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FTO AND PLIN2 GENES POLYMORPHISM IN RELATION TO GROWTH TRAITS OF PIGS

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Abstract. *FTO* and *PLIN2* genes are considered as a markers for fat-related traits in pigs. However, high expression of these genes in muscle tissues and earlier studies suggest, that they can be also associated with growth. Therefore the aim of this study was to analyze polymorphism in *FTO* (g.400C > G) and *PLIN2* (g.98G > A) genes in relation to growth traits in pigs. The study were conducted on 578 sows including following breeds: Duroc, Hampshire, Polish Landrace, Pietrain, Puławska and Polish Large White. *FTO* and *PLIN2* genes polymorphism was determined by use PCR-RFLP and ACRS-PCR methods respectively. Association analysis was performed for Polish Landrace, Puławska and Polish Large White separately and for joined group of 6 breeds. The results show that *FTO* genotypes were related only to test daily gain in Polish Large White pigs ($p < 0.05$). *PLIN2* gene variants, however were associated with test daily gain in Polish Landrace, Puławska ($p < 0.01$) and joined group ($p < 0.05$), average daily gain in Puławska ($p < 0.01$), feed:gain ratio in joined group ($p < 0.05$) and age at slaughter and number of days on test in Puławska ($p < 0.01$) and joined group ($p < 0.01$ and $p < 0.05$ respectively). Obtained results show that *PLIN2* gene is more related to growth traits in pigs than *FTO*, but needs further analysis and explanation.

Key words: *FTO*, *PLIN2*, polymorphism, growth traits, pigs.

INTRODUCTION

One of the method for QTL (quantitative trait loci) identification is evaluation the effect of the polymorphism in genes (known as candidate genes) on the level of certain quantitative traits in different breeds and lines of pigs. This analysis usually involves genes whose product (protein) is known to be involved in physiological processes associated with a given production trait (Żak and Pieszka 2009).

Fat mass and obesity associated gene (*FTO*) plays an important role in controlling of appetite and energy consumption in human and mice. *FTO* gene expression is significantly highest ($p < 0.05$) in pig backfat, when compare to other tissues, whereas in *m. longissimus dorsi* shows the second highest level ($p < 0.05$) (Fu et al. 2013). Although *FTO* gene has been proposed as a marker for fat deposition (Fontanesi et al. 2009) it may be also

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considered as a marker for growth traits in pigs because its high expression in muscles. It was proved by Fan et al (2009) who analyzed single nucleotide polymorphism (SNP) detected in exon 3 (g.400C > G) of porcine *FTO* gene. The study showed that individual genotypes were significantly associated with average daily gain on test and total lipid percentage in muscle ($P < 0.01$) of Berkshire × Yorkshire pigs. Later it was also demonstrated that g.400C > G variants influence some carcass and fat-related and growth traits including average daily gain, feed conversion ratio and feed consumption (Dvořáková et al. 2012).

Perilipin 2 (*PLIN2*) stimulates long chain fatty acid uptake and regulates metabolism of intracellular lipid droplets (Gao and Serrero 1999; Conte et al. 2016). Expression analysis showed the presence of *PLIN2* transcripts in all seven investigated pig tissues with high expression found in adipose tissue and skeletal muscle (Davoli et al. 2011). Other study, that covered fat-type and lean-type pigs indicated that *PLIN2* was expressed similarly in backfat of both groups, however *PLIN2* transcripts were more abundant in muscle of fat-type than in that of lean-type pigs (Tempfli et al. 2016). Similarly to *FTO* gene *PLIN2* has been proposed as a marker for fat-related traits, especially intramuscular content (Yang et al. 2017), but mentioned expression studies indicate that it could be also take into consideration as a marker for growth traits in pigs. This hypothesis is supported by results of Davoli et al. (2011) who detected SNP in porcine 3'-untranslated region (UTR) of *PLIN2* gene (g.98G > A). Association analysis showed that its different variants are related ($P < 0.01$) to average daily gain, feed conversion ratio, lean cuts and ham weight estimated breeding values in Duroc pigs. Further study also confirmed that *PLIN2* SNP is associated with early growth rate and carcass length in the same breed (Gol et al. 2016).

Because *FTO* and *PLIN2* genes are promising markers for growth traits, the aim of this study was to analyze their variants (g.400C > G and g.98G > A respectively) in relation growth traits of pigs reared in Poland.

MATERIAL AND METHODS

The study were performed on 578 sows that belong to following breeds: Duroc ($n = 14$), Hampshire ($n = 7$), Polish Landrace ($n = 269$), Pietrain ($n = 31$), Puławska ($n = 68$) and Polish Large White ($n = 189$). Pigs were kept in three Pig Test Stations (SKURTC_h) of the National Research Institute of Animal Production, located in Chorzelów, Pawłowice and Mełno (Poland). Conditions of rearing and feeding were unified for all animals. Pigs were introduced to stations at the age of 12 weeks and fed *ad libitum* according to SKURTC_h scheme till reach 100 (± 3) kg weight of body (Różycki and Tyra 2009). After feeding period pigs were slaughtered and tissues for DNA analysis were collected.

During the test following growth traits were estimated for each pig: test daily gain (TDG), average daily gain (ADG), feed:gain ratio (FCR), daily feed intake (DFI), age at slaughter (AS) and number of days on test (NDT). TDG was calculated by subtracting off-test weight and on-test weight, then dividing by NDT.

Genomic DNA was isolated from collected tissues using ReliaPrep™ gDNA Tissue Miniprep System (Promega, USA) and Genomic Mini (A&A Biotechnology, Gdynia, Poland) kits.

Polymorphism of porcine *FTO* gene (g.400C > G) was detected by use of PCR-RFLP method. Following pair of primers for PCR were designed based on FM244720.1 sequence: F 5'-GCC GGT GTG TAT AGG TCC AG-3', R 5'-GGA TCC ATG AAG CTC AAC AAA-3' (Primer3Plus; Untergasser et al. 2012). PCR mixes for *FTO* and *PLIN2* genes were prepared in the same way and contains: GoTaq® Master Mix (Promega, USA), 10pmol of each primer, 40–70ng of DNA and PCR grade water fill up to 10µl. For *FTO* gene, following PCR conditions were applied: 95°C/5min, 30 cycles of 95°C/40s, 54°C/40s, 72°C/40s and 72°C/5min. After PCR, amplicons were digested overnight at 37°C by *RsaI* restriction enzyme (Thermo Scientific, USA) and separated in 2.5% agarose gels stained with ethidium bromide.

Polymorphism in pig *PLIN2* gene (g.98G > A) was detected by means of ACRS-PCR method. Primers for PCR were designed manually based on GU461317.1 sequence and its properties were estimated by use of OligoAnalyzer 3.1 software (Integrated DNA Technologies, USA): F 5'-TTT TGC CTC TGT TGC CAC TGT TTG CCA GCT-3', R 5'-GTG AGA CAA ACC AGT GCT GAG GCC-3'. Cycling conditions were as follows: 95°C/5min, 32 cycles of 95°C/1min, 63°C/1min, 72°C/2min and 72°C/5min. Obtained amplicons were digested overnight at 37°C by *PvuII* restriction enzyme (Thermo Scientific, USA). Next, restriction fragments were separated in 3% agarose gels stained with ethidium bromide.

Analyzed growth and feeding traits of pigs were assessed in a fixed model using the least squares method of the General Linear Model procedure in SAS/STAT software (SAS Institute Inc., USA). For association study the following statistical model was used:

$$Y_{ijk} = \mu + b_i + g_j + (bg)_{ij} + e_{ijk}$$

where:

Y_{ijk} – the observation;

μ – the overall mean;

b_i – the fixed effect of i breed;

g_j – the fixed effect of j genotype group of *FTO* or *PLIN2* gene;

$(bg)_{ij}$ – interaction between g_j genotype group and b_i breed (when significant);

e_{ijk} – random residual error.

Analyses were conducted for Polish Landrace, Polish Large White and Puławska breeds separately and for all pigs together (6 breeds).

RESULTS AND DISCUSSION

PCR-RFLP method allowed to determine *FTO* variants based on following lengths of restriction fragments: CC – 123bp, CG – 123, 92, 31bp, GG – 92, 31bp (Fig. 1). All genotypes and alleles of *FTO* gene were present in studied breeds, except Hampshire, where GG genotype was absent. C allele was predominant in all breeds, excluding Duroc.

Proposed method – ACRS-PCR, successfully enabled to determine *PLIN2* variants based on following restriction fragment lengths: AA – 127bp, AG – 127, 99, 28bp, GG – 99, 28bp (Fig. 2). In analyzed breeds, AA genotype was present only in half of them (Duroc, Hampshire and Puławska). In Duroc it reached highest frequency, however GG genotype was absent in this breed. In other breeds GG genotype and G allele were predominant.

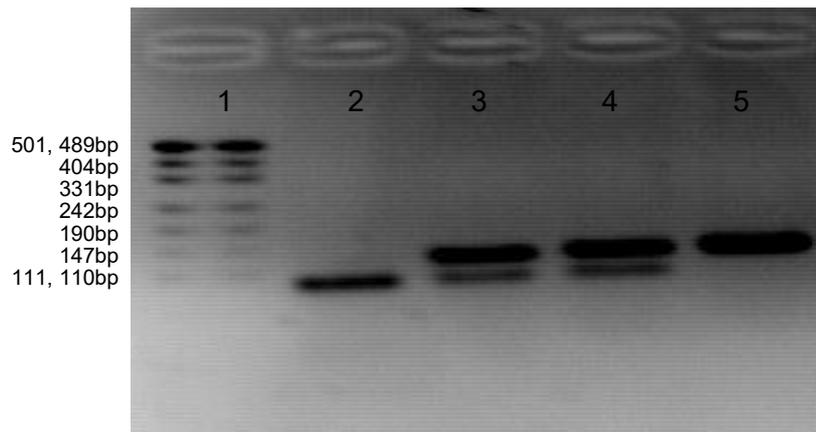


Fig. 1. *FTO* genotype determination by means of electrophoresis in 2.5% agarose gel. Lane 1 – pUC19/*MspI* DNA marker (Thermo Scientific, USA), lane 2 – GG genotype, lanes 3, 4 – CG genotype, lane 5 – CC genotype. Band of 31bp is not visible

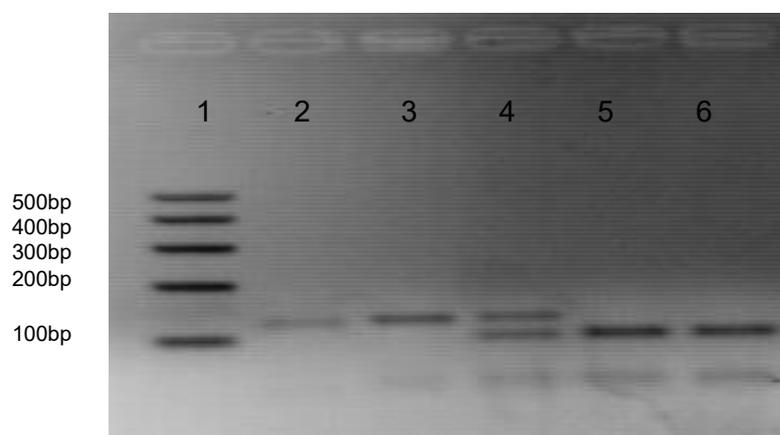


Fig. 2. *PLIN2* genotype determination by means of electrophoresis in 3% agarose gel. Lane 1 – DNA marker M100-500 (DNA Gdańsk, Poland), lanes 2, 3 – AA genotype, lane 4 – AG genotype, lanes 5, 6 – GG genotype. Band of 28bp is not good visible

Association between *FTO* genotypes and growth traits in analyzed pigs is presented in Table 1. We found that different variants of this gene were correlated only with test daily gain in Polish Large White. Sows with CC genotype were characterized by highest values of this trait in relation to those with GG ($p \leq 0.05$). The same tendency was observed for joined group but was not confirmed statistically. Other studies, however shown that GG genotype is favorable for average daily gain in Pietrain \times (Czech Landrace \times Czech Large White) ($p = 0.02$) and Meishan \times Pietrain ($p = 0.04$) crossbreeds as well as in joined group including 7 crossbreeds and one pure breed ($p = 0.02$) (Fan et al. 2009; Dvořáková et al. 2012). In our study we found, however that CC or CG genotype was associated with highest values of this trait but differences were not significant. In Meishan \times Pietrain, Berkshire \times Yorkshire and joined group, CG genotype was also correlated with highest feed conversion ratio ($p = 0.008$, $p = 0.04$, $p = 0.003$ respectively), whereas in our study we observed this non-significant tendency for homozygous genotypes.

Table 1. Association between *FTO* gene polymorphism and growth traits in different pig breeds and in joined group (LSM \pm SE)

Trait	Genotype	Polish Landrace (n = 269)	Polish Large White (n = 189)	Puławska (n = 68)	Joined group (n = 578)
Test daily gain [g/day]	CC	909 \pm 26.3	900 \pm 17.09 ^a	804 \pm 14.29	863 \pm 12.32
	CG	910 \pm 24.7	880 \pm 17.80	811 \pm 15.39	863 \pm 12.41
	GG	904 \pm 27.9	875 \pm 32.4 ^a	814 \pm 28.1	853 \pm 16.24
Average daily gain [g/day]	CC	617 \pm 19.2	592 \pm 8.3	549 \pm 10.6	577 \pm 8.0
	CG	620 \pm 18.0	584 \pm 8.6	542 \pm 11.4	582 \pm 8.0
	GG	619 \pm 20.4	583 \pm 15.7	548 \pm 20.8	576 \pm 10.5
Feed:gain ratio [kg]	CC	2.641 \pm 0.069	2.669 \pm 0.034	2.794 \pm 0.037	2.677 \pm 0.031
	CG	2.645 \pm 0.065	2.714 \pm 0.035	2.788 \pm 0.040	2.690 \pm 0.031
	GG	2.648 \pm 0.074	2.747 \pm 0.064	2.765 \pm 0.072	2.695 \pm 0.041
Daily feed intake [kg]	CC	2.389 \pm 0.071	2.389 \pm 0.042	2.237 \pm 0.027	2.295 \pm 0.031
	CG	2.418 \pm 0.066	2.381 \pm 0.043	2.280 \pm 0.029	2.308 \pm 0.032
	GG	2.382 \pm 0.075	2.386 \pm 0.079	2.245 \pm 0.053	2.284 \pm 0.042
Age at slaughter [days]	CC	173 \pm 5.7	175 \pm 2.5	185 \pm 3.5	181 \pm 2.4
	CG	171 \pm 5.4	177 \pm 2.6	186 \pm 3.8	180 \pm 2.4
	GG	170 \pm 6.1	177 \pm 4.7	185 \pm 6.9	181 \pm 3.2
Number of days on test [days]	CC	83.2 \pm 2.459	81.9 \pm 1.481	88.3 \pm 1.672	86.2 \pm 1.147
	CG	82.3 \pm 2.307	83.4 \pm 1.543	85.6 \pm 1.801	86.0 \pm 1.156
	GG	83.3 \pm 2.609	83.9 \pm 2.809	87.0 \pm 3.283	86.8 \pm 1.513

n – the number of animals analyzed in given group; values marked with the same letter differ statistically at $p \leq 0.05$ (^a)

Table 2 presents results of association study for *PLIN2* polymorphism. In Polish Landrace we found that *PLIN2* variants were associated with test daily gain ($p \leq 0.01$), whereas in Puławska with test daily gain, average daily gain, age at slaughter and number of days on test ($p \leq 0.01$). In the first breed AG genotype was associated with highest test daily gain, while GG in the second. These differences may results from the high genetic distance between mentioned breeds. Puławska is polish native breed, under conservation breeding, classified between meat and meat fat type of utility. Polish Landrace, however is commercial breed that represents meat type. It is hard to discuss obtained results with others because of presence only two genotypes in breeds under association study. Davoli et al. (2011) shown that *PLIN2* SNP is correlated with average daily gain, feed conversion ratio, lean cuts and hams weight estimated breeding values. In Italian Duroc pigs, AA genotype was characterized by highest average daily gain ($p = 0.003$), whereas GG genotype by highest feed conversion ratio ($p = 0.002$). In our study we found statistically significant association with average daily gain only in Puławska breed ($p \leq 0.01$), where GG genotypes was favorable for this trait. Further studies shown that A allele influence only early growth (up to 120 day) in Duroc breed (Gol et al. 2016). In our study average daily gain was measured since introduction of sows to stations at the age of 12 weeks till slaughter (174–202 days), so only partially covered early growth. Similarly to Davoli et al. (2011) we noticed tendency that GG genotype is correlated with highest values of feed:gain ratio in each breed and joined group, but it was confirmed only in all animals together ($p \leq 0.05$). In Puławska breed we found that GG genotype was associated with better gains, what was naturally correlated with lowest age of slaughter and number of days on test ($p \leq 0.01$). In the joined group reverse tendency was noticed. Highest test daily gain ($p \leq 0.05$) and lowest age of slaughter

($p \leq 0.01$) and number of days on test ($p \leq 0.05$) were observed in animals with AA genotype. This tendency may result from the presence of AA genotype in joined group or/and from different utility types of analyzed pigs.

Table 2. Association between *PLIN2* polymorphism and growth traits in different pig breeds and in joined group (LSM \pm SE)

Trait	Genotype	Polish Landrace (n = 269)	Polish Large White (n = 189)	Puławska (n = 68)	Joined group (n = 578)
Test daily gain [g/day]	AA	–	–	–	896 \pm 41.4 ^{ab}
	AG	938 \pm 30.8 ^A	886 \pm 29.8	761 \pm 38.8 ^A	862 \pm 16.8 ^b
	GG	915 \pm 24.4 ^A	892 \pm 15.8	817 \pm 11.0 ^A	858 \pm 13.1 ^a
Average daily gain [g/day]	AA	–	–	–	580 \pm 26.7
	AG	605 \pm 22.4	579 \pm 14.4	505 \pm 28.8 ^A	562 \pm 10.8
	GG	608 \pm 17.8	589 \pm 7.6	564 \pm 8.2 ^A	583 \pm 8.5
Feed:gain ratio [kg]	AA	–	–	–	2.599 \pm 0.104 ^{ab}
	AG	2.644 \pm 0.064	2.688 \pm 0.031	2.785 \pm 0.100	2.693 \pm 0.033 ^a
	GG	2.650 \pm 0.081	2.705 \pm 0.059	2.791 \pm 0.029	2.702 \pm 0.042 ^b
Daily feed intake [kg]	AA	–	–	–	2.321 \pm 0.106
	AG	2.478 \pm 0.083	2.357 \pm 0.072	2.091 \pm 0.072	2.312 \pm 0.043
	GG	2.405 \pm 0.066	2.388 \pm 0.038	2.272 \pm 0.020	2.294 \pm 0.034
Age at slaughter [days]	AA	–	–	–	181 \pm 8.0 ^{Ab}
	AG	174 \pm 6.7	179 \pm 4.3	202 \pm 9.5 ^A	186 \pm 3.2 ^b
	GG	174 \pm 5.3	177 \pm 2.3	179 \pm 2.7 ^A	189 \pm 2.5 ^A
Number of days on test [days]	AA	–	–	–	83.6 \pm 3.854 ^{ab}
	AG	81.1 \pm 2.877	84.5 \pm 2.578	96.1 \pm 4.476 ^A	86.10 \pm 1.560 ^b
	GG	82.7 \pm 2.279	82.4 \pm 1.367	86.3 \pm 1.274 ^A	86.3 \pm 1.226 ^a

n – the number of animals analyzed in given group; values marked with the same letter differ statistically at $p \leq 0.05$ (^{ab}) or $p \leq 0.01$ (^A).

CONCLUSIONS

In our study we have tested hypothesis that *FTO* and *PLIN2* genes polymorphism may be associated with growth traits in pigs. Analysis were performed on three breeds separately and joined group that included six breeds (n = 578). Results shown that *FTO* variants were associated only with one trait (test daily gain) in Polish Large White ($p \leq 0.05$), while *PLIN2* variants in most of analyzed traits. *PLIN2* genotypes were correlated with test daily gain, average daily gain, feed:gain ratio, age of slaughter and number of days on test ($p \leq 0.05$ or $p \leq 0.05$) in individual pig breeds and joined population. We observed also reverse distribution of the growth traits regarding to *PLIN2* genotypes in Puławska breed and joined group. To conclude, *PLIN2* gene seems to be more promising marker for growth traits in pigs, but needs further studies.

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POLIMORFIZM GENÓW *FTO* I *PLIN2* W ODNIESIENIU DO CECH WZROSTU ŚWIŃ

Streszczenie. Geny *FTO* i *PLIN2* są rozważane jako markery cech związanych z cechami otluszczenia świń. Wysoka ekspresja tych genów w tkance mięśniowej i poprzednie badania sugerują, że mogą być one powiązane również ze wzrostem. W związku z powyższym celem niniejszych badań była analiza polimorfizmu w genach *FTO* (g.400C > G) i *PLIN2* (g.98G > A), w odniesieniu do cech wzrostu świń. Badania przeprowadzono na 578 lochach ras: duroc, hampshire, polska biała zwisloucha, pietrain, puławska i wielka biała polska. Polimorfizm genów *FTO* i *PLIN2* został określony za pomocą metod PCR-RFLP i ACRS-PCR. Analizy zależności wykonano oddzielnie dla ras polska biała zwisloucha, puławska i wielka biała polska oraz dla grupy łączącej 6 ras. Wyniki wykazały, że genotypy *FTO* były powiązane tylko z przyrostem dziennym świń rasy polska biała zwisloucha ($p < 0,05$). Warianty genów *PLIN2* były natomiast związane z przyrostem dziennym świń ras polska biała zwisloucha, puławska ($p < 0,01$) i w grupie łączącej 6 ras ($p < 0,01$), ze średnim przyrostem dziennym świń rasy puławska ($p < 0,01$), ze zużyciem paszy na 1 kg przyrostu w grupie łączącej 6 ras ($p < 0,05$) i z wiekiem w dniu uboju, a także z liczbą dni tuczu w czasie testu świń rasy puławska ($p < 0,01$) i w grupie łączącej 6 ras (odpowiednio $p < 0,01$ i $p < 0,05$). Uzyskane wyniki wskazują, że gen *PLIN2* jest bardziej powiązany z cechami wzrostu świń niż gen *FTO*; wymaga to jednak kolejnych analiz i wyjaśnienia.

Słowa kluczowe: *FTO*, *PLIN2*, polimorfizm, cechy wzrostu, świnie.

