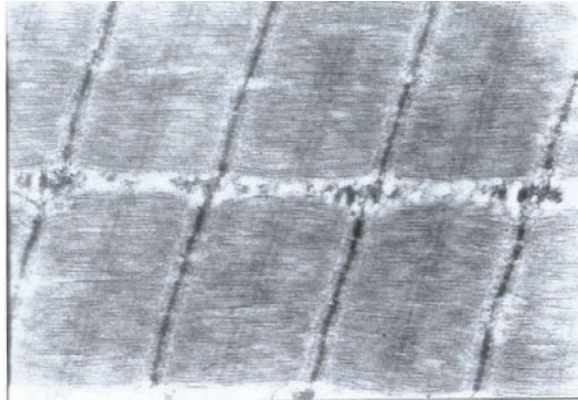


Figure 1  
Image of MLD ultrastructure muscle tissue (normal sarcomeres) of Pulawska breed pig  $\times 14\,000$   
*Ultrastrukturbild des Muskelgewebes MLD von Pulawischweinen (normale Sarkomer)  $\times 14\,000$*

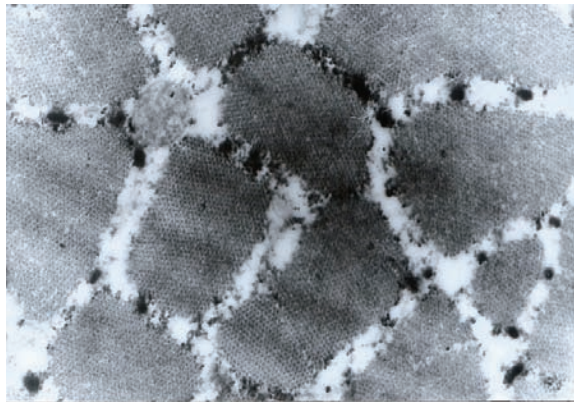


Figure 2  
Electron microphotographs of normal muscle fibre (MS) of Pulawska breed  $\times 14\,000$   
*Aufnahme mit Elektronenmikroskop normaler Muskelfasern (MS) von Pulawischweinen  $\times 14\,000$*

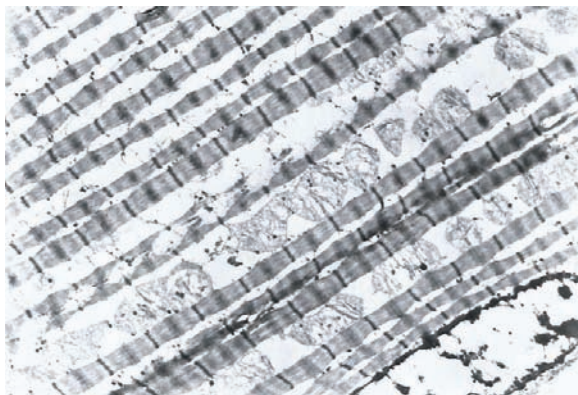


Figure 3  
Image of MS ultrastructure muscle tissue (sarcomeres, mitochondrion, nucleus) of Wild boar  $\times 4\,000$   
*Ultrastrukturbild Muskelgewebe MS (Sarkomer, Mitochondrien, Kern) des Wildschweines  $\times 4\,000$*

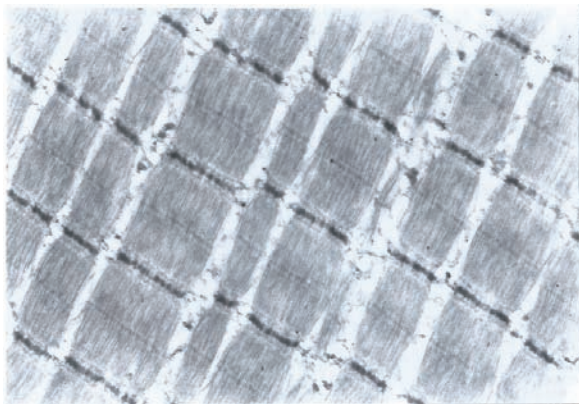


Figure 4  
Structure of muscle fibres MLD of Wild boar (sarcomeres, isotropic and anisotropic bands)  $\times 10\ 000$   
*Muskelgewebestruktur MLD des Wildschweines (Sarkomer, isotrope und anisotrope Streifen)  $\times 10\ 000$*

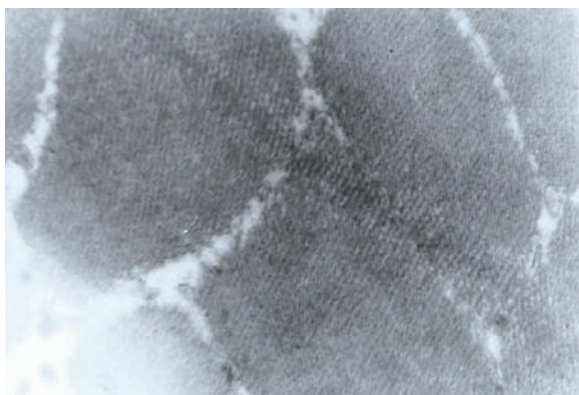


Figure 5  
Electron microphotographs of normal muscle fibre (MLD) of Pulawska  $\times$  (Hampshire  $\times$  Wild boar)  $\times 24\ 000$   
*Elektronenmikroskopaufnahme normaler Muskelfasern (MLD) Pulawi  $\times$  (Hamshire  $\times$  Wildschwein)  $\times 24\ 000$*

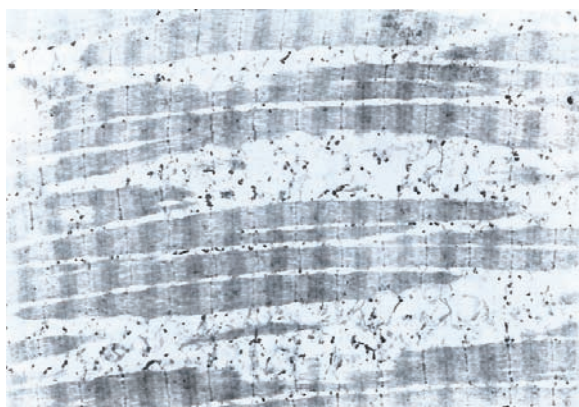


Figure 6  
Structure of muscle fibres MS of Pulawska  $\times$  (Hampshire  $\times$  Wild boar)  $\times 4\ 000$   
*Muskelfaserstruktur (MS) Pulawi  $\times$  (Hampshire  $\times$  Wildschwein)  $\times 4\ 000$*

## Discussion

Hydrogen ion concentration proves to be one of physical parameters employed as a criterion for assessment of meat technological quality. The muscles of the analyzed genetic groups were shown to have good quality – pH values over 6.0. Generally, pH value of meat is recognized a reliable predictor of ultimate quality of pork. However, owing to the fact that only some parts of a muscle may be watery, pH measurement may occasionally provide confusing estimates about meat quality. VAN LAACK *et al.* (2001) demonstrated that pH measured 24h post slaughter did not show any significant differences between normal and watery meat. Besides, pH measurements do not categorize the quality of pork carcasses appropriately if they are obtained from animals presented to slaughter with fatigued pig symptoms. As for pork meat, there is a close relationship between meat colour and pH, i.e. the higher pH value corresponds to the darker meat. The present researches have supported this assumption. A lower pH value means higher drip loss in pork. According to VAN LAACK *et al.* (2001) at pH exceeding 5.7 value, the decline rate of hydrogen ion concentration does not affect the pork quality and thus, no problems associated with porcine PSE and RSE occur.

Each measure of pork quality is dynamic in nature and has undergone various improvements made over time. The products, however, are evaluated in the light of the prevailing economic and social conditions as well as market competition with other new goods. Therefore, the meat sensory assessment influenced by animal breed, age and nutrition has been highlighted in this respect being the basis for consumers' purchasing decision (BAROWICZ 2006, DRANSFIELD 2001). In the present researches, sensory profiling indicated the pork produced from Pulawska breed pigs to be most juicy and tender. The highest ratings for intensity of overall flavour and palatability had the pork obtained from Pulawska pigs and crossbreds. The parameters determining the foods quality include mainly the basic morphological traits of muscle fibres, that is among others, their diameter and area of transverse cross-section as well as sarcomer length and width.

SWATLAND (2003) reports that they may correlate to the sensory attributes of meat, tenderness predominantly. The present investigations have demonstrated that meat from wild boars and crossbreds had the lowest muscle fibre diameters, which is likely to arise from its higher amount in the total red fibre number. Compared to other genetic groups, there were observed markedly shorter myofibril and sarcomer diameters in wild boar muscle tissue, which seems to correspond to meat tenderness in the sensory evaluation. ŻOCHOWSKA-KUJAWSKA *et al.* (2006) found that wild boar muscles were characterized by lower tenderness and juiciness as against pig muscles but according to the assessment conducted by the expert panel, their texture desirability was rated higher. Besides, wild boar muscles were shown to have smaller fibre area, thicker peri- and endomysium and a lower intramuscular fat concentration.

For nearly 50 years, numerous researches have been oriented to the issues concerning quality traits of pork and the results reveal substantial differentiation in meat quality. It is mainly associated with the differences occurring between porcine genotypes and environmental conditions.

Evaluation of the ultrastructural properties of muscles indicated that a muscle type and genotype of investigated individuals proved to be the basic factor determining the



microstructure. Markedly higher yield of sarcosomes was characteristic of the MS muscle. Contractile fibres in the wild boar muscles were separated by greater amount of sarcoplasm as compared to other groups. The analysis of muscle tissue microstructure demonstrated that the studied genetic groups had a normal structure of muscle tissue and did not show any degenerative lesions. The muscles visualized a well preserved sarcomere structure, proper cell nuclei and mitochondria as well as regular cross striation giving evidence for the muscle decontraction state (KÜCHENMEISTER and KUHN 2003). A lack of degenerative changes was most likely to rise from a fact that glycolysis process proceeded properly and meat acidity increased gradually. Numerous authors (BIEREDER *et al.* 1999, BOGUCKA and KAPELANSKI 2004, FEDDERN *et al.* 1995, KLOSOWSKA and FIEDLER 2003, KLOSOWSKA *et al.* 2005, SOSNICKI 1996, WOJTYSIAK and MIGDAL 2007) found that in pigs of reduced adaptive abilities to the changed environmental conditions, wavy arrangement of myofibrils was observed as well as giant muscle fibres that did not show cross striation at the longitudinal cross-section. Besides, the authors confirmed the presence of muscle fibres with granular or follicular degeneration of sarcoplasm, the changes in a cell nucleus shape and location, the atrophy and disappearance of glycogen granules and mitochondrial cristae as well as mitochondrial swelling. There were also reported some changes in contractile structural units consisting in the longitudinal or transverse degeneration of sarcomeres.

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