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Effect of g.2728G>A and g.3996T>C Polymorphisms at the Leptin Gene *Locus* on Microstructure and Physicochemical Properties of *longissimus lumborum* Muscle of Polish Landrace Pigs*

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The influence of *Hind*III (g.2728G>A) and *Bgl*II (g.3996 T>C) polymorphisms at the leptin gene *locus* on muscle fibre characteristics and meat quality of *longissimus lumborum* muscle was studied in 146 barrows of the Polish Landrace breed. Leptin gene polymorphism was identified by PCR-RFLP. Fibre type percentage, fibre diameter and the following technological parameters of meat were also determined: pH_{45}, pH_{24}, L*a*b* colour, drip loss, water holding capacity, shear force and intramuscular fat content. Polymorphism was not detected in the *locus* studied in the Landrace pig herd analysed with the *Bgl*II restriction enzyme (g.3996 T>C). For the *Hind*III enzyme (g.2728G>A), there was a high frequency of GG homozygotes (0.78) and G allele (0.89), but the AA genotype was not present. Moreover, the genotypes of leptin gene RFLP-*Hind*III polymorphism had no effect on intramuscular fat content and muscle fibre type percentage, but had a significant effect on muscle fibre size. Heterozygous GA fatteners had a significantly larger ($P<0.05$) diameter of type IIB and type I fibres compared to homozygous GG fatteners. Generally, meat quality parameters were comparable among the examined genotypes except for water holding capacity (which was the lowest for the GG genotype) and colour lightness (L*) (which was the lightest for GA genotype). Moreover, regardless of genotype, large differences were observed between each animal in the distribution of intramuscular fat.

Key words: Leptin gene polymorphisms, microstructure, meat quality, pig.

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Meat quality parameters in farm animals, determined by both environmental and genetic factors, are of economic importance to breeders. The advances in molecular biology over the last few decades have made it possible to identify genes regulating feed intake, growth, meat quality or body fat content. Breeders use two methods for identification of quantitative trait loci (QTL) – candidate genes and genome scanning (ROTHSCHILD 2003). Out of over 20 candidate genes associated with growth and especially with fatness, a mention should be made of leptin, which in addition to leptin, agouti and carbopeptidase E receptors is part of the central melanocortin system, which plays a key role in maintaining the body's energy homeostasis (BOLZE & KLINGENSPOR 2009; ŚWITOŃSKI *et al.* 2010). Leptin is a protein secreted from white adipocytes and results in a decrease in feed intake, loss of body weight, loss of fat depots and an increase in energy metabolism. In pigs, several polymorphisms were described for the leptin gene at the positions 867C>T, 1112A>G, 3469C>T, 3714G>T, A2845T, 3996T>C and 2728G>A (STRATIL *et al.* 1997; JIANG & GIBSON 1999; KENNES *et al.* 2001). The 3469T>C polymorphism was observed to have a significant effect on the fat depth and daily weight gain in Large White and Landrace pigs (JIANG & GIBSON 1999; KENNES *et al.* 2001), and on the lean meat content in Landrace pigs (KULIG *et al*., 2001). SZYDŁOWSKI *et al.* (2004) observed a weak association between the 3469T>C polymorphism and intramuscular fat content in Polish Large White pigs and loin weight in the synthetic line 990. The 2845A>T polymor-

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phism may be associated with feed intake in Landrace pigs (KENNES *et al.* 2001).

The relationship between polymorphism of the leptin gene and histological characteristics (muscle microstructure) is still not clear. It is well known that many characteristics of muscle tissue such as size, proportion of muscle fibres, collagen and intramuscular fat content directly affect meat quality (RYU & KIM 2005; LEPETIT 2008; OSHIMA *et al.* 2009; YANG *et al.* 2010). Therefore, the purpose of this paper is to evaluate *Hind*III (g.2728G>A) and BglII (g.3996T>C) polymorphisms at the leptin gene *locus* of Polish Landrace pigs and their relationship with histological characteristics of muscle and several other traits that affect meat quality.

Material and Methods

A total of 146 Polish Landrace barrows (only CC genotypes in analysis of mutation g.1843C>T in the ryanodine receptor 1 gene) were kept in individual pens equipped with automatic drinkers and fed *ad libitum*. All animals were reared under the same environmental and production regime. Animals were fattened from 50 to 105 kg body weight using a complete diet containing 13.3 MJ metabolizable energy and 161 g crude protein. This level was calculated based on diet composition, using tabular data for individual components (NORMY ŻYWIENIA ŚWIŃ 1993). When the pigs had attained the appropriate slaughter weight they were electrically stunned and exsanguinated. Pigs were slaughtered at a commercial slaughterhouse. Feed was withdrawn 12 h before slaughter but water was freely available in lairage.

DNA was isolated from whole blood drawn into sterile tubes containing EDTA. Isolation was carried out using a MasterPure™ Genomic DNA Purification Kit (Epicentre Technologies, USA), according to manufacturer's instructions. Polymorphisms at the leptin (*LEP*) *locus* g.2728G>A and g.3996T>C were identified using the PCR-RLFP method described by KENNES *et al.* (2001). Primers were synthesized by Genomed (Poland) and the PCR (Eppendorf Thermal Cycler, Germany) reactions were performed for each polymorphism in 25 μL reaction mixtures containing 150 ng of genomic DNA, standard PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.15 μM of each primer, and 2.0 units of Taq DNA polymerase (MBI Fermentas, Lithuania). The following cycling protocol was used: 94°C for 3 min, followed by 38 cycles of 94°C for 60 s, 65°C for 60 s and 72°C for 60 s, and a final extension step at 72°C for 6 min. The PCR products were digested overnight with 3 units of restriction enzyme *Hind*III (g.2728G>A) and 1.5 units of the enzyme *Bgl*II (g.3996T>C) (MBI Fermentas, Lithuania) at 37°C. The result was read from 1.5% agarose gels stained with ethidium bromide and visualized using the UVI-KS4001/Image PC system (Syngen Biotech, Poland).

Shortly after slaughter (15-30 min) samples for immunohistochemical analysis were taken from the right carcass side from the *m. longissimus lumborum* at the level of the 5th lumbar vertebra and deep within the muscle. Muscle samples were immediately cut into 1 cm^3 pieces (parallel to the muscle fibres) and frozen in isopentane that was cooled using liquid nitrogen and the samples were stored at -80°C until histochemical analyses were performed. 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 slow myosin heavy chain on the same section. First, sections were air-dried for 1 h and incubated for 1 h at 37°C with medium for the determination of NADH-TH (DUBOVITZ *et al.* 1973). Next, on the same section, immunohistochemical staining with monoclonal antibodies against 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 the 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). On the same section three different fibre types were recognized, namely, type I (red, slow twitch oxidative) which were blue-brown granulated; blue granulated type IIA (intermediate, fast-twitch oxidative glycolytic); and unstained type IIB (white, fast-twitch glycolytic) fibres. A minimum of 300 fibres were counted in each section using a NIKON E600 light microscope.

Moreover, frozen sections were also stained with Oil red O (Sigma, Germany) to evaluate lipid content. Intramuscular fat content and distribution was evaluated based on 10 randomly chosen areas (1000 m^2) in each preparation.

The percentage and diameter of muscle fibre types and percentage of intramuscular fat were quantified with an image analysis system using the Multi Scan v. 14.02 computer program.

The samples for meat quality assessment were taken from the right carcass side from the *longis-*

simus lumborum muscle at the level of the 2nd 4th lumbar vertebrae. The meat colour was assessed by the L* (lightness), a* (redness) and b* (yellowness) system (CIE 1976) using a Minolta colorimeter (Chroma Meter CR-310, Minolta Camera C, Osaka, Japan). The sample values were means from three measurements. The pH was measured using a Matthäus (Germany) pH meter with a glass electrode standardized for pH 4.0 and 7.0 according to Polish Standard PN-77/a-82058, with automatic correction for muscle temperature. Intramuscular fat content (IMF) was determined in duplicate according to the methods of BUDSŁAWSKI & DRABENT (1972). Water holding capacity (WHC) was determined as the amount of free water according to the filter paper press method of GRAU & HAMM (1953). For determination of drip loss two chops from each muscle were weighed (e=0.001g) 45 min postmortem (initial weight). Next, the chops were stored in a sealed plastic bag at 4°C. After 24 h of storage, the chops were blotted, and reweighed (final weight). Drip loss percentage was calculated by the following equation: [(initial weight – final weight) / initial weight] × 100. For measurements of tenderness, samples of meat (80 g) were packed separately in plastic bags and cooked in a water bath at 95°C until the core temperature reached 80°C. Then the samples were cooled, weighed for thermal loss determination, and prepared for the shear force measurements. One core (1.27 cm in diameter) was excised from each sample parallel to the muscle fibre orientation, through the thickest portion of the cooked muscle. Shear force was determined as maximum force (kg/cm^2) perpendicular to the fibres, using an INSTRON 5542 equipped with a Warner-Bratzler blade.

Associations of the pig genotypes with parameters of meat quality were calculated by analysing variance of quantitative traits, which included the percentage (%) and diameter (μm) of muscle fibre types and physico-chemical properties of *longissimus lumborum* muscle using GLM of Statistica v.8.0PL (StatSoft, 2009).

Results and Discussion

In the present study, we identified g.2728G>A and g.3996T>C polymorphisms at the leptin gene *locus* of the Polish Landrace population of pigs using *Hind*III and *Bgl*II restriction enzymes. Analysis of the genetic structure of Landrace pigs using *Bgl*II restriction enzyme (g.3996T>C) revealed no polymorphism at the *locus* studied. All the pigs were TT homozygotes.

The identification of g.2728G>A polymorphism using *Hind*III enzyme showed a high frequency of GG homozygotes (0.78) and the G allele (0.89), but no presence of the AA genotype. An earlier study by KENNES *et al.* (2001), which analysed this mutation in Duroc, Landrace and Yorkshire populations showed equally high G allele frequencies of 0.91, 0.85 and 1.0, respectively.

Identification of the RFLP-*Hind*III polymorphism made it possible to investigate its possible influence on the composition of muscle fibres and quality parameters of the meat from the analysed fatteners.

The effect of genotypes of the leptin gene on muscle fibre traits of *m. longissimus lumborum* of Polish Landrace pigs is shown in Table 1 and Fig. 1A-B.

The data revealed that the genotypes of the leptin gene had no effect on muscle fibre type percentage, but it affected the size of muscle fibres. Both type I and type IIB fibres from pigs with the GA genotype were characterized by considerably greater diameter compared to GG fatteners. These results were not confirmed by the literature data. However, the differences found in the present study in the diameter of muscle fibres of both types indicate a higher gain in muscle tissue of *m. longissimus lumborum* in GA genotype pigs compared to GG pigs. This was also indicated by KENNES *et al.* (2001), who showed that *LEP*/*Hind*III polymorphism is associated with the growth rate of fattened animals. In addition, the greater muscle fibres in GA pigs found in the present study would indicate that these animals have a greater loin eye. It must be remembered, however, that loin eye size is determined not only by the size but also by the amount of muscle fibres. The increase in the diameter of muscle fibres, especially white fibres (IIB) with 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 heterozygous (GA) animals.

The intramuscular fat content of meat is an important factor to be considered when evaluating the consumption quality of meat. DASZKIEWICZ *et al.* (2005) reported that a certain amount of intramuscular fat, which results in the marbling of meat and relaxation of connective tissue, is essential for the sensory traits of meat to be favourable. The results of the present analysis did not show a significant effect of the leptin gene g.2728G>A polymorphism on the intramuscular fat content (IMF) of *m. longissimus lumborum* from Polish Landrace pigs (Table 1). Meanwhile, KENNES *et*

Table 1

Percentage (%) and diameter (μm) of muscle fibre types and physico-chemical properties of *longissimus lumborum* muscle as related to leptin gene polymorphism

Item	GG N=114	GA N=32	SEM	*P-value*
Fibre type percentage (%)				
IIB	71.09	71.53	0.92	0.37
IIA	15.27	14.38	0.59	0.25
I	13.64	14.09	0.63	0.08
Fibre diameter (μm)				
IIB	56.68	65.26	0.79	0.02
IIA	42.49	44.32	1.12	0.14
I	47.45	55.93	0.63	0.03
pH_{45}	6.68	6.71	0.02	0.31
pH_{24}	5.63	5.65	0.01	0.47
Colour				
L*	53.07	54.26	0.68	0.04
a*	14.35	14.18	1.19	0.69
b*	3.57	4.08	0.84	0.12
Drip loss (%)	2.63	2.48	0.31	0.38
WHC (%)	37.62	39.78	0.34	0.02
Shear force (kg/cm^2)	6.64	6.35	0.63	0.09
IMF %	1.72	1.86	0.27	0.42
IMF-H %[1]	1.23	1.39	0.41	0.25

[1]intramuscular fat content evaluated histochemically

al. (2001) suggest that lower frequency of the A allele (0.09) in Duroc pigs is related to the highest fatness of animal carcasses (0.66) compared to Landrace pigs, in which the frequency of this allele was 0.15 and carcass fatness level was 0.20. Different results were obtained by JIANG & GIBSON (1999), who studied C→T substitution at position 3469 (exon III) of the leptin gene, identified by STRATIL *et al.* (1997). Experiments with four different populations of pigs (Duroc, Hampshire, Landrace, Large White) showed a relationship between higher frequency of the C allele and lower fatness of Large White pigs. Meanwhile, KENNES *et al.* (2001), who studied the same polymorphism at the leptin gene *locus* obtained results suggesting that in the group of analysed animals (Duroc, Landrace, Yorkshire) the higher frequency of the C allele was responsible for greater carcass fatness. This indicates that the inconsistency of the mentioned results may be due to differences in the gene pools of the analysed populations (breeds), as evidenced by different genotype effects. It should also be recalled that these mutations are localized in the non-coding regions of the leptin gene. 3'UTR is an untranslatable region, but it is conjectured that these mutations may determine mRNA stability or translation efficiency, although this hypothesis needs to be confirmed. They may occur as molecular markers conjugated with specific regions controlling growth and feed intake (KENNES *et al.* 2001). These mutations may also determine the level of gene expression, as reported by TE PAS *et al.* (1999), who showed that point mutation in the 3' region of the myogenin gene has a significant effect on carcass meat content. It is also important that the quantity and quality of fat raw material results from the interaction between genetic type and many other factors such as the animal's age and sex, management and feeding conditions, and location of adipose tissue. The latest research (VILLALBA *et al.* 2009) on g.3469C>T polymorphism confirms the association between the leptin gene polymorphism and the level of carcass fatness according to the age of pigs and location of adipose tissue. Based on the measurements of Duroc pigs at different developmental stages, it was found that the genotype effect changes during individual growth and thus animal's age may influence the effect being estimated. In the investigated population, it was found that in the case of g.3469C>T leptin gene polymorphism, 160-day-old TT pigs had a 0.096 mg/kg higher intramuscular fat content compared to TC pigs, whereas at 220 days TT pigs had a 1.64 mg/kg lower intramuscular fat content compared to TC animals. In addition, GARDAN *et al.* (2006) discovered that the leptin

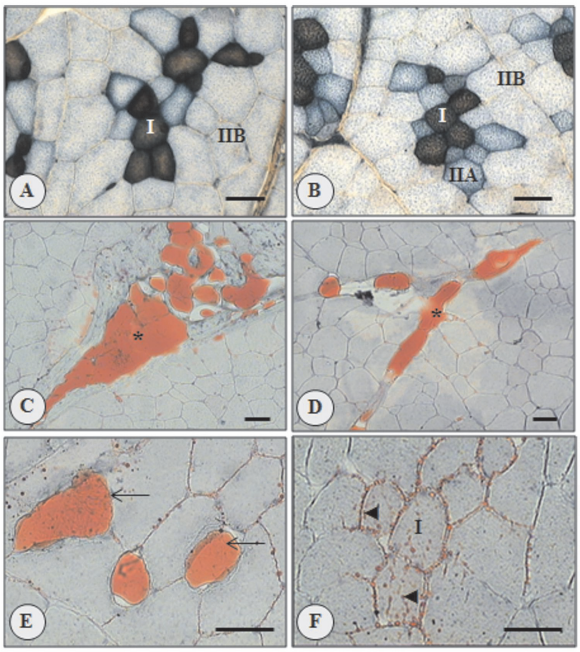

Fig. 1. Exemplary cross-sections of *m. longissimus lumborum* of Polish Landrace fatteners: NADH-TH and immunohistochemical MHCslow staining: I – red fibres; IIA – intermediate fibres; IIB – white fibres; A – genotype GA; B – genotype GG; C, D – intramuscular fat between muscle bundles (*); E – inside muscle bundles (↑); F – intracellular fat (▲). Scale bar 50 μm.

mRNA level between 160 and 210 days of age changes for intramuscular fat adipocytes but no such differences were found for subcutaneous fat adipocytes. It is believed that this results from differences in metabolism and from dissimilar hormonal expression profiles of different types of adipose tissue.

From the viewpoint of technological usefulness and fitness for human consumption, quality of pork is significantly affected not only by intramuscular fat content but also its distribution (Table 1, Fig. 1C-F). The lack of significant differences in intramuscular fat percentage, histochemically determined in the present study between the analysed GG and GA genotypes is due to large differences in intramuscular fat distribution and content between individual animals. Similar to what has been shown in the present study, intramuscular fat occurs not only between but also inside muscle bundles, forming clusters of lipid droplets located between single muscle fibres (LESEIGNEUR-MEYNIER & GANDEMER 1991) but also surrounding mainly type I (red) muscle fibres (ESSÉN-GUSTAVSSON *et al.* 1994). Intramuscular fat is also composed of small lipid droplets located inside red fibres (type I) in the form of intracellular fat. This is consistent with ESSÉN-GUSTAVSSON *et al.* (1994) who showed, in an analysis of the microstructure of pig muscles, that lipids are also deposited in type I and IIA fibres, i.e. in fibres with higher oxidative capacity, and to a lesser extent in white fibres (type IIB) with glycolytic metabolism.

The comparison of meat quality traits (Table 1) did not reveal differences in pH_{45min} and pH_{24h} between the analysed genotypes. Our results are similar to those reported by BORGES & GOULART (2002), who identified g.3469T>C polymorphism in the populations of Landrace, Large White and Pietrain pigs but also did not find the effect of animal genotype on pH of meat. In addition, in the present study, no significant differences in shear force values were identified between GA and GG genotypes. In the literature there are only a few studies on the influence of polymorphism at the leptin gene *locus* on meat quality of pigs. An earlier study with two sheep populations (Dorset, Suffolk) revealed the association between 103A>G polymorphism at the leptin gene *locus* and greater tenderness of *longissimus muscle* in the Suffolk breed (BOUCHER *et al.* 2006). On the other hand, marked differences were noted in the meat colour as affected by genotypes. L^* values were higher in GA than in GG genotypes, which suggests poorer quality of meat raw material from GA pigs. The lighter meat colour in GA pigs is probably related to the greater diameter of type IIB fibres in these animals. This is confirmed by LARZUL *et al.* (1997) and RYU & KIM (2005), who showed positive correlations between parameters of L^* colour and parameters of type IIB muscle fibres. Meanwhile, BORGES & GOULART (2002), who identified 3469T>C polymorphism in the leptin gene in Landrace, Large White and Pietrain pigs, showed no relationship between animal genotype (TT, TC) and meat colour. This may be due to the fact that meat colour, like other meat traits, is affected by several factors such as IMF content, pH and water binding in muscle, the interaction of which determines the variation of this parameter.

Some of the major quality traits of pork, important for technological processing, are water holding capacity and drip loss. In our study we did not find the effect of polymorphism, identified using *Hind*III, on drip loss. Significantly higher water holding capacity was found, however, in m. *longissimus lumborum* of pigs with the GA genotype compared to GG animals. SAFFLE & BRATZLER (1959) hold that muscles with a higher content of intramuscular fat have higher water holding capacity, which is probably due to the relaxation of muscle fibre microstructure by intramuscular fat, which allows for higher water activity in tissue (HAMM 1960). This finding is reflected in the results of the present study, in which GA pigs showed higher water holding capacity in m. *longissimus lumborum* and a tendency towards greater deposition of intramuscular fat compared to GG pigs.

Analysis of the microstructure and technological parameters of ML in the pigs studied revealed the effect of g.2728G>A polymorphism at the leptin gene *locus* on muscle fibre diameter and meat colour and water holding capacity. The results obtained do not provide a basis for definite conclusions because each of the analysed traits is most often modulated by the interaction of a pool of different genes and environmental factors. It is also possible that the leptin gene may influence other muscle parameters for which no genotype effect was found.

References

BOLZE F., KLINGENSPOR M. 2009. Mouse models for the central melanocortin system. Genes Nutr. **4**: 129-134.

BORGES G. S. DO N., GOULART L.R. 2002. Influence of obesity gene in quantitive traits of swine. Genet. Mol. Biol. **25**: 29-35.

BOUCHER D., PALIN M. F., CASTONGUAY F., GARIÉPY C., POTHIER F. 2006. Detection of polymorphisms in the ovine leptin (LEP) gene: Association of a single nucleotide polymorphism with the muscle growth and meat quality traits. Can. J. Anim. Sci. **86**: 31-35.

BUDSŁAWSKI J., DRABENT Z. 1972. Food Analysis Methods. WNT, Warszawa, 1-223. (In Polish).

DASZKIEWICZ T., BĄK T., DENABURSKI J. 2005. Quality of pork with different intramuscular fat (IMF) content. Pol. J. Food Nutr. Sci. 14/15, **1**: 31-36.

DUBOVITZ V., BROOKE M. H., NEVILLE H. E. 1973. Muscle biopsy. A Modern Approach, W. B. Saunders Company LTD London, Philadelphia, Toronto.

ESSEN-GUSTAVSSON B., KARLSSON A., LUNDSTROM K., ENHALT A. 1994. Intramuscular fat and muscle fiber lipid contents in halotan gene free pigs high a low protein diets and its relation to meat quality. Meat Sci. **38**: 269-277.

GARDAN D., GONDRET F., LOUVEAU I. 2006. Lipid metabolism and secretory function of porcine intramuscular adipocytes in comparison with subcutaneous and perirenal adipocytes. Am. J. Physiol. Endocrinol. Metab. 1-38.

GRAU R., HAMM R. 1953. Eine einfache Metode zur Besttimmung der Wasserbindung in Muskel. Naturwissen. **40**: 29.

HAMM R. 1960. Biochemistry of meat hydration. Adv. Food Res. **10**: 355-463.

JIANG Z. H., GIBSON J. P. 1999. Genetic polymorphism in the leptin gene and their association with fatness in four pig breeds. Mamm. Genome **10**: 191-193.

KENNES Y. M., MURPHY B. D., POTHIER F., PALIN M.-F. 2001. Characterization of swine leptin (LEP) polymorphisms and their association with production traits. Anim. Genet. **32**: 215-218.

KULIG H., GRZESIAK W., SZATKOWSKA I. 2001. Effect of leptin gene polymorphism on growth and carcass traits in pigs. Arch. Tierz., Dummerstorf **44**: 291-296.

LARZUL C., LEFAUCHEUR L., ECOLAN P., GOGUE J., TALMANT A., SELLIER P., LE ROY P., MONIN G. 1997. Phenotypic and genetic parameters for *longissimus* muscle fibre characteristics in relations to growth, carcass, and meat quality traits in Large White pigs. J. Anim. Sci. **75**: 3126-3137.

LEPETIT J. 2008. Collagen contribution to meat toughness: Theoretical aspects. Meat Sci. **80**: 960-967.

LESEIGNEUR-MEYNIER A., GANDEMER G. 1991. Lipid composition of pork muscle in relation to the metabolic type of the fibers. Meat Sci. **29**: 229-241.

Normy Żywienia Świń. 1993. Feeding standards for pigs and nutritive values of feeds. Institute of Animal Physiology and Nutrition of the Polish Academy of Sciences. Warszawa. Omnitech Press. (In Polish).

OSHIMA I., IWAMOTO H., NAKAMURA Y-N., TAKAYAMA K., ONO Y., MARAKAMI T., SHIBA N., TABATA S., NISHIMURA S. 2009. Comparative study of the histochemical properties, collage content and architecture of the skeletal muscle of wild boar crossbred pigs and commercial hybrid pigs. Meat Sci. **81**: 382-390.

ROTHSCHILD M. F. 2003. Approaches and challenges in measuring genetic diversity in pigs. Arch. Zootec. **52**: 129-135.

RUUSUNEN M., PUOLANNE E. 2004. Histochemical properties of fibre types in muscle of wild and domestic pigs and the effect of growth rate on muscle fibre properties. Meat Sci. **67**: 533-539.

RYU Y.C., KIM B.C. 2005. The relationship between muszle fiber characteristics, postmortem metabolic rate, and meat quality of pig *longissimus dorsi* muscle. Meat Sci. **71**: 351-357.

SAFFLE R. L., BRATZLER L. J. 1959. The effect of fatness of some processing and palatability characteristics of pork carcasses. Food Technol. **13**: 236.

StatSoft (2009), Data Analysis Software System, Statistica version 8.0 StatSoft, Poland

STRATIL A., PEELMAN L., VAN POUCKE M., CEPTICA S. 1997. A HinfI PCR-RFLP at the porcine leptin (LEP) gene. Anim. Genet. **28**: 371-372.

SZYDŁOWSKI M, STACHOWIAK M, MAĆKOWSKI M, KAMYCZEK M, ECKERT R., RÓŻYCKI M., ŚWITOŃSKI M. 2004. No major effect of the leptin gene polymorphism on porcine production traits. J. Anim. Breed. Genet. **121**: 149-155.

ŚWITOŃSKI M., STACHOWIAK M., CIESLAK J., BARTZ M., GRZES M. 2010. Genetics of fat tissue accumulation in pigs: a comparative approach. J. Appl. Genet. **51**: 153-168.

TE PAS M. F., SOUMILLION A., HARDERS F. L., VERBURG F. J., VAN DEN BOSCH T. J., GALESLOOT P., MAUWISSEN T. H. 1999. Influences of myogenin genotypes on birth weight, growth rate, carcass weight, backfat thickness, and lean weight of pigs. J. Anim. Sci. **77**: 2352-2356.

VILLALBA D., TOR M., VIDAL O., BOSCH L., REIXACH J., AMILLS M., SŔNCHEZ A., ESTANY J. 2009. An age-dependent association between a leptin C3469T single nucleotide polymorphism and intramuscular fat content in pigs Liv. Sci. **121**: 335-338.

YANG Y., ZHAO C., XIAO S., ZHAN H., DU M., WU C., MA C. 2010. Lipid deposition, composition and oxidative stability of subcutaneous adipose tissue and *Longissimus dorsi* muscle in Guizhou mini-pig at different developmental stage. Meat Sci. **84**: 684-960.