A relationship between the PCR-RFLP polymorphism in porcine *MYOG*, *MYOD1* and *MYF5* genes and microstructural characteristics of *m. longissimus lumborum* in Pietrain × (Polish Large White × Polish Landrace) crosses

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ABSTRACT: Muscle fibre formation takes place during embryonic development and is regulated by the *MyoD* gene family, which consists of four genes, *MYOD1, myogenin, MYF5* and *MRF4*. A relationship was studied between *MYOD1, myogenin* and *MYF5* genotypes and microstructural characteristics of the *m. longissimus lumborum* in pigs – crosses: Pietrain × (Polish Large White × Polish Landrace). The data included 115 unrelated animals slaughtered at about 105 kg live body weight. Within 45 min after exsanguination, samples were taken from the *m. longissimus lumborum*, frozen in liquid nitrogen and later analysed for the diameter of slow-twitch oxidative, fast-twitch oxidative and fast-twitch low-oxidative fibres, their proportion in a bundle, the proportion of pathological changes and number of fibres per unit area. The *RYR1* and *MyoD* genotypes were determined using the PCR-RFLP technique. A significant or highly significant relation was observed between the diameter of all types of muscle fibres and genotype *RYR1* – the highest values were recorded for homozygotes *TT* (genetically stress-sensitive). A relation between *MyoD* genotypes and microstructural characteristics of the *m. longissimus lumborum* was analysed on a group of 93 animals of the genotype *CC* or *CT* at locus *RYR1*. Sex appeared to have no significant effect on the muscle microstructural traits in this group of animals. The content of fast-twitch oxidative fibres (FTO) was significantly related to the *MYF5* genotype, whereas that of fast-twitch low-oxidative fibres (FT) was affected by the *MYOD1* and *MYF5*/*Dde*I genotypes. The proportion of angular fibres in a bundle was related to *MYF5*/*Hinf*I genotype. The results showed that *MyoD* genes could be considered as candidate genes for some microstructural characteristics of *m. longissimus lumborum* in pigs.

Keywords: *MyoD* genes; muscle fibres; pig; muscle microstructure

Skeletal muscle is a tissue of major economic importance for meat production. The differences in muscle size are due to differences either in the composition of myofibres and their diameters or to their number per unit area. The typing of myofibres is usually based on histochemical staining of myosin ATPase, pre-incubated at pH 9.4 (Brooke and Kaiser, 1970). The oxidative capacity of muscle fibres can be assessed by staining for NADH-tetrazolium reductase (NADH-TR) or succinate dehydrogenase (SDH). A combination of staining for myosin ATPase at pH 9.4 and NADH-TR or SDH separates the fibres into slow-twitch high-oxidative, fast-twitch high-oxidative and fast-twitch low-oxidative (or fast-twitch glycolytic) ones (Karlsson *et al*., 1999).

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Domestic pigs are selected on the basis of their ability to grow rapidly and the skeletal muscles of these pigs contain a higher percentage of fast-glycolytic, white muscle fibres than those of pigs growing slowly (Kłosowska *et al*., 1994; Karlsson *et al*., 1999). An increase in the carcass lean content of some pig breeds (e.g. Pietrain, Landrace) appeared to be associated to a greater degree with muscles containing a low percentage of oxidative and a high percentage of large diameter glycolytic fibres (see for the review – Karlsson *et al*., 1999) as well as with greater histopathological changes (Kłosowska *et al*., 1995). Intensive selection for lean growth in pigs may have caused a considerable genetic change in fibre type composition, which resulted in a higher proportion of glycolytic fibres and in an increase in fibre diameter in domestic pigs compared to native breeds (Rahelic and Puac, 1981; Weiler *et al*., 1995). It was also shown that the higher meat content in some pig breeds was related with a greater number of muscle fibres (Dwyer *et al*., 1993; Kłosowska *et al*., 1998). Changes in fibre type ratios affect metabolic properties of a muscle and thereby meat quality. The understanding of the growth and development of skeletal muscle is one of the most important goals in animal science as genetic variability and heritability are sufficiently high to use muscle fibre number and muscle fibre size as criteria in selection (Rehfeldt *et al*., 2000).

Muscle fibre formation takes place during embryonic development and is regulated by the *MyoD* gene family, which consists of four genes, *MYOD1* (*MYF3*), *myogenin* (*MYOG* or *MYF4*), *MYF5* and *MRF4* (*MYF6*) (Olson, 1990; Te Pas and Visscher, 1994). The *MYF5* and *MYOD1* genes are involved in myoblast proliferation. The expression of *MYOG* gene is associated with the fusion of mononucleated myoblasts into multinucleated myofibres. The myogenin gene knock-out mice show no muscle fibre development and heterozygous myogenin knock-out mice show half the number of muscle fibres observed in wild type of mice (Hasty *et al*., 1993). The *MYF5* gene is activated in the early embryo and a direct involvement of this gene in the initial muscle cell determination event was suggested. Knock-out mouse experiments showed that both genes, *MYF5* and *MYOD1*, do affect muscle development, although they are redundant to a certain extent. Mice lacking both *MyoD1* and *Myf-5* myogenic regulatory factors were born alive but they died soon after birth (Rudnicki *et al*., 1993). The *MYF6* gene is expressed principally in postnatal myofibres. The postnatal expression of *MYOD1*, *MYF5* and *MYOG* genes was found only in satellite cells and was specifically related to satellite cells activity (Grounds *et al*., 1992; Koishi *et al*., 1995). It has been shown that in adult rat the *MYOD1* gene was expressed principally in white muscles, while *MYOG* in red muscles (Hughes *et al*., 1993).

The *MyoD* genes are also interesting as candidate genes with an expected, significant effect principally on the muscle deposition in various animal species, including pigs. The currently known mutations of the porcine *MYOG*, *MYOD1* and *MYF5* genes were identified in the non-coding regions of those genes. The studies hitherto presented in literature referred principally to the effect of *MyoD* genes polymorphism on the growth rate and carcass content of lean meat in pigs (Te Pas *et al*., 1999a,b; Cieślak *et al*., 2000, 2002). However, no analysis was made of their effect on the microstructural characteristics of muscles.

The objective of this study was to investigate the relation between *MYOG*, *MYOD1* and *MYF5* genotypes and microstructural characteristics of the *m. longissimus lumborum* in Pietrain × (Polish Large White × Polish Landrace) crosses.

MATERIAL AND METHODS

Animals

A total of 115 unrelated pigs (56 females and 59 castrated males) – crosses of Pietrain and (Polish Large White × Polish Landrace) were slaughtered at about 105 kg live body weight. Rearing and feeding conditions were equal for all animals.

Genotyping of *RYR1* and *MyoD* polymorphisms

Blood samples were collected at slaughter into tubes containing K_2EDTA and genomic DNA was isolated from leukocytes by the method according to Kawasaki (1990) and Coppieters *et al*. (1992). *RYR1* genotypes were identified by the method according to Fujii *et al*. (1991). Polymorphism in the myogenin gene (*MYOG*) was determined at the 3′ end using the *Msp*I restriction enzyme, by the method according to Soumillion *et al*. (1997), while in intron 1 of *MYOD1* gene with enzyme *Dde*I, as described by Knoll *et al*. (1997). In the *MYF5* gene

two polymorphisms, identified with enzymes *Hinf*I (in intron 1) and *Dde*I (in intron 2), were analysed by the methods according to Te Pas *et al*. (1999a) and Stratil and Čepica (1999), respectively.

Determination of *m. longissimus lumborum* microstructure

For histological examinations muscle samples (approximately 0.5 × 0.5 × 1.5 cm) were taken with scalpel about 45 min post mortem from the middle part of *m. longissimus lumborum* (between 4th and 5th vertebra). The samples were immediately frozen in liquid nitrogen, stored until the time of analysis, cut in a cryostat into 10 μm thick sections and, in order to identify muscle fibre types [slow-twitch high-oxidative (STO), fast-twitch high-oxidative (FTO) and fast-twitch low-oxidative (FT)], subjected to a double reaction for the activity of NADH-TR oxidoreductase and myofibrillar ATPase (Wegner *et al*., 1993).

From each animal ten muscle bundles, containing on average between 440 and 550 muscle fibres, were randomly selected for an evaluation of proportions between muscle fibre types. All fibres within a bundle were counted and measured. In order to determine the various degenerative characteristics of the muscle, the sections were stained by the method according to van Gieson (Dubowitz *et al*., 1973) and an evaluation was made of the proportion of various pathological changes (atrophied and angular fibres, necrotic fibres with phagocytosis). Mean diameters (in μm) of all fibres of the same type were evaluated using a Leica Q500 MC image analysis system. The total number of the muscle fibres was calculated per unit area (mm^2).

Statistical analysis

The least squares method of the GLM procedure in the SAS statistical package (SAS 8.2; 2001) was used to analyse the relationship between *MyoD* genotypes and the *m. longissimus lumborum* microstructural traits. The fixed effects associated with sex and genotype were included in the linear model. Body weight at slaughter was added to the model as a covariate according to the following formula:

$$Y_{ijklm} = \mu + S_i + H_j + M_k + \beta(BWS_{ijkl} - BWS) + e_{ijklm}$$

where: Y_{ijklm} = character value for the $_{ijklm}$th animal

μ = overall mean

S_i = effect of ith sex (i = f, m)

H_j = effect of jth *RYR1* genotype (j = *CC*, *CT*, *TT*)

M_k = effect of kth genotype at the *MYOG* or *MYOD1* or *MYF5* loci (k = *AA*, *AB*, *BB*)

$\beta(BWS_{ijkl} - BWS)$ = linear regression for body weight at slaughter

e_{ijklm} = random error

RESULTS AND DISCUSSION

The mean diameter of STO, FTO and FT fibres, as identified in this study, was 47.9 μm, 47.4 μm and 62.5 μm, respectively. These results are comparable with the values found by other authors for the same muscle, although it is necessary to emphasize that different pig breeds and muscle sampling methods were used (Sosnicki, 1987; Maltin *et al*., 1997).

The mean proportions of STO, FTO and FT fibres in a bundle, observed in the present study, were similar to those presented by other authors for different pig breeds (Sosnicki, 1987; Brocks *et al*., 2000).

Effect of *RYR1* genotype on microstructural characteristics of *m. longissimus lumborum*

The literature data indicate various opinions concerning the effect of the *RYR1* genotype on muscle microstructural characteristics. Essen-Gustavsson *et al*. (1992) and Fiedler *et al.* (1999) demonstrated that the fibre diameter in *m. longissimus dorsi* was larger in pigs susceptible to stress, when compared to that observed in stress-resistant animals. In turn, Gallant (1980) and Ackermann and Salomon (1991) did not observe such a relation.

The effect of the *RYR1* genotype on the microstructural characteristics of *m. longissimus lumborum* in pigs tested in this study is shown in Table 1. The genotype at the *RYR1* locus showed a significant or highly significant effect on the diameter of all types of muscle fibres. The largest diameters of STO and FTO fibres were found in the muscle of animals of *TT* genotype (stress sensitive). This relation between the fibre diameter and genotype at the *RYR1* locus was reflected in the number of fibres per unit area being the lowest in the *m. longissimus lumborum* of animals of *TT* genotype. The proportion of STO, FTO and FT fibres in a bundle appeared to be inde-

Table 1. Mean value and least-squares means for diameter of STO, FTO and FT fibres and content of normal and pathological fibres in *m. longissimus lumborum* in the Pi × (PLW × PL) crosses as altered by genotype at the locus *RYR1*

Factor	Mean (115) ($\bar{x}$ ± SD)	*RYR1* genotype (*n*)		
		CC (35) LSM ± SE	*CT* (58) LSM ± SE	*TT* (22) LSM ± SE
Diameter of fibres:				
STO	47.9 ± 9.4	**46.1 ± 0.9^{A}**	**48.2 ± 0.8^{AB}**	**50.5 ± 1.3^{B}**
FTO	47.4 ± 10.5	**45.0 ± 1.1^{a}**	**48.3 ± 0.9^{ab}**	**48.6 ± 1.4^{b}**
FT	62.5 ± 15.4	**60.7 ± 1.2^{a}**	**63.6 ± 1.0^{b}**	**62.8 ± 1.6^{ab}**
Content of fibres in bundle:				
STO	16.4 ± 4.6	16.4 ± 0.8	16.6 ± 0.7	18.1 ± 1.2
FTO	16.4 ± 5.6	16.5 ± 0.9	16.5 ± 0.9	16.9 ± 1.4
FT	67.2 ± 7.7	67.1 ± 1.3	66.9 ± 1.0	65.0 ± 1.9
Pathological	9.9 ± 5.6	**9.8 ± 0.9^{A}**	**9.2 ± 0.8^{A}**	**14.2 ± 1.4^{B}**
Giant	1.9 ± 2.9	**1.6 ± 0.4^{A}**	**1.2 ± 0.3 ^{A}**	**5.8 ± 0.6^{B}**
Angular	0.7 ± 1.7	0.5 ± 0.3	0.9 ± 0.3	0.5 ± 0.5
Number of fibres/mm^2	179.3 ± 30.0	**189.7 ± 5.3^{A}**	**177.53 ± 4.4^{a}**	**157.8 ± 8.1^{Bb}**

Pi – Pietrain; PLW – Polish Large White; PL – Polish Landrace

^{AaBb}within rows means bearing different superscripts differ significantly at: small letters = $P \leq 0.05$, capitals = $P \leq 0.01$

pendent of the *RYR1* genotype. In turn, the content of various types of pathological fibres in a bundle, as well as that of giant fibres considered separately, was higher in *TT* animals (stress sensitive) compared to both the remaining *RYR1* genotypes ($P \leq 0.01$). Thus, the relation between the genotype at the *MyoD* loci and the value of *m. longissimus lumborum* microstructural characteristics was evaluated for 93 animals of both sexes being of genotype *CC* or *CT* at locus *RYR1*. The effect of the genotype *TT* at locus *RYR1* on the traits considered was eliminated in this way.

The opinions concerning the effect of sex on muscle fibre diameter and other properties are not uniform – some authors reported the absence of such an effect (Miller *et al*., 1975; Kłosowska *et al*., 1994; Larzul *et al*., 1997), while others reported significantly lower diameters of type I, IIA and IIB fibres in boars as compared to sows (Karlsson *et al*., 1999). The effect of pig sex on the diameter and content of all types of normal and pathologically changed fibres considered in this study in *m. longissimus lumborum* appeared to be insignificant in the case of animals of the genotype *CC* or *CT* at the *RYR1* locus (Tables 2 and 3).

Relation between the *MyoD* genotype and fibre diameter as well as the proportion of STO, FTO, FT and pathological fibres in a muscle fibre bundle

The genotype at the *MyoD* loci was proved to have no effect on muscle fibre diameter and on the number of fibres per unit area in *m. longissimus lumborum* of the tested pigs (Table 2). The presented studies did not show any significant effect of the *MYOG* genotype on the microstructural characteristics of *m. longissimus lumborum*, either. However, the absence of one of the homozygous genotypes (*AA*) may have affected the results of the statistical analysis. It should be mentioned here that the *AA* genotype shows a lower frequency within European pig breeds and lines as compared to that noticed within native breeds: Meishan (Soumillion *et al*., 1997), Zlotnicka Spotted (Cieślak *et al*., 2000), Mangalica (Anton *et al*., 2002). The only European breed showing a high frequency of *AA* genotype (77.5%) was the Great Yorkshire (Soumillion *et al*., 1997). Te Pas *et al.* (1999b) demonstrated that the *MYOG* genotype explained 5.8% of the total phenotypic variation of lean weight in Yorkshire pigs. This carcass trait is

Table 2. Mean value and least square means for diameters (μm) of STO, FTO and FT fibres and number of fibres per unit area (mm^2) in *m. longissimus lumborum* in Pi × (PLW × PL) crosses as altered by sex and *MyoD* genotypes

Factor	Genotype	Number of animals	Diameter of the fibres (μm)			Number of fibres/mm^2
			STO	FTO	FT	
Sex	males	46	46.7 ± 1.2	46.3 ± 1.4	61.5 ± 1.6	187.7 ± 7.4
	females	47	48.1 ± 1.9	47.9 ± 2.1	63.5 ± 1.4	176.3 ± 5.7
MYOG	*AB*	43	47.6 ± 1.0	46.6 ± 1.3	62.1 ± 1.2	182.0 ± 7.6
	BB	50	47.4 ± 1.0	47.7 ± 1.2	62.7 ± 1.3	182.2 ± 6.8
MYOD1	*AA*	46	47.7 ± 1.1	47.3 ± 1.1	63.6 ± 1.1	177.3 ± 4.4
	AB	43	46.5 ± 1.3	46.5 ± 1.2	61.0 ± 1.7	187.1 ± 6.1
	BB	4	50.8 ± 3.1	51.2 ± 3.4	65.3 ± 4.0	175.5 ± 5.8
MYF5/*Dde*I	*AA*	33	46.8 ± 1.3	47.6 ± 1.1	63.2 ± 1.4	179.6 ± 5.6
	AB	53	47.7 ± 0.9	46.6 ± 1.4	61.6 ± 1.2	185.1 ± 6.6
	BB	7	49.9 ± 1.9	49.7 ± 1.7	65.3 ± 2.5	173.3 ± 12.8
MYF5/*Hinf*I	*AA*	32	48.6 ± 1.6	47.6 ± 1.8	63.2 ± 1.1	182.2 ± 6.0
	AB	38	47.2 ± 1.5	47.7 ± 1.2	62.5 ± 1.3	177.8 ± 4.5
	BB	23	46.0 ± 1.3	45.6 ± 1.6	61.8 ± 2.0	187.9 ± 5.5

Pi – Pietrain; PLW – Polish Large White; PL – Polish Landrace

related to the number of fibres in muscles of pigs, thus the authors suggested that the causal mutation in the coding or regulatory sequences of the myogenin gene should be identified.

The genotype of animals at *MYOD1* locus significantly affected the proportion of FT fibres in a bundle in *m. longissimus lumborum*. The animals with *AB* genotype showed the highest content of this type of fibres in a bundle, when compared with both the remaining *MYOD1* genotypes. A positive molecular heterosis, described for several human genes by Comings and MacMurray (2000), may explain this phenomenon. The authors suggested the occurrence of an optimal gene expression in heterozygotes when the subjects heterozygous for a specific genetic polymorphism showed a significantly higher effect on a quantitative trait than the subjects homozygous for either allele. On the other hand, this point mutation being located in the intron of *MYOD1* gene may not be a causal mutation. Thus, one may suggest that it is linked to another mutation in the coding or regulatory regions of the gene being a causal mutation for the variability in a proportion of FT fibres in a bundle in *m. longissimus lumborum*. However, introns have been shown to affect transcriptional efficiency of numerous genes in a variety of organisms (see for the review – LeHir *et al*., 2003; Greenwood and Kelsoe, 2003). It should be mentioned here that a relationship between the fibre type composition and meat quality has been reported. Increasing the proportion of glycolytic fibres can lead to paler meat through a decrease in both myoglobin content and *post mortem* pH (Henckel *et al*., 1997; Larzul *et al*., 1997). Thus, the *MYOD1* genotype influencing the content of FT fibres in a bundle may indirectly affect the meat quality traits. However, a further study on the pig breed or crosses showing a higher frequency of *BB* genotype is needed to evaluate its effect on the microstructure of *m. longissimus lumborum*.

In the present study a significant effect was observed of genotype at loci *MYF5*/*Dde*I and *MYF5*/*Hinf*I on the proportion of FTO fibres in a bundle. Animals with *BB* genotype at *MYF5*/*Dde*I locus and those of *AA* genotype at MYF5/*Hinf*I locus showed a significantly higher proportion of FTO fibres than animals with the two remaining genotypes at each of the loci considered. Moreover, a significant differentiation in the percentage of FT fibres in a bundle was shown to depend on the *MYF5*/*Dde*I genotype. Animals with genotype *AA* had the high-

Table 3. Mean value and least square means for content (%) of STO, FTO, FT and pathologically changed fibres in *m. longissimus lumborum* in Pi × (PLW × PL) crosses as altered by sex and *MyoD* genotypes

Factor	Genotype	Number of animals	Content of fibres (%)					
			STO LSM ± SE	FTO LSM ± SE	FT LSM ± SE	Pathological LSM ± SE	Giant LSM ± SE	Angular LSM ± SE
Sex	males	46	16.6 ± 1.0	17.2 ± 1.1	66.2 ± 1.5	9.0 ± 1.2	1.2 ± 0.4	0.5 ± 0.5
	females	47	16.1 ± 1.1	15.8 ± 1.2	67.9 ± 1.6	9.4 ± 1.1	1.4 ± 0.3	0.7 ± 0.6
MYOG	*AB*	43	16.4 ± 0.8	16.6 ± 1.1	67.0 ± 1.6	9.3 ± 0.9	1.3 ± 0.4	0.4 ± 0.3
	BB	50	16.5 ± 0.7	16.5 ± 1.2	66.9 ± 1.5	9.0 ± 0.9	1.3 ± 0.2	0.7 ± 0.3
MYF3	*AA*	46	17.1 ± 0.8	17.7 ± 1.2	**65.1 ± 1.4^{ab}**	8.6 ± 0.9	1.6 ± 0.4	0.7 ± 0.3
	Ab	43	15.9 ± 1.1	15.1 ± 1.3	**69.2 ± 1.4^{a}**	9.1 ± 1.0	0.9 ± 0.3	0.4 ± 0.3
	BB	4	15.8 ± 2.5	20.4 ± 3.6	**63.8 ± 2.3^{b}**	9.8 ± 2.3	1.8 ± 1.0	0.4 ± 0.9
MYF5/*Dde*I	*BB*	33	17.0 ± 1.0	**16.2 ± 1.3^{ab}**	**68.1 ± 1.8^{a}**	9.1 ± 0.9	1.1 ± 0.3	0.9 ± 0.4
	AB	53	16.0 ± 0.8	**16.0 ± 1.1^{a}**	**66.8 ± 1.4^{ab}**	9.0 ± 0.8	1.5 ± 0.4	0.4 ± 0.2
	Bb	7	14.5 ± 1.9	**21.1 ± 2.1^{b}**	**61.4 ± 3.2^{b}**	8.0 ± 1.9	0.5 ± 0.8	0.6 ± 0.4
MYF5/*Hinf*I	*AA*	32	16.1 ± 1.0	**19.2 ± 1.4^{a}**	64.5 ± 1.5	9.5 ± 1.0	1.4 ± 0.4	**0.5 ± 0.2^{AB}**
	AB	38	17.0 ± 0.8	**16.3 ± 1.1^{ab}**	65.5 ± 1.3	8.7 ± 0.9	1.4 ± 0.3	**0.3 ± 0.3^{A}**
	BB	23	16.3 ± 1.3	**14.3 ± 1.9^{b}**	69.1 ± 1.1	9.9 ± 1.5	1.3 ± 0.6	**1.5 ± 0.5^{B}**

Pi – Pietrain, PLW – Polish Large White; PL – Polish Landrace

^{AaBb}within column and locus means bearing different superscripts differ significantly at: small letters – $P \leq 0.05$, capitals – $P \leq 0.01$

est proportion of FT fibres. Thus, one may suggest that the genotype at the *MYF5* locus influencing the proportion of FTO and FT fibres in a bundle may also affect the metabolic properties of muscle and thereby meat quality. It should be mentioned here that only 7 animals of *BB* genotype at the *MYF5*/*Dde*I locus were present in the examined group of animals but the absence of this genotype was also observed in all commercial pig breeds genotyped by Stratil and Čepica (1999).

Several studies have shown the presence of giant fibres in *post mortem* muscle (Cassens *et al*., 1969; Kłosowska *et al*., 1985; Handel and Stickland 1986; Essen-Gustavsson, 1995; Fiedler *et al*., 1999). The proportion of giant fibres observed in the present study (about 1% in animals with *CT* and *CC* genotype at the *RYR1* locus – Tables 1 and 2) was similar to that reported by Handel and Stickland (1986) in stress-resistant pigs. These authors showed that giant fibres were present in muscles of about 85% of normal pigs and were identified from birth to 128 days of age. They suggested that these ultrastructural changes of muscles might result from some defects in the muscle fibres during development, such as an inadequate amount of sarcoplasmic reticulum, eliciting hypercontractile activity and consequential structural and metabolic anomalies within the fibres. These authors also concluded that giant fibres did not appear to be a result of degenerative changes within the muscle. In the present study the genotype at the *MyoD* loci was proved to have no effect on the proportion of pathologically changed fibres in the bundle or on giant fibres analysed separately.

Only several papers discussing the occurrence of pathological fibres in pig muscles have been found in the literature. Bader (1987) concluded that the lesions of muscle fibres did not necessarily originate at the time of slaughter but could occur even during the animals' life. Wegner and Ender (1990) determined the occurrence of angular fibres during the postnatal development of Landrace pigs and found a higher frequency of that type of pathologically changed fibres during the phase of intensive growth of young animals than during subsequent phases of more moderate growth. A similar opinion was presented by Hausman and Campion (1986) as well

as Kłosowska *et al*. (1994) concluding that the high frequency of angular fibres in the muscle of pigs susceptible to stress was a result of a considerable increase in the muscle growth rate.

The presented analysis showed a significant effect of genotype at the *MYF5*/*Hinf*I locus on the proportion of angular fibres in a bundle in *m. longissimus lumborum*. The highest content of these fibres was observed in animals with *BB* genotype. It was documented in the previous study that the tested porkers of *AA* genotype at the locus *MYF5*/*Hinf*I showed the greatest loin eye area ($P < 0.01$) when compared to *AB* or *BB* genotypes (Cieślak *et al*., 2000). Moreover, growth rate of these animals was not related to the *MYF5*/*Hinf*I genotype (not published). Thus, summarizing a hypothesis on the origin of angular fibres and the results of our studies, it is rather difficult to conclude that a higher content of this type of pathological fibres in a bundle could be conditioned by a higher growth rate of muscle related to the genotype at the *MYF5*/*Hinf*I locus.

CONCLUSIONS

No relation between genotype at the loci *MYOG*, *MYOD1*, *MYF5* and fibre diameters as well as the number of fibres per unit area was observed in the pigs being crosses of Pietrain × (Polish Large White × Polish Landrace);

The *MYOD1*and *MYF5* genotype affecting the proportion of FTO and FT fibres in a bundle, may also influence the metabolic properties of muscle and thereby meat quality;

The highest content of angular fibres was characteristic for animals with *BB* genotype at the *MYF5*/*Hinf*I locus.

The porcine loci *MYOD1* and *MYF5* could be considered as candidate genes for some characteristics of *m. longissimus lumborum* microstructure.

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ABSTRAKT

Závislost mezi polymorfismem PCR-RFLP v genech *MYOG*, *MYOD1* a *MYF5* prasat a mikrostrukturálními charakteristikami svalu *m. longissimus lumborum* u kříženců plemen pietrain × (polské bílé ušlechtilé × polská landrase)

Tvorba svalových vláken probíhá během embryonálního vývoje a je regulovaná rodinou genů *MyoD*, kterou tvoří čtyři geny: *MYOD1*, *myogenin*, *MYF5* a *MRF4*. Byla sledována závislost mezi genotypy *MYOD1*, *myogenin* a *MYF5* a mikrostrukturálními charakteristikami svalu *m. longissimus lumborum* u prasat – kříženců plemen pietrain × (polské bílé ušlechtilé x polská landrase). Byly získány údaje o 115 nepříbuzných zvířatech, která byla poražena při dosažení tělesné hmotnosti kolem 105 kg. Za 45 minut po vykrvácení byly odebrány vzorky ze svalu *m. longissimus lumborum*, které byly zmrazeny v kapalném dusíku; později byla provedena jejich analýza zaměřená na zjištění průměru pomalu stažitelných oxidačních, rychle stažitelných oxidačních a rychle stažitelných glykolytických vláken, jejich podílu ve snopci, podílu patologických změn a počtu vláken na jednotku plochy. Genotypy *RYR1* a *MyoD* byly stanoveny pomocí metody PCR-RFLP. Byla zjištěna významná nebo vysoce významná závislost mezi průměrem všech typů svalových vláken a genotypem *RYR1*; nejvyšší hodnoty byly zaznamenány pro homozygoty *TT* (geneticky citlivé na stres). U souboru 93 zvířat genotypu *CC* nebo *CT* na lokusu *RYR1* byla provedena analýza závislosti mezi genotypy *MyoD* a mikrostrukturálními charakteristikami svalu *m. longissimus lumborum*. Pohlaví nemělo u této skupiny zvířat významný vliv na mikrostrukturální znaky svaloviny. Obsah rychle stažitelných oxidačních vláken byl významně závislý na genotypu *MYF5*, zatímco rychle stažitelná glykolytická vlákna byla ovlivněna genotypy *MYOD1* a *MYF5*/*Dde*I. Podíl vláken hranatého tvaru ve snopci závisel na genotypu *MYF5*/*Hinf*I. Výsledky naznačily, že geny *MyoD* lze považovat za kandidátní geny pro některé mikrostrukturální charakteristiky svalu *m. longissimus lumborum* u prasat.

Klíčová slova: geny *MyoD*; svalová vlákna; prase; svalová mikrostruktura

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