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Effect of the fat mass and obesity associated (FTO) gene polymorphism on carcass traits in pigs

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ABSTRACT

The aim of this study was to evaluate the effect of FTO single nucleotide polymorphism (g.276T>G) on carcass traits in Large White pigs. In several studies have been suggested that the FTO gene influenced significantly regulation of energy balance and feed intake in pigs and therefore can be considered as genetic marker for their production traits. We used 150 boars (73) and sows (77) from Large White breed. Genotyping of animals was carried out by PCR-RFLP method with using restriction enzyme TaiI. We identified three genotypes TT (20.66%), TG (66.67%) and GG (12.67%). Associations of FTO gene polymorphism with back fat thickness, lean meat percentage, thigh percentage and MLT area were analyzed. The results of the statistical analyses did not confirmed the effect of the FTO mutation (g.276T>G) on selected carcass traits in analyzed population of Large White pigs.
(Keywords: carcass traits, FTO, SNP g.276T>G, pig)

INTRODUCTION

Knowledge on the genetic background of fat tissue accumulation is important in livestock production. Several fatness traits are considered in pig breeding improvement, most frequently back fat thickness and intramuscular fat content. The fatness traits are related to meat quality or fattening efficiency (*Switonsky et al.*, 2010). The breeding goals in modern pig breeds often include decreased back fat, abdominal fat weight and optimal level of intramuscular fat. Therefore, the knowledge on the contribution of the pig FTO gene to fat accumulation variability is important for meat production (*Szydlowski et al.*, 2012).

The amino acid sequence of the FTO gene showed high conservation among human, pig and other important domestic animals. The FTO gene is highly expressed in the hypothalamic-pituitary-adrenal axis, suggesting this gene may participate in the central control of energy homeostasis or in the development of fat tissue (*Zhang et al.*, 2009). The pig FTO gene was mapped to SSC6 (*Fontanesi et al.*, 2009). Several studies reported relationship between phenotypes and genetic variants of FTO gene in different breeds of pigs. *Fan et al.* (2010) found in Yorkshire pig experimental population significant associations between SNPs in FTO gene and residual feed intake. *Szydlovsky et al.* (2012) reported for FTO gene multiple significant associations with back fat thickness, abdominal fat weight and lean meat content in Polish Landrace pigs and therefore can be associated with fatness traits in purebred pigs selected for low fatness. Results of *Dvořáková et al.* (2012) study show that in commercial pig populations FTO

influences back fat depth. *Zhang et al.* (2009) found in 6 Chinese native pigs breeds population significant association only between FTO gene and intramuscular fat content. The association analyses of *Fontanesi et al.* (2010) confirmed the effect of the FTO mutation on obesity-related traits (visible intermuscular fat, back fat thickness and lean cuts) in the Italian Duroc pigs.

The aim of this study was to analyse the effect of FTO g.276T>G single nucleotide polymorphism on carcass traits in Large White pigs.

MATERIAL AND METHODS

Animals used in the study included boars (n=73) and sows (n=77) of Large White pigs from the Experimental Centre of Farm Animals, Department of Animal Husbandry, Slovak University of Agriculture in Nitra.

Genomic DNA was extracted from blood samples using protocol according to *Miller et al.* (1988). Concentrations of DNA were estimated by spectrophotometer measurement by the optical density at wave length of 260 nm. For genotyping of animals was used PCR-RFLP method. A 397 bp fragment of intron 4 in porcine FTO gene was amplified by PCR using forward and reverse primers according to *Fontanesi et al.* (2010). The polymerase chain reaction was performed in a 25 µl reaction mixtures, containing: 10 x PCR reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 4 pM of each primer, 1 U Tag DNA polymerase (Fermentas), 50 ng genomic DNA. Thermal cycling conditions included: an initial denaturation step at 95 °C for 2 min, followed by 40 cycles of 95 °C for 30 sec, 64 °C for 40 sec, 68 °C for 50 sec and a final extension at 68 °C for 7 min. PCR products of FTO gene were subsequently digested with 1 µl of FastDigest *Tai*I (Fermentas) restriction enzyme at 37 °C in time 15 min and separated by horizontal electrophoresis in 3% agarose gels in 0.5 x TBE (130 V for 40 min) stained with GelRed (Biotium) prior to visualization under UV light.

The allele and genotype frequencies of FTO gene were estimated by direct counting and examined for deviation from Hardy – Weinberg equilibrium using Chi-square (χ^2) test. The analyzed carcass traits – back fat thickness (BFT), lean meat percentage (LM), thigh percentage (TP) and MLT area were measured by standard technical norm STN 466164. Estimates of the effects were tested by t-test for significant deviation from zero. Association analysis of the SNP g.276T>G in FTO gene was performed using GLM (General Linear Model) procedure of SAS Enterprise Guide 4.2 software (*SAS Institute Inc.*, 2009) with the following model:

$$Y_{ijk} = \mu + G_i + S_j + W_k + e_{ijk}$$

where: Y_{ijk} – dependent variable (analyzed carcass traits), μ – the general mean, G_i – genotype, S_j – sex, W_k – live weight (kg) as covariate, e_{ijk} – random error.

RESULTS AND DISCUSSION

Three genotypes were identified in the analyzed group of pigs, TT (n=31), TG (n=100) and GG (n=19). Allele T showed higher frequency than allele G (0.54 vs. 0.46). The population was in Hardy-Weinberg equilibrium (P>0.05). Allele and genotype frequencies are presented in *Table 1*. These results are similar to the reported by *Fontanesi et al.* (2009) and *Dvořaková et al.* (2012), where the g.276T allele was the predominant in Large White, Duroc, Landrace, Hampshire and Pietrain pigs. *Fontanesi et al.* (2010) also confirmed lower occurrence of g.276G allele in Italian Duroc and

commercial pig populations. *Table 2* and *3* shows average values of analyzed carcass traits in relation to specific genotype in analyzed population of pigs.

Table 1

Alleles and genotypes frequencies of FTO g.276T>G marker in pigs

Genotype frequency			Allele frequency		χ^2 test
g.276TT	**g.276TG**	**g.276GG**	**g.276T**	**g.276G**	1.23^{-}
20.66	66.67	12.67	0.54 ± 0.029	0.46 ± 0.029	

P>0.05

The observed associations of individual genotypes of FTO gene with the values of analyzed carcass traits are presented in *Table 3*. In the group of evaluated pigs statistical analyses shows only non-significant associations between the variability of back fat thickness, lean meat percentage, thigh percentage and MLT area and different FTO g.276T>G genotypes. Statistically significant effect was found only for sex (P<0.0001) and live weight (P<0.01). The highest value of BFT was found for heterozygous animals. In group of GG homozygote was observed the best value of any others evaluated carcass traits, but differences were low and non-significant (P>0.05).

Table 2

Basic statistical variation measurements carcass traits in pigs

	n	**mean**	**SD**	**min**	**max**
BFT (mm)	150	18.86	4.39	8.67	28.67
LM (%)	150	53.82	2.62	45.14	63.23
TP (%)	150	21.89	1.50	17.59	28.82
MLT area (cm^2)	150	41.79	5.62	26.90	62.50

Table 3

The effect of FTO g.276T>G genotypes on carcass traits in pigs

Trait	**Genotype**								
	g.276TT			**g.276TG**			**g.276GG**		
	n	**mean**	**SD**	**n**	**mean**	**SD**	**n**	**mean**	**SD**
BFT (mm)	31	18.82	3.96	100	19.06	4.54	19	17.85	4.38
LM (%)	31	53.86	2.61	100	53.76	2.78	19	54.08	1.69
TP (%)	31	22.12	1.54	100	21.77	1.55	19	22.19	1.10
MLT area (cm^2)	31	42.82	5.71	100	41.09	5.59	19	43.74	5.21

P>0.05

Important QTL for carcass and meat quality traits localized on SSC6 have been reported in many studies (*Fan et al.*, 2009; *Zhang et al.*, 2009; *Fontanesi and Russo*, 2012;

Szydlovsky et al., 2012), but the effect of g.276T>G single nucleotide polymorphisms in FTO gene was assessed only in a few studies. *Fontanesi et al.* (2009) have identified this FTO polymorphism in intron 4 in associations with intermuscular fat deposition in the Duroc breed and with feed conversion rate in Italian Large White pigs. These results have been confirmed in subsequent analyses on Italian Duroc ($P<0.01$) and commercial pig populations ($P<0.05$) (*Fontanesi et al.*, 2010).

CONCLUSIONS

The SNP g.276T>G in FTO gene was analysed for associations with carcass traits in Large White pigs. In our study it doesn't confirmed the findings of other authors on the effect of FTO gene on production traits in pigs. The associations with analyzed carcass traits showed only statistically non-significant results, but different studies between FTO polymorphisms and production traits indicated that this gene has a major role in the variation of fatness traits. One of the causes can be genetic background effect and limited population size of evaluated animals. To be able to consider FTO gene as genetic marker in assisted selection programs in commercial as well as purebred pig population it is needed to confirm its effect on economically important traits.

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