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An association between genotypes at the porcine loci *MSTN (GDF8)* and *CAST* and microstructural characteristics of *m. longissimus lumborum* : a preliminary study

Abstract

The aim of the study was to evaluate the association between the microstructure characteristics of *longissimus lumborum* (*LL*) muscle and the genotype at porcine loci *MSTN* and *CAST*. The study was carried out on 132 unrelated pigs – 93 crosses of Pietrain and (Polish Large White x Polish Landrace) and 39 Stamboek castrated males. Crosses Pi x (PLW x PL), with an equal proportion of castrated males (n=46) and females (n=47), were of genotype CC or CT at the locus *RYR1*, whereas Stamboek pigs were of genotype *CC* at this locus. The diameters of slow-twitch oxidative (STO), fast-twitch oxidative (FTO) and fast-twitch glycolytic (FTG) fibers, their per cent share in a bundle and number of fibers per 1 mm^2 were determined. Moreover, the analyses covered the frequency of occurrence of pathological fibers, including giant and angular fibers. The parameters examined, characterising the microstructure of *LL* muscle, were not found to be related to the presence of the C→T polymorphism in exon 3 of the *MSTN* gene, identified by enzyme *Taq*I.   However, it was shown that the diameters of the STO, FTO and FTG fibres in the *LL* muscle were significantly smallest in the Stamboek pigs with genotype FF at locus *CAST* compared to both the remaining genotypes. Among the crosses Pi x (PLW x PL) this genotype was not observed. The content of FTG fibres in a bundle proved to be related to the *CAST* genotype in Stamboek pigs. The frequency of pathological fibres in the *LL* muscle was the lowest in pigs with genotype EE at the locus *CAST*, but only in the case of the crosses Pi x (PLW x PL) was this relation statistically significant. The studies should be continued to determine whether such relations occur in other pig breeds. The frequency of pathological fibres affects meat quality and thus the polymorphism of the *CAST* gene could be of importance in selection.

Key Words: pig, muscle microstructure, *MSTN* (*GDF8*), *CAST*, *m. longissimus lumborum*

Zusammenfassung

Titel der Arbeit: **Der Zusammenhang zwischen Genotypen der Schweine-Loci *MSTN* (*GDF8*), *CAST* und der mikrostrukturellen Charakteristik des Muskels *longissimus lumborum*: Vorläufige Untersuchungen**
Ziel der Untersuchung war es, den Zusammenhang zwischen der mikrostrukturellen Charakteristik des Muskels *longissimus lumborum* (*LL*) und dem Genotyp der Schweine-Loci *MSTN* und *CAST* zu prüfen. Die Untersuchungen wurden an 132 nicht-verwandten Schweinen, darunter 93 Kreuzungsprodukten Pietrain x (Polish Large White x Polish Landrace) und 39 Stamboek-Kastraten, durchgeführt. Die Kreuzungstiere Pi x (PLW x PL), die sich etwa zur Hälfte aus Kastraten (n=46) und Sauen (n=47) zusammensetzten, waren vom CC- oder CT-Genotyp im Locus *RYR1*, dagegen die Stamboek-Schweine vom CC-Genotyp in diesem Locus.
Der Faserdurchmesser der Typen STO (slow-twich oxidative), FTO (fast-twich oxidative) und FTG (fast-twich glycolytic) und die Fasertypenanteile sowie Muskelfasergesamtanteil per 1mm^2 wurden bestimmt. Ausserdem umfasste die Analyse die Häufigkeit des Auftretens von pathologischen Fasern, inklusive Riesenfasern und angulären Fasern. Es konnte keine Beziehung zwischen den untersuchten Parametern der Mikrostruktur des *LL* Muskels und dem C→T Polymorphismus im Exon 3 des *MSTN*-Genes, der durch das *Taq*I-Enzyme identifiziert wurde, festgestellt werden. Auf der anderen Seite konnte nachgewiesen werden, dass der Durchmesser von STO, FTO und FTG Fasern im *LL*-Muskel bei Stamboek-Schweinen vom Genotyp FF im *CAST*-Locus, signifikant kleiner war als bei den anderen untersuchten Genotypen. Unter den Pi x (PLW x PL)-Kreuzungstieren konnte dieser Genotyp nicht beobachtet werden. Der Anteil an FTG Fasern war verbunden mit dem *CAST*-Genotyp bei

Stamboek-Schweinen. Die niedrigste Häufigkeit von pathologischen Fasern im *LL* Muskel wurde bei Schweinen vom EE Genotyp im *CAST*-Locus festgestellt, aber diese Beziehung war nur bei Pi x (PLW x PL)-Kreuzungstieren statistisch signifikant. Die Untersuchungen sollten fortgesetzt werden um zu prüfen, ob diese Beziehungen auch bei anderen Schweinerassen existieren. Da die Häufigkeit von pathologischen Fasern die Fleischqualität beeinflusst, kann der Polymorphismus der *CAST*-Gene in der Selektion von Bedeutung sein.

Schlüsselwörter: Schwein, Muskelfaser, *MSTN, CAST, m. longissimus lumborum*

Introduction

The skeletal muscle is a tissue of major economic importance for meat production, what in turn means that meat production capacity is related to the number of muscle fibers and their growth rate. The number of muscle fibers per unit area declines with the increasing lean meat percentage. At the same time the fiber diameter increases markedly (LENGERKEN et al., 1994). An increase in muscle body can occur by both an increase in myofiber diameter (hypertrophy) and in the number of myofibers (hyperplasia). Myofiber formation takes place during embryonic development and no significant increase in the number of myofibers occurs after birth (SWATLAND and KIEFFER, 1974). Histochemical and biochemical properties of a muscle, such as fiber type composition, fiber area, oxidative and glycolytic capacities, are factors that have been found to affect meat quality (FIEDLER et al., 1993, 1998, 1999). The genetic variability and heritability of muscle fiber number and size is sufficiently high to include these traits in selection, beside the commonly used criteria for improving lean meat content and meat quality (REHFELDT et al., 2000). These traits, as all quantitative traits, are most probably polygenic and affected by non-genetic environmental factors.

Several candidate genes, affecting muscle mass in farm animals, may be selected on the basis of their participation in the processes of muscle development. The myostatin gene (*MSTN; GDF8*) mutations are responsible for muscular hypertrophy in several breeds of cattle (GROBET et al., 1998). Double-muscled animals are characterized by an increase in muscle mass due to a general skeletal-muscle hyperplasia - that is, an increase in the number of muscle fibers rather than in their individual diameter (HANSET, 1991). Myostatin is a member of the TGFβ superfamily of growth and differentiation factors and plays an important role in controlling the development of skeletal muscles as a negative regulator during muscle growth (MCPHERRON et al., 1997). It affects meat quality in cattle (KOBOLÁK and GÓCZA, 2002).

The myogenic bHLH (basic-helix-loop-helix) family of transcription factors plays an important regulatory role in development of skeletal muscle. There are four members of this family in vertebrates, MyoD1, myogenin, myf-5 and MRF4 (myf-6). The myogenic bHLH genes (*MyoD* genes) are exclusively expressed in skeletal muscle (TE PAS and VISSCHER, 1994). Calpastatin was first reported as an endogenous inhibitory protein acting on calpain, the calcium-dependent cysteine proteases in animal cells. The calpain-calpastatin system plays an important role in normal, postnatal skeletal muscle growth. Recent studies, however, have shown that calpain activity is required for myoblast fusion and cell proliferation in addition to cell growth. Hence, the calpain system may also affect the number of skeletal muscle cells (fibres) by altering the rate of myoblast proliferation and modulating myoblast fusion (see for review GOLL et al., 1998). BARNOY et al. (1996) reported that the fusion of rat L8 line myoblasts was accompanied by a dramatic change in the calpain/calpastatin ratio.

Their results showed that calpastatin level is regulated during myoblast differentiation and that its diminution is important in determining the activity of the calpain required for myoblast fusion.

In a previous study, KŁOSOWSKA et al. (2004) demonstrated a significant effect of the genotype at the porcine loci *MYF3* and *MYF5* on the content of FTO, FTG and angular fibres in a bundle in *m. longissimus lumborum*. The objective of this study was to examine a relation between genotypes at the *MSTN* and *CAST* loci and microstructural characteristics of the *m. longissimus lumborum* in Piétrain x (Polish Large White x Polish Landrace) crosses and Stamboek pigs.

Material and methods

Animals

The study was carried out on 132 unrelated pigs. Ninety three animals with an almost equal proportion of females and castrated males were crosses of Pietrain and (Polish Large White x Polish Landrace), while 39 castrated males were of the Stamboek line (Dutch Large White x Dutch Landrace). The animals were slaughtered at about 105 kg live body weight. Rearing and feeding conditions were equal for all animals.

Genotyping of *RYR1*, *MSTN* and *CAST* polymorphisms

Polymorphism at the porcine loci *RYR1*, *MSTN* and *CAST* was defined in all experimental animals. Blood samples were collected at slaughter into tubes containing K_2EDTA and genomic DNA was isolated from leukocytes according to the method by KAWASAKI (1990). *RYR1* genotypes were identified according to the method by FUJII et al. (1991). Genotypes at the *MSTN* and *CAST* loci were determined as described by STRATIL and KOPEČNY (1999) and ERNST et al. (1998), respectively, using symbols of the alleles at these loci according to the authors.

Determination of the microstructure of *m. longissimus lumborum*

For histological examinations muscle samples (approximately 0.5 x 0.5 x 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 frozen immediately in liquid nitrogen, stored up to the time of analysis, cut in a cryostat into 10 µm thick sections and, in order to identify muscle fiber types [slow-twitch oxidative (STO), fast-twitch oxidative (FTO) and fast-twitch glycolytic (FTG)], subjected to a double reaction for activity of NADH-TR oxidoreductase and myofibrillar ATPase (WEGNER et al., 1993).

From each animal ten muscle bundles, containing on an average between 440 and 550 muscle fibers, were randomly selected for an evaluation of proportions between muscle fiber types. All fibers within a bundle were counted and measured.

In order to determine the various degenerative characteristics of the muscle, the sections were stained according to the method by van Gieson (DUBOWITZ et al., 1973) and an evaluation was made of the share of various pathological changes (atrophied and angular fibers, necrotic fibers with phagocytosis).

Mean diameters (in µm) of all fibers of the same type were evaluated using a Leica Q500 MC image analysis system. The total number of the muscle fibers was calculated against an area unit (mm^2).

Statistical analysis

The least squares method of the GLM procedure in the SAS statistical package (SAS 8.2; 2001) was used to analyze the relationship between *MSTN* and *CAST* genotypes and the *m. longissimus lumborum* microstructure traits. The fixed effects associated with sex and genotype were included into the linear model. Body weight at slaughter was added to a 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 $_{ijklm}$th animal;
	μ	- the overall mean;
	S_i	- effect of *i*th sex;
	H_j	- effect of *j*th *RYR1* genotype;
	M_k	- effect of *k*th genotype at the tested *MSTN* and *CAST* loci;
	$\beta(BWS_{ijkl}$	- BWS) – linear regression for body weight at slaughter;
	e_{ijklm}	- random error

Results

The microstructure characteristics of the *longissimus lumborum* muscle was determined on a total of 132 unrelated pigs: 93 porkers (46 castrated males and 47 females) being crosses of Pietrain and (Polish Large White x Polish Landrace) [Pi x (PLW x PL)] and 39 Stamboek pigs (castrated males). The crosses Pi x (PLW x PL) were of CC or CT genotype, while the Stamboek pigs were of CC genotype as regards locus *RYR1*.

The *MSTN/Taq*I and *CAST/Rsa*I genotypes, identified in the pigs from the both populations examined, are presented in Table 1. In both the crosses Pi x (PLW x PL) and Stamboek pigs no TT homozygotes as regards locus *MSTN/Taq*I were observed. Moreover, in the pigs being crosses Pi x (PLW x PL), homozygote FF as regards locus *CAST/Rsa*I was also not observed.

Table 1
Frequency of *MSTN/Taq*I and *CAST/Rsa*I genotypes in crosses of Pietrain and (Polish Large White x Polish Landrace) [Pi x (PLW x PL)]and Stamboek pigs (Die Häufigkeit von *MSTN/Taq*I- und *CAST/Rsa*I-Genotypen bei Kreuzungstieren Piétrain x (Polish Large White x Polish Landrace) und Stamboek-Schweinen)

Locus	Geno-type	Number (n) and frequency (%) of genotypes in pigs			
		Pi x (PLW x PL)		Stamboek	
		n	%	n	%
*MSTN/Taq*I	CC	27	46.6	31	79.5
	CT	31	53.4	8	20.5
*CAST/Rsa*I	EE	48	51.6	10	25.6
	EF	45	48.4	18	46.2
	FF	0	0.0	11	28.2

Table 2 presents a characteristics of the microstructure of the *m. longissimus lumborum* (*LL*) muscle in the pigs examined. In the crosses Pi x (PLW x PL) no significant effect was observed of sex on the microstructure traits of the *LL* muscle, whereas the diameter of FTG fibres was the only muscle microstructure characteristic affected by the *RYR1* genotype (P≤0.05).

The diameters of the STO fibres proved to be significantly, while of the FTO and FTG fibres highly significantly greater in crosses Pi x (PLW x PL) as compared to Stamboek pigs. In turn, the number of fibres per area unit (mm^2) was significantly higher in Stamboek pigs than in crosses. The per cent share of STO and FTG fibres showed significant differences between the pig populations examined (P≤0.05).

Table 2
Least-squares means (LSM)and their standard errors (SE) for diameter of STO, FTO and FTG fibres and content of normal and pathological fibres in *m. longissimus lumborum* in crosses of Pi and (PLW x PL) and Stamboek pigs (Mittelwerte der kleinsten Quadrate (LSM) und Standardfehler (SE) für den Faserdurchmesser der STO, FTO und FTG Fasern sowie Fasertypenanteile (%) und Muskelfasergesamtanzahl per 1mm^2 im Muskel *longissimus lumborum* bei Kreuzungstieren und Stamboek-Schweinen)

Characteristic	Pi x (PLW x PL) (n=93)										Stamboek (n=39)	
	males (n=46)		females (n=47)		*RYR1* genotype				Mean (n=93)			
					CC (n=35)		*CT* (n=58)					
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Diameter of fibres (µm):												
STO	46.7	1.2	48.1	1.9	46.1	0.9	48.2	0.8	**47.2^a**	**1.6**	**44.8^b**	**2.1**
FTO	46.3	1.4	47.9	2.1	45.0	1.1	48.3	0.9	**47.0^A**	**1.9**	**38.2^B**	**1.8**
FTG	61.5	1.6	63.5	1.4	**60.7^a**	**1.2**	**63.6^b**	**1.0**	**62.6^A**	**1.5**	**50.0^B**	**1.2**
Share of fibres in a bundle (%):												
STO	16.6	1.0	16.1	1.1	16.4	0.8	16.6	0.7	**16.3^a**	**1.1**	**13.3^b**	**0.6**
FTO	17.2	1.1	15.8	1.2	16.5	0.9	16.5	0.8	16.5	1.2	16.5	0.9
FTG	66.2	1.5	67.9	1.6	67.1	1.3	66.9	1.0	**67.1^a**	**1.6**	**70.2^b**	**0.9**
Pathological	9.0	1.2	9.4	1.1	9.8	0.9	9.2	0.8	9.2	1.2	6.5	0.9
Giant	1.2	0.4	1.4	0.3	1.6	0.4	1.2	0.3	1.3	0.4	1.7	0.8
Angular	0.5	0.5	0.7	0.6	0.5	0.3	0.9	0.3	0.6	0.6	0.4	0.1
Number of fibres/mm^2	187.7	7.4	176.3	5.7	189.7	5.3	177.5	4.4	**181.9^A**	**6.5**	**230.5^B**	**7.7**

^{AaBb}within rows and *RYR1* genotype or between breeds, means bearing different superscripts differ significantly at: small letters - P≤0.05, capitals - P≤0.01.

Due to the significant and highly significant differences in fibre diameters and share of fibres STO and FTG in a bundle between the pig groups examined, the effect of genotypes *MSTN* and *CAST* on the microstructure traits of the *LL* muscle was analysed separately for each pig population. In the case of both pig population examined it was shown that the microstructure traits of the *LL* muscle observed in pigs of genotype CC at the locus *MSTN* did not differ significantly from those determined for pigs of genotype CT at the same locus.

A significant relation between the *CAST* genotype and the diameters of STO, FTO and FTG fibres in the *LL* muscle was observed in Stamboek pigs (Table 3). The diameters of STO and FTO fibres were significantly, while of FTG fibres highly significantly smaller in animals of genotype FF than of all the remaining genotypes. This relation between the fibre diameter and genotype at the *CAST* locus was reflected in the number of fibres per unit area being the lowest in the *m. longissimus lumborum* of animals of FF genotype. Moreover, animals with FF genotype at *CAST* locus showed a significantly higher share of FTG fibres in a bundle than animals with EE genotype at this locus. Pigs differing in the *CAST* genotype did not differ as regards the per cent share of STO and FTO fibres in a bundle (Table 4). Pathological fibres occurred more often in Pi x (PLW x PL) pigs of EF than EE genotype. A similar tendency was observed in the Stamboek line, where the EE homozygotes demonstrated a lower

frequency of this type of fibres than animals of the remaining two genotypes. However, those differences did not prove significant.

Table 3
Least square means (LSM) and their standard errors (SE) for diameter of STO, FTO and FTG fibres and number of fibres per mm^2 in the *m. longissimus lumborum* of crosses of Pi and (PLW x PL) and Stamboek pigs in reference to *CAST* genotype (Mittelwerte der kleinsten Quadrate (LSM) und Standardfehler (SE) für den Faserdurchmesser der STO, FTO und FTG Fasern sowie Muskelfasergesamtanzahl per 1mm^2 im Muskel *longissimus lumborum* bei Kreuzungstieren und Stamboek-Schweinen in Abhängigkeit vom *CAST*-Genotyp)

Breed/line	*CAST* genotype	Number of animals	Diameter of fibres (μm)						Number of fibres/mm^2	
			STO		FTO		FTG			
			LSM	SE	LSM	SE	LSM	SE	LSM	SE
Pi x (PLW x PL)	EE	48	47.95	1.95	48.19	1.57	63.62	1.15	176.71	5.68
	EF	45	46.85	1.29	46.06	1.28	61.29	1.32	185.81	7.98
	FF	0	-	-	-	-	-	-	-	-
Stamboek	EE	10	43.23[ab]	1.15	**39.35[a]**	**1.49**	**53.28[Aa]**	**1.82**	**229.8[a]**	**8.50**
	EF	18	**44.37[b]**	**1.08**	38.47[ab]	1.11	**50.56[a]**	**1.36**	232.7[ab]	9.92
	FF	11	**40.57[a]**	**1.40**	35.03[b]	1.44	**45.89[Bb]**	**1.76**	253.5[b]	10.98

P$\leq$ 0.01; small letters   P$\leq$ 0.05
[a, b] within columns means bearing different letters differed significantly: capital letters

Table 4
Least square means and their standard errors (LSM±SE) for share of fibres STO, FTO, FTG and pathologically changed in a bundle in *m. longissimus lumborum* (*LL*) as related to the *CAST* genotype in crosses of Pi and (PLW x PL) and Stamboek pigs (Mittelwerte der kleinsten Quadrate (LSM) und Standardfehler (SE) für den Fasertypenanteile (%) im Muskel *longissimus lumborum* bei Kreuzungstieren und Stamboek-Schweinen in Abhängigkeit vom *CAST*-Genotyp).

Breed	*CAST* geno-type	Num-ber of ani-mals	Per cent share of fibres in a bundle (LSM±SE)						
			value	STO	FTO	FTG	Patho-logic	Giant	Angu-lar
Pi x (PLW x PL)	EE	48	LSM	16.72	17.10	66.17	**7.75[a]**	1.233	0.482
			SE	1.15	1.58	1.51	**0.87**	0.481	0.291
	EF	45	LSM	16.38	15.51	68.11	**10.71[a]**	1.054	0.373
			SE	1.22	1.42	1.62	**1.17**	0.391	0.214
	FF	0	-	-	-	-	-	-	-
Stamboek	EE	10	LSM	14.03	17.32	**68.52[a]**	5.49	0.700	0.267
			SE	0.79	1.58	**1.54**	1.41	0.841	0.242
	EF	18	LSM	14.00	15.87	**70.05[b]**	7.03	1.351	0.259
			SE	0.59	1.18	**1.15**	1.05	0.627	0.181
	FF	11	LSM	12.24	15.08	**72.94[a]**	8.06	2.151	0.758
			SE	0.76	1.53	**1.47**	1.34	0.802	0.231

Discussion

The mean diameter of STO, FTO and FTG fibers in *m. longissimus lumborum*, as identified in this study, was comparable to 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). The mean proportions of STO, FTO and FTG fibers in a bundle, observed in the present study, were also similar to those presented by other authors for different pig breeds (SOSNICKI, 1987).

In literature, one may find diverse opinions concerning the effect of the *RYR1* genotype on muscle microstructure characteristics. FIEDLER et al. (1993, 1998) and

KŁOSOWSKA et al. (2004) demonstrated that the fiber diameter in *m. longissimus dorsi* was larger in pigs susceptible to stress (TT genotype), when compared to those occurring in stress-resistant animals. In turn, ACKERMANN and SALOMON (1991) did not observe such a relation. Moreover, KŁOSOWSKA et al. (2004) reported that the content of various types of pathological fibers in a bundle, as well as that of giant fibers considered separately, was highest in TT animals (stress susceptible) compared to both the remaining *RYR1* genotypes (P≤0.01). Thus, to eliminate the effect of the genotype TT at locus *RYR1* on the traits considered, the relation between the genotype at loci *MSTN* and *CAST* and the *m. longissimus lumborum* microstructure characteristics was evaluated on pigs of genotype CC or CT at locus *RYR1*.

Opinions about the effect of sex on muscle fiber diameter and other properties are not uniform - some authors reported a lack of such an effect (BIEREDER et al., 1999), while others reported significantly lower diameters of type I, IIA and IIB fibers in boars as compared to sows (KARLSSON et al., 1999). In the present study, the effect of pig sex on diameter and content of all types of normal or pathologically changed fibers in the *m. longissimus lumborum* appeared to be insignificant within tested pigs Pi x (PLW x PL).

The results obtained demonstrated that the microstructure traits of the *LL* muscle were not significantly related to the *MSTN* genotype. Similarly, no differences were observed in the carcass quality traits between the *MSTN* genotypes (CIEŚLAK et al., 2003). The replacement C→T in exon 3 of the *MSTN* gene does not result in an amino acid substitution (STRATIL and KOPEČNY, 1999). Thus it is probable, that this mutation has no effect on the myostatin function in the processes of myogenesis, similar to that observed in mice and cattle. In the case of those species known are *MSTN* gene mutations, which lead to disturbances in the control of the proliferation range of myoblasts, causing an increased number of muscle fibers and thus an increased mass of skeletal muscle (MCPHERRON et al., 1997; GROBET et al., 1998).

The studies presented demonstrated that the diameter of STO, FTO and FTG fibres in the *longissimus lumborum* muscle in Stamboek pigs was significantly lower in animals of genotype FF at locus *CAST* in relation to animals of genotypes EE or EF. The fact that in pig being crosses Pi x (PLW x PL) there were no animals of genotype FF made a complete analysis in those crosses impossible. It should be mentioned here that this genotype was also absent in Polish Landrace and Pietrain pigs tested in our previous study (KURYŁ et al., 2003).

Moreover, the present study indicated a significant relation between the frequency of occurrence of pathological fibres and the *CAST* genotype in Pi x (PLW x PL) pigs, what may arise from the larger number of animals of this population analysed. However, the lack of Pi x (PLW x PL) pigs of genotype FF renders it impossible to ascertain whether this genotype would be as unfavourable for this trait as genotype EF. A similar tendency was observed among Stamboek pigs but it did not prove significant.

A relation between eye-muscle area and *CAST* genotype has been presented in an earlier study by KURYŁ et al. (2003). Stamboek pigs of genotype EE showed a significantly higher value of this characteristic than those of the two remaining genotypes at this locus. Moreover, EE genotypes at *CAST* locus showed a significantly lower share of FTG fibres in a bundle than animals with FF genotype at this locus. Thus, one may suggest that the genotype at the *CAST* locus influencing the proportion

of FTG fibres in a bundle may also affect the metabolic properties of muscle and thereby meat quality. Summarizing the results of the previous study and those presented here, one may suggests that genotype EE is the most profitable both for the size of eye muscle area and for the microstructure of *m. longissimus lumborum*.

BALCERZAK et al. (1998) have shown that the m-calpain-calpastatin equillibrium was very important for a myoblast fusion development. The phenomenon of myoblast fusion appears to require m-calpain activity via the cleavage of fibronectin network and cytoskeletal components such as talin and desmin. This cleavage could modify membrane and cytoskeleton organization for the myoblasts to fuse. The point mutation leading to *CAST* gene variants, identified in this study with *Rsa*I enzyme, was present in intron 6. Thus, this mutation cannot be considered as a causal mutation for variability in the *m. longissimus lumborum* microstructure characteristics, showed in the present study (diameter of STO, FTO and FTG fibers as well as percentage of FTG and pathological fibers). One may suggest that this mutation is linked to another mutation within the *CAST* gene, which changes the sequence of protein and thus affects the activity of calpastatin as a m-calpain inhibitor. Such activity is connected principally with B subdomains within domains I-IV (GOLL et al., 2003). The causal mutation could be also localized within the regulatory region of the gene affecting the expression level resulting in the level of calpastatin. A similar suggestion was presented by TE PAS et al. (1999) in a work presenting the results of an analysis of the effect of a point mutation in 3' region of *myogenin* gene on the carcass meat content. On the other hand, it was recently shown in several studies that introns affect and enhance eucaryotic gene expression (LE HIR et al., 2003). Thus, taking into account this hypothesis, the intronic mutation analyzed in the present study could be considered not only as a marker for muscle microstructure characteristics being linked to a hypothetical causal mutation present in another region of the *CAST* gene, but also as the causal mutation itself.

Conclusions

Diameter of the fibres STO, FTO and FTG as well as the percentage of FTG and pathologically changed fibres in *m. longissimus lumborum* was significantly related to the genotype at the porcine *CAST*/*Rsa*I locus (intron 6 of the gene). This relation should be further analyzed in another pig breeds or lines in order to confirm it and to identify a causal mutation(s). No significant relation was found between the *MSTN* gene variants and a value of microstructure characteristics of the muscle *longissimus lumborum*.

Acknowledgement

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