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ANALYSIS OF *FTO* AND *PLIN2* POLYMORPHISMS IN RELATION TO CARCASS AND MEAT QUALITY TRAITS IN PIGS*

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Abstract

The aim of this study was to analyze the association of polymorphisms in alpha-ketoglutarate-dependent dioxygenase (*FTO*) and perilipin 2 (*PLIN2*) genes with carcass and meat quality traits in pigs reared in Poland. The research covered 578 sows that belong to the following breeds: Duroc, Hampshire, Polish Landrace, Pietrain, Puławska and Polish Large White. *FTO* (FM244720:g.400C>G) and *PLIN2* (GU461317:g.98G>A) genes variants were determined by means of PCR-RFLP and ACRS-PCR methods respectively. Association between individual genotypes and analyzed traits was calculated by means of GLM procedure for Polish Landrace, Polish Large White and Puławska breeds separately and for all six breeds together in case of *FTO* gene. The results showed that *FTO* variants were associated with weight of loin without backfat and skin (WL), loin eye area (AL) and meat percentage (MP) in Polish Large White (P≤0.05), mean backfat thickness from 5 measurements (BFT) and pH measured 45 min after slaughter in *m. longissimus dorsi* (pH24 ld) as well as with water holding-capacity (WHC) in Puławska breed (P≤0.01). *PLIN2* genotypes, however were correlated with WL and height of the loin eye (HL) in Polish Large White and Puławska (P≤0.05), AL in Polish Large White (P≤0.01) as well as luminosity (L*) in Puławska (P≤0.05) pigs. We observed most consistent relationships of *PLIN2* SNP with intramuscular fat content (IMF) and WHC. In 3 analyzed breeds *GG* genotype was connected with highest values of these traits (P≤0.05).

Key words: *FTO*, *PLIN2*, SNP, carcass, meat, pigs

For many years, breeding work in herds of pigs was aimed at increasing meat content in the most important cuts (Żak et al., 2008). The breeding programs of modern pig breeds often aim to decrease backfat and abdominal fat weight while keeping the optimum intramuscular fat content (Żak and Pieszka, 2009; Szydlowski et al., 2012). Numerous genes, which are involved in lipid and energy metabolism were

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identified as candidate genes for IMF deposition, since their variants were associated with IMF content (Zhao et al., 2012). Among them, *PLIN2* and *FTO* genes deserve special attention.

Alpha-ketoglutarate-dependent dioxygenase (FTO), also known as fat mass and obesity-associated protein, demethylates various methylated nucleic acids, strongly preferring N6-methyladenosine (m6A) in single stranded RNA (Jia et al., 2011). In human, *FTO* gene variants were associated with body mass index (BMI) and the risk of obesity (Hunt et al., 2008; Qi et al., 2008). Porcine *FTO* is located on chromosome 6 (SSC6: 31,174,569-31,564,906) and consists of 9 coding exons (Ensembl, Sscrofa11.1; Aken et al., 2016). Analysis of porcine *FTO* expression showed that its transcripts were present in all investigated tissues with the highest level in backfat, lung and subcutaneous tissue. Moreover, *FTO* mRNA and protein expression in tissues of high-fat pigs was significantly higher ($P \leq 0.05$) in comparison to low-fat pigs, which may suggest beneficial effects of *FTO* expression on fat deposition (Fu et al., 2013; Chen et al., 2016). Numerous studies were focused on searching for association between different *FTO* variants and performance traits in pigs. One example is an investigation in which twelve variants of porcine *FTO* gene were detected including ten single nucleotide polymorphisms (SNP) and two insertion-deletion polymorphisms. Next, five SNPs were chosen for association analysis between growth and fat-related traits in the Iowa State University Berkshire × Yorkshire pig resource family. Among them, only c.594C>G was associated with backfat traits: lumbar backfat ($P \leq 0.05$), tenth rib backfat and average backfat ($P \leq 0.1$). It had also a highly significant ($P \leq 0.01$) association with total lipid percentage (Fan et al., 2009). Further study has proved that c.594C>G SNP, named later g.400C>G (FM244720:g.400C>G) was significantly associated with backfat depth and muscling traits in commercial pig populations and with fat-related traits in the Meishan × Pietrain F_2 pigs (Dvořáková et al., 2012). Detected polymorphism does not change amino acids sequence (Ala198Ala), however it could influence transcription, splicing, mRNA transport or translation, any of which could alter the phenotype (Goymer, 2007).

Perilipin 2, also called adipose differentiation-related protein (ADRP), is involved in lipid droplets formation, which are responsible for storage of lipids in cells. Porcine *PLIN2* gene consists of 9 exons and is located on chromosome 1 (SSC1: 203,683,875-203,713,121) (Ensembl, Sscrofa11.1; Aken et al., 2016). Zambonelli et al. (2016) showed that *PLIN2* expressed four different isoforms that differ by skipping of the second or fourth exon or longer first exon and lengths of their 3′-untranslated regions (UTRs). Numerous studies focused on expression of *PLIN2* gene in pigs. Analyzing fat-type and lean-type pigs Tempfli et al. (2016) proved that *PLIN2* transcript levels were positively correlated with backfat thickness but negatively with loin diameter and average daily gain during fattening. Morever *PLIN2* expression in muscle was higher for fat-type pigs which may be associated with IMF accumulation. This hypothesis is supported by results of Yang et al. (2017) who detected higher expression of *PLIN2* measured in *longissimus* muscle of Wujin breed in comparison to Landrace. Wujin represents Chinese fatty breed which is characterized by high IMF content. Porcine *PLIN2* gene was also analyzed in relation to its genetic variability.

Davoli et al. (2011) detected in this gene six SNPs – two localized in introns, two in the 3'-UTR and two missense in exons. Association analysis showed that 3'-UTR polymorphism (GU461317:g.98G>A) in Italian Duroc was related to estimated breeding values (EBV) for average daily gain, feed conversion ratio, lean cuts and ham weight ($P \leq 0.01$). Further study showed that g.98G>A SNP also influenced early growth rate, lean growth and carcass lean weight in the same breed (Gol et al., 2016). Polymorphism in 3'-UTR region may interfere with or introduce transcription factor binding sites, which regulate gene expression. Analysis of g.98G>A SNP by use of Tfsitescan (Ghosh, 2000) showed that *G* allele abolishes a binding site for GATA-1 transcription factor.

The objective of this research was to analyze above-mentioned SNPs in *FTO* and *PLIN2* genes in relation to carcass and meat quality traits including mean backfat thickness and IMF in pigs reared in Poland.

Material and methods

Animals

The study included 578 sows derived from six breeds: Duroc (n=14), Hampshire (n=7), Polish Landrace (n=269), Pietrain (n=31), Puławska (n=68) and Polish Large White (n=189). Polish Landrace group derived from 57 boars, Polish Large White from 34, whereas Puławska from 18 boars. Pigs were maintained in Pig Test Stations that belong to the National Research Institute of Animal Production. They are situated in Chorzelów, Pawłowice and Mełno (Poland). Housing and feeding conditions were equal for all the animals. Pigs were introduced to stations at the age of 12 weeks and fed *ad libitum* according to SKURTCh procedure to reach 100 (±3) kg body weight. The slaughter was performed after an electric shock by means of tongs with the following parameters: voltage 380–400V, amperage 1.3A, frequency 50Hz. The duration of the stunning time was from 4 to 8 seconds. After the suspension, carotid arteries and the jugular vein were cut and animals were exsanguinated. For the estimation of meat quality parameters, samples of *m. longissimus lumborum* and *m. semimembranosus* tissues were collected after dissection.

Carcass and meat quality traits

Following carcass traits were determined: slaughter efficiency (SEF), weight of loin without backfat and skin (WL), weight of ham without backfat and skin (WH), mean backfat thickness from measurements at five points (BFT), width of the loin eye (WdL), height of the loin eye (HL), loin eye area (AL), meat percentage (MP) and weight of the main cuts (WMC).

Measured meat quality traits were as follows: intramuscular fat content (IMF), meat color – luminosity (L*), redness (a*), yellowness (b*), pH measured 45 min (pH45) and 24 h (pH24) after slaughter in both muscles and water holding-capacity (WHC). IMF was estimated in *m. longissimus dorsi* by Soxhlet extraction using the SOXTHERM SOX 406 apparatus (Gerhardt, Königswinter, Germany). The pH of

muscles was determined by pH-Star (Matthäus, Eckelsheim, Germany). According to the SKURTCh methodology, the measurement of the pH45 in loin was performed on the right half of carcass at the point between the last thoracic vertebra and the first lumbar vertebra after making a 2 cm incision of *m. longissimus dorsi* and the complete insertion of the glass electrode of apparatus. The measurement was performed on half-carcasses hanging 45 minutes after slaughter. Measurement of the pH45 in ham was conducted in the middle of the exposed *m. semimembranosus*. Analogous measurements on the hanging half-carcasses were performed for pH24, however pH of the loin was estimated at three points on the intersection plane of *m. longissimus dorsi* in the position between mentioned last thoracic vertebra and the first lumbar vertebra. Then average was calculated for these three measurements. Meat color parameters of loin were measured applying CR-310 Chromameter (Konica Minolta, Tokyo, Japan). WHC, however, was established by the Grau-Hamm method (Hamm, 1986).

***FTO* and *PLIN2* polymorphism analysis**

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

Polymorphism in exon 3 of porcine *FTO* (g.400C>G) was determined by means of PCR-RFLP method (polymerase chain reaction-restriction fragment length polymorphism). Following pair of primers for PCR were designed by use of Primer3Plus software (Untergasser et al., 2012) and ENSSSCG00000035949 sequence: forward 5'-GCC GGT GTG TAT AGG TCC AG-3', reverse 5'-GGA TCC ATG AAG CTC AAC AAA3'. Reaction mixes for *FTO* and *PLIN2* genes were performed in total volume of 10µl that contained: GoTaq® Master Mix (Promega, Madison, USA), 10pmol of each primer, 40–70ng of DNA and PCR grade water. Thermal cycling for *FTO* was as follows: initial denaturation at 95°C/5min, 30 cycles of 95°C/40s, 54°C/40s, 72°C/40s and final synthesis at 72°C/5min. Obtained amplicons were digested by *Rsa*I enzyme (Thermo Scientific, Waltham, USA) overnight and separated in 2.5% agarose gels. *FTO* genotypes were determined based on following lengths of restriction fragments: *CC* – 123bp, *CG* – 123, 92, 31bp, *GG* – 92, 31bp.

Polymorphism in 3'-UTR region of porcine *PLIN2* gene (g.98G>A) was analyzed by using ACRS-PCR (PCR-based amplification created restriction site) method. Primer set for PCR was designed manually based on ENSSSCG00000035863 sequence and its properties were determined by OligoAnalyzer 3.1 software (Integrated DNA Technologies, Coralville, USA): 5'-TTT TGC CTC TGT TGC CAC TGT TTG CCA GCT-3', reverse 5'-GTG AGA CAA ACC AGT GCT GAG GCC-3' (mismatched nucleotide underlined). Following cycling conditions were applied: initial denaturation at 95°C/5 min, 32 cycles of 95°C/1 min, 63°C/1 min, 72°C/2 min and final synthesis at 72°C/5 min. Obtained amplicons were digested by *Pvu*II enzyme (Thermo Scientific, Waltham, USA) overnight and separated in 2.5–3% agarose gels. *PLIN2* genotypes were determined based on following restriction fragment lengths: *AA* – 127bp, *AG* – 127, 99, 28bp, *GG* – 99, 28bp.

Statistical analysis

Population parameters – genotype and allele frequency, gene diversity (H_e), polymorphic information content (PIC) and Hardy-Weinberg equilibrium (HWE) were calculated using PowerMarker (3.25) software (Liu and Muse, 2005). Analyzed carcass and meat quality traits in pigs were assessed in a fixed model using the least squares method of the GLM (General Linear Model) procedure in SAS/STAT software (SAS Institute Inc., USA). The following model was applied:

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

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),
βSW – linear effect of slaughter weight as covariate (for slaughter traits only),
e_{ijk} – random residual error.

Primary model included effect of sire, station and year of slaughter, but due to non significant influence, they have been excluded from calculations. Association analyses were performed for Polish Landrace, Polish Large White and Puławska breeds separately as well as for all animals covering six breeds, with regard to *FTO* gene.

Results

Analysis of *FTO* gene showed presence of three genotypes (*CC*, *CG*, *GG*) and two alleles (*C*, *G*) in the studied group of pigs. We noticed some differences between breeds in genotype and allele frequencies. *CC* genotype appeared most often in Duroc, however least often in Polish Landrace. Highest observed and expected heterozygosity were noticed for Polish Landrace. We found also that this breed was not in the state of Hardy-Weinberg equilibrium (P=0.0002). *C* allele was most frequent in each breed, except Duroc.

In case of *PLIN2* gene we observed also three genotypes, namely *AA*, *AG*, *GG*, but *AA* was only present in half of the analyzed breeds – Duroc, Hampshire and Puławska. Although a small sample was analyzed for the breed, *AA* genotype was most frequent in Duroc (0.57). We noticed also loss of HWE (P=0.0126) for Puławska breed. Deviations from HWE can be due to an association between the trait and functional locus or a SNP marker which is in linkage disequilibrium (LD) with a functional locus (Li and Leal, 2009). Genotype and allele frequencies with some population genetic indexes for both polymorphisms are presented in Table 1.

Table 1. Genotype and allele frequencies with some population genetic indexes in different breeds of pigs, calculated for *FTO* (g.400C>G) and *PLIN2* (g.98G>A) polymorphisms

Breed	n	genotypes frequency			alleles frequency		H_e	PIC	χ^2	P
		CC	*CG*	*GG*	*C*	*G*				
FTO										
Duroc	14	0.57	0.36	0.07	0.25	0.75	0.375	0.305	0.032	0.8586
Hampshire	7	0.57	0.43	–	0.79	0.21	0.337	0.280	0.521	0.4706
Polish Landrace	269	0.21	0.61	0.18	0.52	0.48	0.499	0.375	14.041	0.0002
Pietrain	31	0.35	0.55	0.10	0.63	0.37	0.467	0.358	0.940	0.3298
Puławska	68	0.34	0.46	0.13	0.66	0.37	0.448	0.348	0.437	0.5084
Polish Large White	189	0.44	0.49	0.07	0.69	0.31	0.429	0.337	3.369	0.0664
PLIN2										
		AA	*AG*	*GG*	*A*	*G*				
Duroc	14	0.57	0.43	–	0.79	0.21	0.337	0.280	1.041	0.3075
Hampshire	7	0.14	0.14	0.72	0.21	0.79	0.337	0.280	2.321	0.1277
Polish Landrace	269	–	0.09	0.91	0.04	0.96	0.081	0.079	0.537	0.4639
Pietrain	31	–	0.03	0.97	0.02	0.98	0.032	0.031	0.008	0.9273
Puławska	68	0.01	0.06	0.93	0.04	0.96	0.084	0.081	6.225	0.0126
Polish Large White	189	–	0.08	0.92	0.04	0.96	0.085	0.078	0.369	0.5434

H_e – gene diversity, PIC – polymorphic information content, χ^2 – Chi-squared test (Hardy-Weinberg equilibrium).

Table 2. Association between *FTO* polymorphism (g.400C>G) and carcass traits in different pig breeds and in whole population analyzed (LSM ± SE)

Trait	Genotype	Landrace (n=269)	Large White (n=189)	Puławska (n=68)	Whole group (n=578)
SEF (%)	*CC*	77.9±0.633	77.0±0.427	76.1±0.507	77.4±0.312
	CG	78.2±0.594	76.9±0.444	75.4±0.546	77.4±0.314
	GG	77.8±0.671	76.9±0.809	76.6±0.995	77.3±0.411
WL (kg)	*CC*	6.514±0.207	6.263±0.102 a	5.963±0.108	6.350±0.084
	CG	6.529±0.194	6.364±0.107 b	5.703±0.116	6.369±0.085
	GG	6.429±0.219	6.329±0.194 b	6.112±0.212	6.330±0.111
WH (kg)	*CC*	9.226±0.161	9.072±0.092 a	9.066±0.127	9.448±0.075
	CG	9.208±0.151	9.069±0.096 a	8.902±0.136	9.442±0.075
	GG	9.147±0.170	9.294±0.174 b	9.081±0.249	9.431±0.098
BFT (cm)	*CC*	1.524±0.088	1.557±0.043 a	1.373±0.057	1.356±0.038 a
	CG	1.538±0.082	1.495±0.045	1.436±0.061	1.308±0.038
	GG	1.521±0.093	1.411±0.082 b	1.412±0.111	1.236±0.050 b
WdL (cm)	*CC*	10.47±0.211	10.15±0.163	10.34±0.153	10.36±0.158
	CG	10.46±0.198	10.16±0.170	10.34±0.164	10.42±0.107
	GG	10.39±0.224	10.32±0.309	9.96±0.300	10.41±0.087
HL (cm)	*CC*	6.87±0.193	6.78±0.100	7.03±0.101	6.87±0.095
	CG	6.89±0.181	6.96±0.104	6.93±0.109	7.01±0.067
	GG	7.00±0.205	7.00±0.190	6.68±0.199	7.00±0.054
AL (cm)	*CC*	54.6±1.475	53.3±0.896 a	53.7±1.066	56.9±0.702
	CG	55.8±1.384	53.9±0.933	50.5±1.148	57.4±0.707
	GG	55.8±1.566	55.6±1.699 b	54.3±2.093	57.7±0.925
MP (%)	*CC*	60.2±0.849	60.2±0.495 a	61.6±0.508	61.7±0.382
	CG	59.8±0.797	60.6±0.516 a	60.7±0.547	61.7±0.385
	GG	59.8±0.901	61.5±0.939 b	61.4±0.996	61.7±0.503
WMC (kg)	*CC*	24.5±0.361	23.8±0.210	23.6±0.289	24.7±0.170
	CG	24.5±0.339	23.9±0.219	22.9±0.312	24.7±0.171
	GG	24.3±0.383	24.3±0.399	23.8±0.568	24.7±0.224

SEF – slaughter efficiency, WL – weight of loin without backfat and skin, WH – weight of ham without backfat and skin, BFT – mean backfat thickness from 5 measurements, WdL – width of the loin eye, HL – height of the loin eye, AL – loin eye area, MP – meat percentage, WMC – weight of the main cuts, n – the number of animals analyzed in given group; values marked with different letters show significant differences between genotypes at $P \leq 0.05$ (a, b).

During analysis of *FTO* gene variants and carcass traits we found statistically significant associations only in Polish Large White pigs. Animals with *GG* genotypes were characterized by higher values of WL, AL and MP ($P \leq 0.05$) in relation to those with *CC*. An opposite trend was observed for BFT ($P \leq 0.05$) and confirmed also in

the whole group (Table 2). Table 3 presents associations between *FTO* SNP and meat quality traits. We found higher values of pH24, measured in *m. longissimus dorsi* in pigs with *GG* genotype (P≤0.05). It was noticed for Polish Large White breed and the whole group. *GG* genotype was also associated with highest value of WHC (P≤0.01) in Puławska breed.

Table 3. Association between *FTO* polymorphism (g.400C>G) and meat quality traits in different pig breeds and in whole population analyzed (LSM ± SE)

Trait	Genotype	Landrace (n=231)	Large White (n=173)	Puławska (n=67)	Whole group (n=509)
IMF (%)	*CC*	1.136±0.044	1.062±0.054	1.194±0.025	1.184±0.030
	CG	1.168±0.041	1.038±0.056	1.207±0.027	1.196±0.031
	GG	1.185±0.047	1.007±0.097	1.287±0.049	1.210±0.040
L*	*CC*	49.8±0.816	52.7±0.334	53.6±0.334	53.7±0.335
	CG	49.6±0.765	53.2±0.348	54.2±0.359	54.5±0.340
	GG	49.6±0.868	52.5±0.633	54.1±0.655	54.1±0.439
a*	*CC*	18.75±0.555	18.09±0.227	16.77±0.187	18.20±0.219
	CG	18.31±0.520	17.74±0.237	16.80±0.202	17.85±0.222
	GG	18.66±0.591	18.12±0.430	16.78±0.368	18.06±0.287
b*	*CC*	5.349±0.456	3.248±0.194	2.233±0.110	5.213±0.181
	CG	5.145±0.485	3.858±0.203	2.348±0.118	5.112±0.184
	GG	5.145±0.485	3.596±0.368	2.366±0.216	5.220±0.274
pH45 ld	*CC*	6.320±0.059	6.256±0.035	6.324±0.026	6.270±0.027
	CG	6.326±0.056	6.280±0.067	6.225±0.028	6.271±0.027
	GG	6.358±0.063	6.283±0.037	6.359±0.051	6.292±0.036
pH24 ld	*CC*	5.527±0.031	5.584±0.016 a	5.629±0.012	5.610±0.013 a
	CG	5.522±0.033	5.623±0.017	5.620±0.013	5.630±0.013
	GG	5.545±0.030	5.637±0.030 b	5.671±0.023	5.650±0.017 b
pH45 s	*CC*	6.473±0.049	6.373±0.032	6.294±0.023	6.362±0.025
	CG	6.473±0.046	6.401±0.034	6.220±0.024	6.370±0.025
	GG	6.531±0.052	6.376±0.061	6.334±0.044	6.401±0.033
pH24 s	*CC*	5.587±0.031	5.610±0.020	5.635±0.024	5.590±0.015
	CG	5.601±0.029	5.594±0.021	5.659±0.026	5.591±0.015
	GG	5.594±0.033	5.570±0.038	5.645±0.048	5.580±0.020
WHC	*CC*	34.2±1.813	32.6±1.114	32.6±1.107 a	32.6±0.866
	CG	34.4±1.694	32.5±1.123	31.6±1.184 B	31.9±0.868
	GG	33.4±1.913	32.8±1.950	37.7±2.163 Cb	32.9±0.102

IMF – intramuscular fat content, L* – luminosity, a* – redness, b* – yellowness, pH45, pH24 – pH measured 45 min and 24 h after slaughter respectively in *m. longissimus dorsi* (ld) or *m. semimembranosus* (s), WHC – water holding-capacity, n – the number of animals analyzed in given group; values marked with different letters show significant differences between genotypes at P≤0.05 (a, b) or P≤0.01 (B, C).

Table 4. Association between *PLIN2* polymorphism (g.98G>A) and carcass traits in different pig breeds (LSM ± SE)

Trait	Genotype	Landrace (n=269)	Large White (n=189)	Puławska (n=67)
SEF (%)	*AG*	78.8±0.739	77.3±0.740	75.2±1.402
	GG	78.0±0.585	76.9±0.393	75.9±0.399
WL (kg)	*AG*	6.673±0.241	6.178±0.178 a	5.616±0.305 a
	GG	6.512±0.191	6.321±0.094 b	5.890±0.087 b
WH (kg)	*AG*	9.268±0.188	9.316±0.159	9.515±0.343
	GG	9.207±0.149	9.053±0.084	8.948±0.098
BFT (cm)	*AG*	1.612±0.102	1.562±0.076	1.286±0.155
	GG	1.529±0.081	1.524±0.040	1.416±0.044
WdL (cm)	*AG*	10.52±0.247	10.11±0.280	10.17±0.403
	GG	10.44±0.196	10.17±0.150	10.18±0.122
HL (cm)	*AG*	7.07±0.222	7.19±0.173 a	7.45±0.234 a
	GG	6.95±0.179	6.85±0.093 b	6.84±0.081 b
AL (cm)	*AG*	57.0±1.729	53.2±01.54 A	53.1±3.039
	GG	55.4±1.369	55.3±0.821 B	52.4±0.865
MP (%)	*AG*	59.9±0.995	60.8±0.865	62.1±1.377
	GG	59.9±0.788	60.4±0.459	61.0±0.392
WMC (kg)	*AG*	24.8±0.422	24.2±0.366	24.1±0.809
	GG	24.5±0.334	23.8±0.194	23.3±0.230

SEF – slaughter efficiency, WL – weight of loin without backfat and skin, WH – weight of ham without backfat and skin, BFT – mean backfat thickness from 5 measurements, WdL – width of the loin eye, HL – height of the loin eye, AL – loin eye area, MP – meat percentage, WMC – weight of the main cuts, n – the number of animals analyzed in given group; values marked with different letters show significant differences between genotypes at P≤0.05 (a, b) or P≤0.01 (A, B).

In case of *PLIN2* polymorphism, among 3 analyzed breeds we took into consideration only *GG* and *AG* genotypes due to lack or very low frequency of *AA* genotype. Calculation for the whole group, however was not performed because of high disproportion between *A* allele frequency in Duroc pigs and other analyzed breeds (Table 1).

Analyzing carcass traits we found associations between *PLIN2* variants with WL and HL in Polish Large White and Puławska (P≤0.05). In case of WL, pigs with *GG* genotypes were characterized by highest values of this parameter, however in case of HL – by lowest. In Polish Large White we also noticed strong correlations (P≤0.01) for AL with *GG* genotype being favorable for this trait (Table 4). Association analyses for *PLIN2* gene and meat quality traits are listed in Table 5. In case of this traits group we obtained consistent results for 3 breeds in relation to IMF and WHC. Again, pigs with *GG* genotypes represented highest values of these traits (P≤0.05). We noticed the correlation (P≤0.05) between *PLIN2* genotypes and L* in Puławska too.

Table 5. Association between *PLIN2* polymorphism (g.98G>A) and meat quality traits in different pig breeds (LSM ± SE)

Trait	Genotype	Landrace (n=241)	Large White (n=173)	Puławska (n=66)
IMF (%)	*AG*	1.082±0.052 a	1.165±0.093 a	1.090±0.079 a
	GG	1.168±0.041 b	1.219±0.050 b	1.213±0.019 b
L*	*AG*	54.7±0.954	52.1±0.586	51.9±0.899 a
	GG	54.5±0.756	52.9±0.311	54.3±0.256 b
a*	*AG*	18.74±0.652	17.21±0.393	17.45±0.506
	GG	18.42±0.516	18.01±0.208	16.72±0.144
b*	*AG*	3.943±0.535	4.609±0.335	2.299±0.302
	GG	3.364±0.424	3.456±0.178	2.298±0.086
pH45 ld	*AG*	6.253±0.069	6.236±0.061	6.196±0.750
	GG	6.278±0.055	6.271±0.033	6.306±0.210
pH24 ld	*AG*	5.610±0.370	5.644±0.028	5.655±0.032
	GG	5.620±0.290	5.606±0.015	5.621±0.009
pH45 s	*AG*	6.270±0.580	6.431±0.056	6.197±0.064
	GG	6.217±0.460	6.311±0.030	6.272±0.018
pH24 s	*AG*	5.640±0.036	5.715±0.035	5.622±0.067
	GG	5.657±0.028	5.638±0.018	5.641±0.019
WHC	*AG*	33.1±1.676 a	28.5±1.799 a	28.7±3.078 a
	GG	36.8±2.174 b	33.1±1.027 b	34.6±0.876 b

IMF – intramuscular fat content, L* – luminosity, a* – redness, b* – yellowness, pH45, pH24 – pH measured 45 min and 24 h after slaughter respectively in *m. longissimus dorsi* (ld) or *m. semimembranosus* (s) WHC – water holding-capacity, n – the number of animals analyzed in given group; values marked with different letters show significant differences between genotypes at P≤0.05 (a, b).

Discussion

Taking into consideration *FTO* allele frequency, in Polish Large White it was higher (0.69) than observed in Czech Large White (0.45), however lower in Polish Landrace (0.52) when compared to Czech Landrace (0.66) (Dvořáková et al., 2012). Previous study showed that g.400C>G polymorphism in *FTO* gene is associated with marbling score, total lipid percentage in muscle, backfat at last lumbar vertebra and average daily gain on test (P≤0.05) in Berkshire × Yorkshire pigs. Highest values for the first two traits were observed in animals with *GG* genotypes, however for the second two in those with *CG* genotype (Fan et al., 2009). In our study we found statistically significant relationships between *FTO* variants and mean backfat thickness from 5 measurements in Polish Large White and whole group (P≤0.05), however their values were highest for pigs with *CC* genotype. Similar results were presented by Dvořáková et al. (2012), who found that *CC* genotype was associated with highest values for fat in belly (2%) and average backfat depth (mm) calcu-

lated from three measurements in Pietrain × (Czech Landrace × Czech Large White) crossbred pigs. In joined population that consists of commercial pigs from 7 crosses and 1 purebred, *CC* genotype was also favorable for these traits as well as for fat depth (mm) estimated by skinfold caliper. Average backfat depth measurements are more reliable because they are estimated at few points in opposition to backfat at last lumbar vertebra, which is determined at one point. Other traits, associated with g.400C>G SNP, i.e., marbling score and total lipid percentage in muscle represent intramuscular fatness. In our study we did not notice differences between *FTO* genotypes and IMF but found some tendency. In Polish Landrace, Puławska and whole group highest IMF content was observed in animals with *GG* genotype, which is in agreement with earlier results (Fan et al., 2009). Another study also showed that intramuscular fat content in *m. longissimus lumborum et thoracis* is highest in pigs with *GG* genotype, however in the ham with *GG* and *GC* genotypes (Dvořáková et al., 2012). The genetic correlation between IMF and BFT is moderate and provides the opportunity to improve lean growth independent of the IMF (Ibáñez-Escriche et al., 2016). During analysis of German pig breeds Baulain et al. (2000) found positive correlation between IMF and pH (r=0.46). In our study pH measured 45 minutes and 24 hours after slaughter in *m. longissimus dorsi* tend to be highest in animals with *GG* genotype. Statistically significant (P≤0.05) confirmation, however was found only for pH24 in Polish Large White and whole group. Dvořáková et al. (2012) suggested that *GG* genotype is most favorable for muscling traits. In our study it may be referred to Polish Large White breed, where meat percentage, loin eye area, weight of ham and weight of loin were statistically higher (P≤0.05) in pigs with *GG* genotype in relation to those with *CC*.

In case of *PLIN2* allele frequency we observed a similar tendency as Davoli et al. (2011), because in Italian Landrace *G* allele appeared exactly with the same frequency as in Polish Landrace (0.96). On the other hand in Italian Duroc *A* allele was predominant (0.58) but in our study it reached higher value (0.79). First report concerning g.98G>A polymorphism indicated that its individual variants were associated with EBV for weight of ham in Italian Duroc pigs. Highest additive effect was observed for *AA* genotype, lowest for *GG*, however moderate for *AG* (Davoli et al., 2011). Next, it was also demonstrated that *A* allele had a probability of at least 98% of producing carcasses with heavier ham weight (+0.10 kg) in Duroc pigs (Gol et al., 2016). In our study we did not find statistically significant association between *PLIN2* genotypes and weight of ham without backfat and skin but observed similar tendency. Although *AA* genotype did not occur in 3 analyzed breeds or was present with very low frequency, heterozygous genotype was characterized by heavier WH and difference ranged from 0.061 to 0.567 kg. It shows that *A* allele in heterozygous form also increases ham weight, which is in agreement with earlier results. Analyzing other carcass traits we found that *PLIN2* genotypes were associated with the loin parameters WL, HL and AL. Weight of loin shows high genetic correlations with loin muscle area (0.78) (Newcom et al., 2002). It is easily visible in case of Polish Large White and Puławska breeds because highest values of WL and AL were observed for animals with *GG* genotype (P≤0.05 or P≤0.01). In Polish Landrace pigs inverted tendency was observed but it was not confirmed statistically. In case of meat

quality traits we observed significant associations of *PLIN2* SNP with IMF, L* and WHC. These three parameters are most important for Pork Quality System (PQS), which was introduced in Poland in 2009. Its aim is to provide good quality pork with parameters important for consumers and the processing industry such as: correct pink-red color, juiciness and tenderness, lack of excessive drip loss, free of PSE and DFD defects (Hammermeister et al., 2013). In the analyzed pigs IMF and L* were in the range for PQS. Third parameter, however in our study was determined by Grau–Hamm method, whereas in case of PQS it is measured as drip loss. Both parameters are obviously highly correlated (Jennen et al., 2007). When referring IMF and WHC to *PLIN2* variants most consistent results may be noticed. In all analyzed breeds their highest values were observed for animals with *GG* genotype ($P \leq 0.05$). IMF and WHC are not correlated (0.12), so selection for increased IMF based on *PLIN2 GG* variant will not influence WHC value, for which this genotype is unfavorable (Sellier, 1998).

Obtained results show that *FTO* and *PLIN2* genes variants are significantly ($P \leq 0.01$ or $P \leq 0.05$) associated with some carcass and meat quality traits in pigs reared in Poland. These relationships, nevertheless were not consistent among 3 analyzed breeds (*FTO* and *PLIN2*) and joined population that consists of six breeds (*FTO*). Unambiguous results for 3 breeds were confirmed only in case of *PLIN2* gene and two parameters – IMF and WHC. However, due to lack or low frequency of *AA* genotype in Polish Landrace, Polish Large White and Puławska breed it is necessary to carry out further studies preceded by the population structure analysis.

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