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CARCASS AND MEAT QUALITY IN RELATION TO THE POLYMORPHISM IN PORCINE *MYF4* GENE

SUMMARY

The aim of this study was to analyse the associations between polymorphism in porcine myogenin gene (*MYF4*) and economically important traits in relation to the carcass and meat quality of pigs. The genomic DNA samples were taken from in total 180 crossbreeds (Large White x Landrace). The detection of polymorphism in *MYF4* gene were performed in order to evaluate its effect on back fat thickness, proportion of valuable meat parts, MLT area and proportion of thigh. The genotyping of analysed individuals was carried out by means PCR-RFLP method and restriction endonuclease *Msp*I. The allele frequencies were as follows: A 0.75 and B 0.25. A prevalence of AA genotype (59%) compared to AB (33%) and BB genotypes (8%) were detected in analysed crossbreed population. The observed average value of heterozygosity (0.33) and positive value of the Wright's F_{IS} index (0.12) similarly reflected the higher proportion of homozygous genotype in populations. The effect of *MYF4* gene polymorphism on selected phenotypic traits has been tested using one-way ANOVA procedure. The statistical analysis showed only non-significant results. Due to the polygenic character of selected phenotypic traits the involvement of other candidate genes and increase of sample size could clarify the role of *MYF4* gene in porcine carcass and meat quality regulation. The study, which is based on molecular variability of livestock genetic resources, is necessary for the genetic improvement and understanding of relations between markers and trait of interest.

Keywords: genetic variability, myogenin gene, meat quality, SNP, pig.

INTRODUCTION

The pig breeding plays currently a key role in meat production, and the high consumption of pork corresponds to this. The high efficiency of pig breeding is absolutely necessary for competitiveness under today's difficult economic conditions (Weisz *et al.*, 2011). One of goals of animal genetics is to locate and identify loci that are responsible for economical important traits such

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as meat performance, reproduction, and product qualities which are the most significant factors for pork production efficiency (Bulla *et al.*, 2007).

Variation in meat quality is likely to be caused by differences in many genetic and non-genetic (environmental) factors, which interact together and determine the course of metabolic processes in muscle tissue and in the *postmortem* conversion of muscle into meat (Kapelański *et al.*, 2005). 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. Changes in fibre type ratios affect metabolic properties of a muscle and thereby meat quality (Kłosowska *et al.*, 2004).

Histochemical and biochemical muscle fibre properties are the factors that influence the quantitative and qualitative characteristics of pork meat (Stupka *et al.*, 2014). Muscle fibre formation takes place during embryonic development in mammals and is controlled by the *MyoD* gene family, which consists of four genes, *MyoD1* (*MYF*3), myogenin (*MYOG* or *MYF4*), *MYF5* and *MYF6* (*MRF4*) (Horák *et al.*, 2004; Zhang *et al.*, 2007; Ujan *et al.*, 2011). The expression of the *MYF5* and *MYOD1* (*MYF3*) genes plays a fundamental role during myoblast proliferation, while the expression of the *MYOG* (*MYF4*) and the *MYF6* (*MRF4*) genes is linked to the differentiation and maturation of myofibres (Stupka *et al.*, 2012). The myogenin (*MYF4*) gene, which controls the start of myoblast fusion and myofibres formation, was identified on the 9th autosome, in the 9q2.1-2.6 area (Soumillion *et al.*, 1997; Ernst *et al.* 1998). The myogenin is a transcription factor specific to skeletal muscle and fulfils a key function in muscle differentiation by controlling the onset of myoblast fusion and the establishment of myofibres. Genetic variation in the *MYF4* gene can be associated with differences in myoblast and myofibril numbers (Weisz *et al.*, 2011). Ernst *et al.* (1993) and Soumillion *et al.* (1997) detected using endonuclease *Msp*I gene three polymorphic sites in the *MYF4*, in the promoter region, the second intron and the 3' side of the gene.

The aim of this study was to analyse the effect of polymorphism in porcine *MYF4* gene on economically important traits in relation to the carcass and meat quality in population of Large White x Landrace crossbreeds.

MATERIAL AND METHODS

The biological samples were collected in 2014 from in total of 180 crossbreeds of Large White x Landrace (86 boars and 94 sows) from the Experimental Centre of Farm Animals (Department of Animal Husbandry, Slovak University of Agriculture in Nitra, Slovakia). Each of selected individuals included in present study have been farmed in the same conditions and fed with standard feed mixtures.

To extract of genomic DNA from blood samples the protocol according to Miller et al. (1988) has been used. Subsequently, the concentration and purity of genomic DNA were analysed based on the spectrophotometry measurements by

the optical density at wave length of 260 nm. The polymorphism in *MYF4* gene was analysed according to Soumillion *et al.* (1997) using restriction endonuclease *Msp*I. The products of PCR reaction and restriction fragments have been separated and visualised using horizontal electrophoresis in 2% agarose gels (130 V for 50 min) and stained with day GelRed.

The genotypic structure of population and allele frequencies have been determined using Genalex version 6.1 (Peakall and Smouse, 2012). The Hardy-Weinberg equilibrium in population was tested based on the significance between observed and expected genotype frequencies using Chi-square test. The diversity indices including observed (H_o) and expected heterozygosity (H_e), effective allele numbers (N_e), polymorphic information content (PIC), and F_{IS} index was calculated using Genalex version 6.1 (Peakall and Smouse, 2012).

The effect of *MYF4* has been studied in relation to the back fat thickness (BFT), lean meat percentage (LM), thigh percentage (TP) and MLT area. Each of these carcass and meat quality indicators was measured according to Slovak standard technical norm (STN 466164). The associations between locus in *MYF4* gene and selected production parameters have been analysed using One-way ANOVA procedure incorporated in SAS software environment (SAS Institute Inc., 2009).

RESULTS AND DISCUSSION

In analysed population of Large White x Landrace crossbreeds the prevalence of homozygous AA genotype was found (59%), whereas the AB heterozygotes reached the level of 33%. The lowest proportion was observed for BB homozygotes (8%). The A allele was more frequent (0.75) than B allele (0.25). The higher proportion of homozygotes across all evaluated individuals was reflected in the decrease of H_o and H_e heterozygosities (0.33 and 0.39). The differences between observed and expected genotype frequencies have been only non-significant ($P<0.05$) that indicated the Hardy-Weinberg equilibrium in population. The value of N_e (1.59) signalised unbalanced alleles activity and observed value of polymorphic information content (0.38) showed medium level of polymorphism according to Botstein et al. (1980). The positive value of F_{IS} index (0.12) and also relative high value of observed homozygosity (0.67) indicated the deficiency of heterozygous animals compared to the Hardy-Weinberg expectations. The value of F_{IS} index, which is also considered as molecular equivalent of individual inbreeding coefficient with respect to the population, reflected in analysed population the increase of homozygosity resulting probably from the higher animal's relatedness and signalized the risk of inbreeding increase in the future generations.

The effect of *MYF4* gene polymorphism has been analysed in association to four indicators reflected carcass and quality of pork meat: back fat thickness, lean meat percentage, thigh percentage and MLT area. Table 1 shows the observed average values of analysed traits in relation to the *MYF4* genotypes. Contrary to the expectations resulting from previously published studies (Verner

et al., 2007; Civaňova and Knoll, 2007; Stupka *et al.*, 2012) all of the *MYF4* genotypes showed only non-significant (P<0.05) impact on selected production traits.

Table 1. Average values of measured traits in relation to MYF4 polymorphism genotypes

Genotypes	N	Traits (in average)							
		BFT (mm)	P	LP (%)	P	MLT area(cm^2)	P	TP (%)	P
AA	106	17.30±3.51	ns	55.35±1.90	ns	43.76±3.79	ns	22.62±1.24	ns
AB	59	17.40±3.53	ns	55.08±1.77	ns	44.56±4.85	ns	22.62±1.28	ns
BB	15	16.71±3.11	ns	55.56±2.35	ns	43.53±4.03	ns	22.82±1.42	ns

ns – not significant, BFT – back fat thickness, LP – lean meat percentage, MLT area – area of *musculus longisimus thoracis*, TP – thigh percentage (%)

The associations of *MYOD* genes family with pork quality has been previously investigated in various pig populations by several studies (Soumillion *et al.*, 1997; Kapelañski *et al.*, 2005; Cinar *et al.*, 2012; Stupka *et al.*, 2014). The *MyoD* genes were tested as candidate genes with an expected, significant effect principally on the muscle deposition (Kłosowska *et al.*, 2004). The genetic variation in the *MYF4* gene has been associated mainly with differences in myoblast and myofibre numbers (Soumillion *et al.*, 1997; Kim *et al.*, 2009). Te pass *et al.* (2004) revealed that the level of *MYF4* gene mRNA expression is localized mainly in red muscles in animals at slaughter and is associated with muscle fibre type. Gerześ *et al.* (2010) and Stupka *et al.* (2014) reported significant effect of *MYF4* polymorphism on the number of muscle fibres per area unit in pigs. Some authors also showed that the *MYF4* gene had also a tendency to regulate backfat thickness (Civaňova and Knoll, 2007). The animals with AA genotype were associated with increase birth weight, growth rate and content of lean meat (Te Pass *et al.*, 1999). In the Czech Large White population was found the significant association between *MYF4* gene polymorphism and back fat thickness (Verner *et al.*, 2007). Based on this and due to the polygenic character of selected phenotypic traits the involvement of other candidte genes mainly from *MYOD* family and increase of sample size is in the future studies needed in order to clarify the role of *MYF4* gene in porcine carcass and meat quality regulation.

CONCLUSIONS

In analysed population of Large White x Landrace crossbreeds the prevalence of homozygous AA genotype was found, whereas the lowest proportion was observed for BB homozygotes. The lower proportion of heterozygotes within population was reflected in the decrease of genetic variability represented by FIS index and heterozygosity. Moreover, the FIS index as molecular equivalent of inbreeding coefficient signalized for the analysed

population the risk of animal's relatedness increase in the next generations. In the contrary to the expectations resulted from the previously reported studies the MYF4 genotypes showed only non-significant ($P<0.05$) impact on all of the analysed production traits. In the future, the involvement of other candidate genes and increase of sample size could clarify the role of MYF4 gene in porcine carcass and meat quality regulation.

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