THE RELATIONSHIP BETWEEN POLYMORPHISMS IN PORCINE *MYOG*, *MYF-3* AND *MYF-5* GENES AND MICROSTRUCTURAL CHARACTERISTICS OF *LONGISSIMUS* MUSCLE - A PRELIMINARY STUDY

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Background

Domestic pigs today are selected on the basis of their ability to grow rapidly and skeletal muscles of these pigs have higher percentage of fast –glycolycic, white muscle fibers than muscles of the pigs growing slowly (Szentkuti et al., 1990).
The differences in muscle size are due to differences either in myofiber composition and their diameters and number. Mean fibre diameters increase with the age of the animals and most of these fibers have a lower oxidative capacity, (Kłosowska et al., 1988). An increase in lean percentage of some breeds of pigs (Pietrain, Landrace) appeared to be more associated with muscles containing a low percentage of oxidative and a high percentage of glycolytic fibers with large diameters (Nowak et al., 1994). It has been shown also that greater meat content in PIC pigs can be related to a greater number of the muscle fibers (Kłosowska et al., 1998).
The fibers profiles are decisive, after slaughter as regards qualitative and quantitative characteristics of the muscle as meat (Kiessling et al., 1982; Kłosowska , 1984).
Muscle fiber formation takes place during embryonal development and is regulated by the MyoD family, which consists of four genes: *MyoD1* (*Myf-3*), myogenin (*MYOG* or *Myf-4*), *Myf-5* and *Myf-6* (*MRF4*), TePas and Visscher, (1994), Fiedler et al., (2000). TePas and Visscher (1994) hypothesized that naturally occuring genetic variation in *myogenin* could affect muscle fiber number and, thus, lean production characteristics.

Objective

The objective of this study was investigate relationship between *MYOG (Myf-4)*, *MyoD1* (*Myf-3)* and *Myf-5* genotypes and microstructural characteristics of *Longissimus* muscle in pigs.

Methods

Thirty pigs of each breed i.e., Polish Landrace (PL), Pietrain (Pi), Złotnicka Spotted (ZS) and crosses Pi x ZS with equal number of gilts and barrows in each group were slaughtered at about 105 kg live weight. Genomic DNA was isolated from blood samples taken at exsanguination according to Kawasaki (1990) or using WIZARD Kit (Promega, USA). For histological examinations muscle samples were taken from the middle part of m. *longissimus lumborum* about 45 min *post mortem*. The samples were frozen in a liquid nitrogen up to the time of analysis. The muscle samples were cut in cryostat into 10 μm thick sections and subjected to double reaction for activity of NADH-TR oxidoreductase and myofibrillar ATPase (Wegner et al., 1993) to identify muscle fiber types (STO, FTO and FTG). Ten muscle boundles containing average from 439 to 553 muscle fibers per individuals were randomly selected to evaluate the proportion of muscle fibers. The diameters of all fibers of the same type were evaluated using a Leica Q 500 MC image analysis system.

RYR1 genotypes were established according to Fuiji et al., (1991). Polymorphism in *myogenin* gene (*MYOG*) was identified at its 3' end with *Msp*I restriction enzyme according to Soumillion et al., (1997) and that in *Myf-3* gene with *Dde*I according to Knoll et al., (1997). Two polymorphisms in the porcine *Myf-5* gene identified with enzymes *Hinf*I and *Dde*I were analyzed according to Stratil and Cepica (1999).

The statistical package SAS was used to analyse the relationship between MyoD genotypes and the M. *longissimus lumborum* microstructure traits. The fixed effects associated with breed, sex, genotype was included into linear model. Age at slaughter was added to a model as a covariate.

Results and discussion

Fiber type distribution in M. *longissimus lumborum* and fiber type diameters for pig breeds being analysed in this study are shown in Table 1. The longissimus lumborum of the pigs varied in the muscle fibre composition. The lower content of STO fibres had Pi pigs and crosses Pi x ZS comparing to PL and ZS which showed a similar content of this type of fibres in bundle. Higher content of FTO fibers was noticed among ZS pigs, whereas three remaining breeds showed similar content of this type of fibers in bundle. Next, ZS pigs had a lowest content of FTG fibers, whereas remaining breeds showed similar value of this trait. The higher diameter of all fiber types was found in crosses Pi x ZS comparing to other breeds being analysed in this study.A total number of fibers in bundle was also highest in ZS pigs comparing to remaining analysed breeds and crosses Pi x ZS which not differeing in this characteristic.

Two variants and three genotypes were identified for RYR1 gene and each of MyoD genes investigated in this study. The distribution of genotypes among tested breeds is shown in Table 2.

A relationship between M. *longissimus lumborum* fiber types and their diameters and percentage of fibers in bundle on one side and *MyoD* genes genotype on the other is shown in Table 3. A significant effect of some of *MyoD* genotypes on diameter of fibers or their content in bundle was noticed. The diameter of STO fibers was affected with *Myf-3* genotype. The pigs with AA genotype showed significantly lower diameter of this fiber type than both AC and CC genotypes. A significant relationship between

the diameter of FTO fibers and genotype at both *Myf-3* and *Myf-5 loci* was observed. The diameter of FTG fibers was independent on the genotype at the *MyoD loci*. The content of FTO and FTG fibers in bundle was affected with some of MyoD family genes. The pigs with AB genotype identified with both DdeI and HinfI enzymes at Myf-5 locus had a higher content of FTO fibers than both AA and BB genotypes. The content of FTG fibers in bundle was related to MYOG and Myf-5 genotypes. The pigs with AA genotype at MYOG locus had a greater content of FTG fibers than both AB and BB genotypes. Next, the pigs with AA or AB genotype at the Myf-5/DdeI locus had a highly significantly greater content of FTG fibers in bundle than BB individuals.

Conclusions

1. A significant difference in the diameter and content in bundle of STO, FTO and FTG fibers exist between some pig breeds. A higher number of fibers in bundle was noticed in Złotnicka Spotted pigs comparing to Pietrain, Polish Landrace and crosses Pietrain x Złotnicka Spotted pigs
2. A significant relation between polymorphism of genes belonging to *MyoD* family (*MYOG2*, *Myf-3*, *Myf-5*) and muscle fiber types and their diameters and content in the of M. *longissimus lumborum* of the four pig groups.
3. The relationship between MyoD genes polymorfism and microstructural features confirmed the meaning of the MyoD genes in differentiation of the muscle structure.

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Table 1
Percentage of fibers, their diameters and number of fibers in bundle, respectively, among Pietrain (Pi), Polish Landrace (PL) and Zlotnicka Spotted (ZS) and crosses (Pi x ZS) pigs

Specification		Genetic group			
		Pi	PL	ZS	Pi x ZS
Percentage of fibers in bundle	STO	12.84^{A}	15.41^{B}	13.83^{AB}	12.03^{A}
	FTO	17.70^{A}	14.43^{A}	23.77^{B}	15.44^{A}
	FTG	70.50^{A}	70.16^{A}	62.46^{B}	72.53^{A}
Fibre diameters [µm]	STO	44.25^{A}	44.30^{A}	47.85^{B}	51.80^{C}
	FTO	42.97^{A}	42.03^{A}	41.56^{A}	50.20^{B}
	FTG	56.96^{A}	55.89^{A}	57.43^{A}	62.93^{B}
Number of fibers in bundle		44.62^{B}	46.04^{B}	55.30^{A}	43.91^{B}

A,B,C – stastically significant differences, $p<0.001$

Table 2
Frequency (number and percentage) of *MY0G2*, *Myf-3* and *Myf-5* genotypes identified with *Msp*I, *Dde*I and (*Dde*I, *Hinf*I) enzymes, respectively, among Pietrain (Pi), Polish Landrace (PL), Zlotnicka Spotted (ZS) and crosses (Pi x ZS) pigs

Locus genotype		Genetic group							
		Pi		PL		ZS		Pi x ZS	
		n	%	n	%	n	%	n	%
MYOG2	BB	21	70.0	15	50.0	2	6.7	19	63.3
	AB	9	30.0	11	36.7	15	50.0	8	26.7
	AA	0	0	4	13.3	13	43.3	3	10.0
Myf-3	AA	12	40.0	0	0	27	90.0	12	37.9
	AC	11	36.7	11	36.7	3	10.0	15	51.7
	CC	7	23.3	19	63.3	0	0	3	10.3
Myf-5 (DdeI)									
	AA	6	23.1	18	62.1	30	100.0	18	60.0
	AB	13	50.0	11	37.9	0	0	10	33.3
	BB	7	26.9	0	0	0	0	2	6.7
Myf-5 (HinfI)									
	AA	7	24.1	22	73.3	10	34.5	6	20.0
	AB	16	55.2	8	26.7	14	48.3	10	33.3
	BB	6	20.7	0	0	5	17.2	14	46.7

Table 3
A relationship between STO, FTO and FTG fibers diameter and percentage of fiber in bundle in M. *longissimus lumborum* and *MyoD* genes genotype

Locus	Fibers					
	STO		FTO		FTG	
	diameter	percentage	diameter	percentage	diameter	percentage
MYOG2	ns	ns	ns	ns	ns	0.04
Myf-3	0.04	ns	0.05	ns	ns	ns
Myf-5/DdeI	ns	ns	0.05	ns	ns	0.001
Myf-5/HinfI	ns	ns	0.05	0.05	ns	ns