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Association between FTO gene polymorphism and productivity traits in Lithuanian pigs population

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ABSTRACT

FTO in pigs have reported associations of several single nucleotide polymorphisms with some fat-related traits. The purpose of our study was to investigate the FTO gene single nucleotide polymorphism (SNP) (g.400C>G) in the population of pigs and to evaluate the influence of polymorphism on productivity traits. This study of porcine FTO gene g.400C>G SNP was established from the isolated genomic DNA, amplified by nested polymerase chain reaction (PCR) and digested with restriction enzymes, then DNA fragments were separated by agarose gel electrophoresis. Allele C observed with frequency 0.4, allele G – 0.6. The most common genotype was GG, genotype CC was the rarest. CC genotype pigs consume the most feed per kilogram of weight gain compared with other genotypes. The highest values for backfat thickness at the last vertebra at Fat1 and Fat2 were observed in animals with genotype CC. The lowest muscularity (%) was also observed in CC genotype. It was found that almost all pig production traits were significantly influenced by breed. The breed and genotype interaction influence was statistically significant for muscularity of the carcasses ($P<0.05$) and backfat at the Fat2 thickness ($P<0.05$).

Key words: Carcass traits, c.400C>G, FTO gene, Polymorphism, SNP

Single nucleotide polymorphisms (SNPs) in the fat mass and obesity-associated (FTO) gene are associated with higher obesity risk in humans (Zhao *et al*. 2014). These days obesity is one of the most relavant problems in the world and because of this there is a need for research and analysis of candidate genes in good animal models. The porcine model can be considered as a very usefull and exceptional platform of research based on a biomedical model, the reason being that they share a vast amount of similarities between themselves and a man, such as: physiological, anatomical, metabolical and biochemical. Pigs are prone to suffering the same dietary health problems as humans because of being omnivores, because of this reason, pigs are considered as a good model for human obesity research (Madsen *et al*. 2010). Several genes affecting obesity in humans have been shown to exert important effects on fat deposition traits in pigs, suggesting that the transfer of information from one species to another could be useful both to identifying deoxyribonucleic acid (DNA) markers in candidate genes for breeding purposes in pig production and to elucidate biological mechanisms involved in fat deposition and obesity with medical relevance (Fontanesi *et al.* 2010). FTO gene have a relatively large effect on human obesity as well as on body composition in rodents and, more recently, in livestock (Jevsinek *et al.* 2016).Obesityisanimportant cause of morbidity and mortality in industrialized countries. Obesity can increase the number of serious illnesses such as cardiovascular disease, hypertension, and different types of cancer or type 2 diabetes mellitus (Madsen *et al*. 2010). It was confirmed that FTO gene have a role in fatness among species (Fontanesi and Russo 2013).

Thehuman FTO gene is located in chromosome 16 (16q12.2) it is a large gene consisting of 9 exons spanning more than 400kb and encoding a 505 amino acid protein (Sebert *et al*. 2014, Tung and Yeo 2011, Zhang *et al*. 2011). FTO gene encoded protein molecular weight is 58 kDA (Sebert *et al*. 2014).

Theporcine FTO gene has been mapped to the p arm of chromosome 6 or SSC6 (SSC for *Sus scrofa*) (Dvoøáková *et al*. 2012). In the pig, the FTO gene was assigned between SW1302 and SWR1130 on SSC6, where it is adjacent to the region that a number of QTL (Quantitative Trait Locus) for back fat and average daily gain traits were mapped (Fan *et al.* 2009).

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FTO gene SNP (g.400C>G) CC and CG genotype was associated with higher values for average back fat depth and in pigs with genotype GG associated with higher values of average daily gain, feed conversion and muscle depth (Dvoøákováet *al*. 2012).

Duringthelastdecades, the international pig industry has been focusing on the selective breeding towards genotypes with high growth rate, improved feed efficiency and increased meat content (Balatsky *et al.* 2016).Fat deposition is a crucial aspect of pig meat quality as fat content influences both organoleptic and nutritive characteristics of fresh meat, meat products and consumer acceptance (Braglia *et al.* 2014). Analysing obesity genes and animals with useful selection features can help develop faster growing, lower feed expenses and better carcass traits of pigs.

The aim of our research was to investigate the FTO gene single nucleotide polymorphism (g.400C> G) in the populationof pigsand to evaluate the influence of polymorphism on productivity traits.

MATERIALS AND METHODS

Animals: Pig hair samples were collected at a State Pig Farming Station in Lithuania. 140 pigs were examined: Lithuanian White (LW) (n = 16), Large White (LLW) (n = 18), Yorkshire (Y) (n = 20), Yorkshire (Y) and Landrace (L) cross (n = 22), Yorkshire (Y) x Landrace (L) x L (Landrace) cross (n = 44), Large White (LLW) x Landrace (L) x Landrace (L) cross (n = 20). 5–6 pig hair bulbs were used per sample.

DNA extraction: DNA was extracted from pig hair samples.Lysis mixture was prepared (Dithiothreitol 7.5 µl, Chelex 200 µl, Proteinase K 20 mg/ml) and poured over the tube contents. Samples were vortexed for 30 s, centrifuged at 13500 rpm for 10 s, then incubated for 30 min at 56°C temperature. Before PCR reaction DNA inactivation was performed. Tubes with 10 µl of DNA were heated in a thermocycler for 10 min at 94° C.

Restriction fragment length polymorphism-polymerase chain reaction (PCR-RFLP): For fragments amplification two PCR reactions were carried out. Forward and reverse primers were used according toDvoøáková *et al*. (2012). Both reactions were followed by 35 cycles. The first polymerase chain reaction was performed in a 25 µl reaction mixture – 10 µl genomic DNA and 15 µl prepared PCR mixture (high quality deionized water, buffer solution without $MgCl_2$, $MgCl_2$, dNTP, forward primer (5'-TCAAGAAGCCTTCCTCGCACTG–3'), reverse primer (5' -TGGGGATCCATGAAGCTCAACA–3'), BSA, Taq polymerase. The resulting PCR product – 435 bp. The second PCR reaction was performed in a 20 µl reaction mixture (2 µl of first PCR reaction product and 18 µl second PCR reaction mixture. FTO gene primers for the second reaction: forward (5' - GGCTCTGATGCAAAGTACA-3'), reverse (5' - CCATGAAGCTCAACAAAGTTAG -3'). The resulting PCR product - 259 bp. PCR products of FTO gene were digested with 0.5 µl of RsaI (10 U/µl, 1000 U) restriction enzyme at 37 °C for 16 h and separated by horizontal electrophoresis in 3% agarose gels in 0,5X TBE (Tris-borate-EDTA) buffer (110 V for 45 min) stained with Ethidium Bromide prior to visualization under UV lightusing Bio-Imaging Systems. After restriction 243 and 16 bp fragments were obtained for C allele and 216 bp, 27 bp, 16 bp for G allele.

Estimation pigs productivity traits: Polymorphisms influence evaluated for these productivity traits: all hot carcass weight (1) and yield as well as hot carcass without the head weight (2) and yield; age at 100 kg; average daily gain; feed conversion; carcass length; bacon length; dorsi area; ham weight, backfat thickness at 6–7 rib, backfat thickness at 10 rib, backfat thickness at the last rib, backfat thickness at the last lumbar vertebra; „Piglog 105" (Piglog 105 Users Guide, 1991) data: at two points set backfat thickness, loin muscle depth then according to the data muscularity was calculated. This productivity data was received from the State Pig Farming Station in Lithuania.

Statistical analysis: It was performed using SPSS Statistics 20 statistical software and Microsoft Excel spreadsheets. In order to evaluate reliability of the mean difference comparable groups' characteristics averages and error averages were calculated. The results were considered statistically significant at $P<0.05$. Allele and genotype frequencies were calculated with GenAlEx 6.0 (Peakall *et al*. 2006).

RESULTS AND DISCUSSION

Allelic variants for FTO gene single nucleotide polymorphism (g.400C>G) were determined by nested PCR-RFLP in samples choosen from different breeds. Fig. 1 shows the electrophoretic pattern of specific amplified products. After the first PCR reaction a product of 435 bp

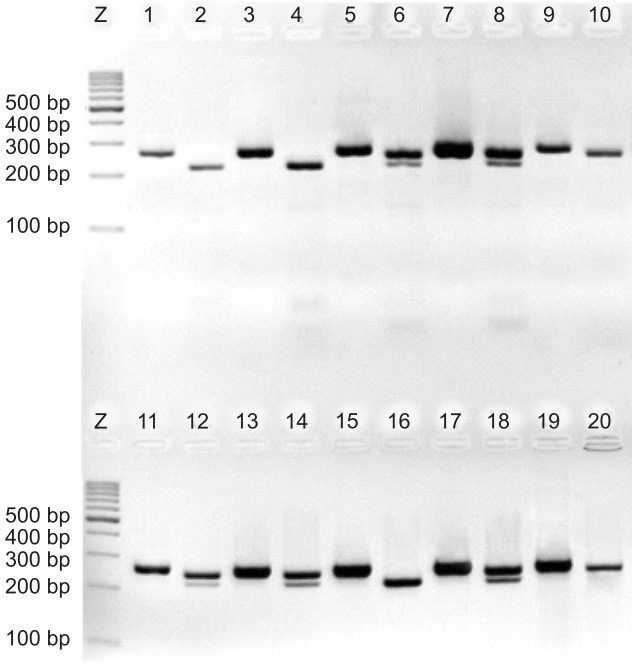

Fig. 1. Genotyping of FTO polymorphism by PCR-RFLP

Table 1. Genotypes and alleles frequencies

Genotype	N	Frequency	Allele	Frequency
CC	34	0.243	C	0.4
CG	44	0.314	G	0.6
GG	62	0.443		
Total:	140	1		1

and after the second reaction a product of 259 bp were obtained. After electrophoresis both alleles (C and G) were detected in agarose gel and all possible variants of the genotypes were detected (Fig. 1).

Following the reaction with the RsaI restriction enzyme fragments of different lengths were obtained: C allele - 243 bp and 16 bp fragment length, G allele - 216 bp, 27 bp, 16bp fragment. 10 and 20 wells – CC genotype; 6, 8, 12, 14 and 18 wells – CG genotype; 2, 4 and 16 wells – GG genotype. The small fragments are difficult to estimate, but genotypes are easily identified by 243 bp and 216 bp fragments. • – molecular sign.

Genotype and allele frequencies were calculated for the studied population (Table 1). The most frequent genotype was GG and it accounted for 44.3% of the test population. The rarest genotipe was CC. It accounted for 24.3% of pigs. G allele in the analyzed pig population was more frequentthan C allele.AccordingDvoøákovà*et al*. (2012) allele C isassociatedwithhighervaluesforfatdeposition, whileallele G withhighervaluesformeattraits.

Genotype and allele frequency distribution varied in the studied pig population (Table 2). The most common genotypes in Lithuanian White (LW) pigs were CC (50%) and GG (37.5%). Major part of Large White (LLW) pigs was GG genotype (66.7%). Yorkshire (Y) had greater portions of CC and CG genotype (respectively 50% and 40%). In Yorkshire pigs C allele was predominant (70%). Yorkshire (Y) and Landrace (L) cross had CG and GG genotype established at the same frequency (0.4545). Yorkshire (Y) x Landrace (L) x L (Landrace) cross also had CG and GG genotype predominance at the same frequency. In Large White (LLW) x Landrace (L) x Landrace (L) cross pigs GG genotype was the most frequent (75%). G allele was predominant (81%).

The largest amount of feed per kilogram of daily weight gain was found in pigs with CC genotype. Pigs with CC genotype on average consumed feed for 1 kg of weight gain 230 g and 210 g more than pigs with GG and CG genotypes accordingly (P<0.05) (Table 3). Dvoøáková *et al*. (2012) associated SNP g.400C>G C allele with higher feed consumption; average daily gain and feed conversion were higher in pigs with GG genotype.

Studies on pig FTO have reported associations of several single nucleotide polymorphisms with some fat-related traits (Dvoøáková *et al*. 2012, Fan*et al*. 2009, Fontanesi *et al*. 2013, Moravèikova*et al*. 2014, Zhang*et al*. 2009).Severalstudiesestablishedrelationship 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. In an Italian Duroc population and a Berkshire´Yorkshire population related traits were also confirmed (Fontanesi *et al*. 2010, Fontanesi *et al*. 2013). 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 (Jacino *et al.* 2015). Results of Dvoøáková *et al.* (2012) study show that in commercial pig populations FTO influences backfat depth. Zhang *et al.* (2009) found in 6 Chinese native pigs breeds population significant association only between FTO gene and intramuscular fat content. Genotype, breed and their interaction influence on pigs productivity traits were studied (Table 4). Breed had the highest influence on almost all productivity traits in

Table 2. FTO gene genotypes and alleles frequencies of the studied pure and crossbred pigs

Breed	N	Genotype frequency			Allele frequency	
		CC	CG	GG	C	G
LW	16	0.500	0.125	0.375	0.5625	0.4375
LLW	18	0.222	0.111	0.667	0.2775	0.7225
Y	20	0.500	0.400	0.100	0.700	0.300
Y × L	22	0.091	0.4545	0.4545	0.318	0.682
Y × L × L	44	0.166	0.417	0.417	0.375	0.625
LLW × L × L	20	0.125	0.125	0.750	0.1875	0.8125

Table 3. Fattening traits of pigs in different genotypes

Genotype	N	Age at finish (days)	Age at 100 kg (days)	Average daily gain (g)	Feed conversion (kg/kg)
CC	34	172.8± 2.74	175.6± 3.61	767.0± 24.24	2.63± 0.061 a
CG	44	174.1± 2.66	176.1± 2.98	799.9± 19.91	2.42± 0.062 b
GG	62	173.6± 1.88	176.3± 2.56	769.3± 17.20	2.40± 0.050 b

a,b – values with different letters in the columns differ significantly (P<0.05)

P – most significant probability of t test between two genotype classes

Table 4. Genotype, breed and their interaction influence on productivity traits (%)

Factor/Trait	Genotype	Breed	Genotype × breed
Age at finish (days)	0.1	54.8***	6.3
Age at 100 kg (days)	0.03	41.1***	8.6
Hot carcass weight 1 (kg)	5.7	13.7	5.1
Yield 1 (%)	0.7	23.6*	7.7
Hot carcass weight 2 (kg)	5.7	13.7	5.1
Yield 2 (%)	0.7	24.1*	7.8
Average daily gain (g)	9.1*	42.9***	3.9
Feed conversion (kg/kg)	1.6	36***	12.7
Carcass length (cm)	1.6	36***	12.7
Bacon length (cm)	2.6	39.3***	12.5
Dorsi area (cm^2)	4.3	18.3	7.5
Ham weight (kg)	5.4	21.9*	8.9
Muscle depth at Fat2 (mm)	2.1	40.3***	8.2
Muscularity (%)	6.6**	57.5***	10.9*
Backfat thickness at 6-7 rib (mm)	12.7*	11.7	12.6
Backfat thickness at 10 rib (mm)	12.6**	18.3	14.7
Backfat thickness at the last rib (mm)	7.1	22.6*	16.6
Backfat thickness at the last lumbar vertebra (mm)	7.3*	37.6***	10.8
Backfat thickness at Fat1 (mm)	6.9**	56.7***	10.6
Backfat thickness at Fat2 (mm)	6.8**	55.3***	13.7*

*P<0.05; ** P<0.01;***P<0.001

pigs. Muscularity of the carcasses and backfat at the Fat2 thickness were influenced statistically significantly by breed and genotype interaction (P<0.05). Genotype in this research had a significant influence on the pig daily gain, backfatthickness at 6–7rib, backfatthickness at the last lumbar vertebra (P<0.05), muscularity, backfat thickness at the 10 rib, backfat thickness at Fat1 and Fat2 points (P<0.01). The association analyses of Fontanesi *et al.* (2010) confirmed the effect of the FTO on obesity-related traits (visible intermuscular fat, back fat thickness) in the Italian Duroc pigs. Although different populations were used and different traits were recorded, in all cases genotypes at some of the SNP were associated mostly with fatness traits. While Fan *et al.* (2009) did not find any association between polymorphisms in exon 3 and the 5' untranslated region of porcine FTO, respectively, and average back fat thickness in the Berkshire × Yorkshire F2 population. A polymorphism in the 5' regulatory region of FTO has been shown to be associated with intramuscular fat content in a Jinhua × Pietrain F2 population (Zhang *et al*. 2009). Fan *et al.* (2009) identified two DNA markers in the FTO gene that were ssociated with total lipid percentage in muscle of a Berkshire × Yorkshire F2 population. Moreover, it was reported that different genotypes of FTO affected intermuscular fat deposition in Italian Duroc pigs and feed conversion rate in Italian Large White pigs (Fontanesi *et al*. 2009). Results showed that FTO mRNA expression in muscle was significantly affected by both breed and developmental stage in pigs. The results indicated that FTO is involved in the genetic variation of intramuscular fat content at differentbreedsof pigs through a distinct genetic mechanism. It might be that the FTO protein differs with different phenotypes and splice variants, which could be more related to intramuscular fat content in different breeds (Tao *et al*. 2013).

Carcass traits of pigs are very important for pork production. Different genotypes porcine carcass traits werean alyzed. Ourresultsshowsignificant difference for hot carcass weight averages between CC and CG genotypes (P<0.05). According Sebert *et al*. (2014) GG genotype was associated with higher muscularity and lean cuts valueshowever the mean differences were not statistically significant but in our research the highest values for muscularity was observed in pigs with CG genotype (58.04±0.5%). Significant difference established between CC and CG genotype muscularity (P<0.05). According to Dvoøáková *et al.* (2012) highest muscularity values were found in pigs with CG genotype also. Whileloinmuscledepth at Fat2 was highest in animals with GG genotype (49.84±1.30 mm) and lowest with CC genotype (47.47±1.65 mm).CG genotypevaluesfor dorsi area were ~2.59 cm^2 and CC genotype ~2.39 cm^2 lower than GG genotype (42.03±1.1cm^2). CG genotypehamweight values were about 0.22 kg and CC genotype about 0.33 kg lower than GG genotypehamweight (12.18±0.1 kg).

In addition, the study of Fan *et al.* (2010) revealed a significant effect of SNP g.400C>G on average back fat depth, with genotypes CC and CG having higher effects. In our study the lowest backfat thickness at 6–7 and 10 ribs values were found in pigs with CG genotype (P<0.05). CG genotype backfat thickness at 6–7 rib was 3.7 mm lower than in CC genotype (18.6±0.95 mm) and 3.5 mm lower than backfat thickness in GG genotype (18.4±0.8 mm). Backfat thickness values in pigs with CG genotype (13.4±0.93 mm) at the 10 rib were 3.2 mm lower than CC genotype and 3.3 mm lower than GG genotype. Significant results were identified for backfat thickness at the last lumbar vertebra and at Fat1 and Fat2 points between CC and CG genotypes (P<0.05). CCgenotypebackfatthickness at the last lumbar vertebra was ~2.86 mm higher than CG genotype. CC genotype pigs had respectively about 2.41 mm and 2.2 mm higher backfat thickness at Fat1 and at Fat2. Dvoøáková *et al.* (2012) highest backfat thickness values identified in CC and CG genotypes.

The purpose of our study was to investigate the FTO gene single nucleotide polymorphism (SNP) (g.400C>G) in the population of pigs and to evaluate the influence of polymorphism on productivity traits. The most common genotype was GG. CC genotype pigs consume the most feed per kilogram of weight gain. The highest values for backfat thickness at the last vertebra at Fat1 and Fat2 were observed in animals with genotype CC. The lowest muscularity (%) was also observed in CC genotype. It was found that almost all pig production traits were significantly influenced by breed. The breed and genotype interaction influence was statistically significant for muscularity of the carcasses

(P<0.05) and backfat at the Fat2 thickness (P<0.05).

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