Association of single nucleotide polymorphisms in leptin (*LEP*) and leptin receptor (*LEPR*) genes with backfat thickness and daily weight gain in Ukrainian Large White pigs

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ABSTRACT

Leptin (*LEP*) and leptin receptor (*LEPR*) genes play an important role in the regulation of fat deposition and other commercially important traits in pigs and this regulation is known to be breed-specific. The aim of the present study was to investigate the presence and frequency of *LEP* polymorphisms g.2845A>T, g.3996T>C, and *LEPR* polymorphisms c.232A>T, c.915C>T and c.2856C>T in Ukrainian Large White pigs, and to determine whether these polymorphisms are associated with the following traits: backfat thickness at the 10th rib, backfat thickness at the 6-7th rib, backfat thickness at sacrum and average daily weight gain. The study was conducted on 108 Ukrainian Large White purebred female pigs. Genotyping of *LEP* and *LEPR* polymorphisms was performed using RCR-RELF technique. The study demonstrated that the *LEP* SNP g.2845A>T was segregating in the population of Ukrainian Large Whiter pigs studied with almost equal frequencies of the alternative alleles being observed. The *LEP* SNP g.3996 T>C was absent in Ukrainian Large White pigs with all the animals having g.3996CC genotype. The *LEPR* SNP c.915C>T was segregating in the pig population studied with c.915C allele frequency dominating. Segregation was also observed for the *LEPR* SNP c.2856 C>T with an almost equal frequency of the alternative alleles. The *LEPR* SNP c.232A>T was polymorphic with the frequency of the alternative alleles c.232A and c.232T being 0.25 and 0.75 respectively. No association was established between any of the traits investigated and the *LEP* SNP g.2845 A>T, *LEPR* SNP c232A>T, and *LEPR* SNP c.915C>T. The *LEPR* SNP c.2856C>T was associated with backfat thickness at the level of 6-7th and 10th ribs with c.2856TT genotype having lower backfat thickness compared to c.2856CC and c.2856CT. The *LEPR* SNP c.2856C>T was also associated with average daily weight gain which was lower in animals with c.2856TT genotype. Results of the study suggest that *LEPR* SNP c.2856C>T can be considered as a genetic marker for subcutaneous fat deposition and average daily weight gain in Ukrainian Large White pigs. This marker can be of particular importance in breeding programmes aiming to modify the carcass structure and pigs growth rate.

Key words: pig, leptin, leptin receptor, polymorphism, fat deposition, genetic marker

1. Introduction

One of the main aims of international pig industry is the production of animals with high growth rate and high meat content. Fat content and distribution in pig carcasses are important meat quality characteristics (Tyra et al., 2013; Pena et al., 2016) which can be regulated by marker-assisted selections based on associations between DNA polymorphisms and the traits of interest.

Leptin (*LEP*) and leptin receptor (*LEPR*) genes play an important role in the regulation of fat deposition (Sook–Ha et al., 2014). Over 400 *LEP* polymorphisms have been reported in pigs (Bidwell et al., 1997; Perez-Montarelo et al., 2012) with some of them being associated with carcass weight, daily weight gain, subcutaneous fat content and composition (Kennes et al., 2001; Baurer et al., 2006; de Oliveira Peixoto et al., 2006). However, data of the literature on associations between *LEP* polymorphisms and meat quality and production traits are controversial and differ between pig breeds.

Leptin receptor gene (*LEPR*) is located on a chromosome 6 in the region associated with intramuscular fat content, back fat thickness, animal growth rate, and carcass conformation (Ovilo et al., 2000; Varona et al., 2002; Ovilo et al., 2005; Galve et al., 2012). Over twenty five *LEPR* SNPs have been identified, some of which have been shown to be associated with meat quality traits in a breed-specific manner (Li et al., 2010; Uemoto et al., 2012; Zhang et al., 2014).

Investigation of associations between *LEP* and *LEPR* polymorphisms and economically important traits are of particular interest in Large White breed which has been extensively used in cross-breeding programmes across the world. In our previous work we demonstrated that traditional Ukrainian pig breeds have breed-specific polymorphisms in cathepsin genes which play an important role in controlling meat quality (Balatsky et al., 2015). However, the presence

and frequency of *LEP* and *LEPR* polymorphisms in Ukrainian Large White pigs and their association with meat quality traits and growth rate remain unclear.

A number of *LEP* polymorphisms have been identified in a regulatory region of the gene. This includes (i) the SNP 2845A>T on the second intron in the area with the regulatory sites for mRNA expression (Kennes et al., 2001; Chorev et al., 2012;) and (ii) the SNP 3996T>C in the area controlling mRNA stability (Conne et al., 2012; Matoulkova et al., 2012). However it remains unknown whether *LEP* SNPs g.2845 A>T and g.3996 T>C are associated with meat quality and pig productivity traits in Ukrainian Large White pigs.

A number of SNPs have also been reported in the *LEPR* gene including SNPs c.232A>T c.915C>T and c.2856C>T which are situated in the areas linked to the regulation of the protein structure and functions. Associations between *LEPR* SNPs and meat quality traits have been reported for Duroc, Polish Landrace, Yorkshire x Landrace crosses and Landrace pigs (Mackowski, 2005; Amills et al., 2008; Kuehn et al., 2009; Uemoto, 2012). However, there is no information on associations between the *LEPR* SNP c232C>T and economically important traits in Large White breed.

The *LEPR* SNP c.2856C>T has been reported to be associated with intramuscular fat and moister content, taste, cholesterol level, flavour, overall liking and the shear force in Korean x Yorkshire cross-breed (Liu et al., 2010) and commercial Canadian cross-breeds (Zhang et al., 2014). No information is available on associations between the *LEPR* SNP c.2856C>T, *LEPR* SNP c.915C>T and productivity traits in Large White pigs.

The aims of this study were: (i) to investigate the presence and frequency of the *LEP* polymorphisms g.2845A>T, g.3996T>C, and *LEPR* polymorphisms c.232A>T, c.915C>T and c.2856C>T in Ukrainian Large White pigs, and (ii) to determine whether there is association between the above polymorphisms and backfat thickness and average daily weight gain as indicators of pigs growth rate.

2. Materials and methods

2.1. Animals and experimental design

The study was conducted on 108 Ukrainian Large White purebred female pigs reared under the same conditions on the farms of the Ukrainian Academy of Agricultural Sciences. Ukrainian Large White breed was developed by genetic selection on the basis of British Large White in order to improve meat quality and carcass composition whilst retaining a high growth rate. (Balatsky et al., 2016).

We recognize that a number of animals used in this study was lower when compared to an average number of animals used in other association studies reported in the literature. This was due to a relatively modest scale of production of Ukrainian Large White breed and associated difficulties with collecting a larger number of samples. All the procedures related to animal handling complied with the European Convention for the Protection of Vertebrate Animals used for Experimental and Others Scientific Purposes. The experimental protocol was approved by the Scientific Committee of the Institute of Pig Breeding and Agro-Industrial Production, National Academy of Agricultural Sciences, Ukraine.

The protocol for association studies was designed following the approach described by Fontanesi et al. (2011). During the growth phase (the live weight between 40 and 60 kg), the pigs were fed the diet containing per dry matter: 12.9 MJ/kg of net energy, 19.1% of crude protein and 1.14 % of lysine. The diet was modified when the animals reached the live weight of 60 kg (the finishing diet) and it contained per dry matter: 12.8 MJ/kg of net energy, 18.0% of crude protein and 1.0% of lysine. The finishing diet was fed until the animals reached 100 kg of live weight. The feed was manufactured by Poltava Feed Mill (Poltava, Ukraine).

All the pigs used in the association studies were tested for the 843 CT mutation in the ryanodine receptor I gene which is associated with pig meat quality defects (Fujii et al., 1991). It

123 was demonstrated that all the animals used in the present study had a CC genotype, e.g. the
124 mutant allele variant was absent.
125
126 *2.2. Analyzes of backfat thickness and average daily weight gain*
127 Backfat thickness was measured by a portable digital Renco Lean-Meter device (Renco
128 Corporation, USA) in the following three locations: (i) at the 10^{th} rib; (ii) at the 6^{th}-7^{th} rib and
129 (iii) at sacrum (Getya et al., 2006).
130 An average daily weight gain was calculated based on the data obtained from the birth and
131 over the whole period of animal life. The age of animals at 100 kg of live weight was recorded.
132
133 *2.3. DNA isolation, amplification and genotyping*
134
135 Blood samples (1 ml) were obtained from the jugular vein in the morning before feeding. The
136 blood samples were mixed with 0.05 M EDTA as an anticoagulant and stored up to seven days at
137 +4 °C until used for DNA isolation. Genomic DNA was isolated by a sorbent method using
138 DiatomTM DNA Prep100 kit (Isogen, Moscow, Russia) following the manufacturer instructions
139 with guanidine thiocyanate as a lysis reagent.
140 Genotyping of the *LEP* and *LEPR* polymorphisms was carried out using the polymerase chain
141 reaction-restriction fragment length polymorphism (PCR–RFLF) assay with primer pairs
142 described in Table 1. PCR reactions were performed in 25 µl (final volume) of the mixture
143 containing genomic DNA, 200 nM of forward and reverse primers, 2.5 mM $MgCl_2$, 0.25 mM of
144 each of the dNTPs and one unit of the recombinant Taq DNA Polymerase (Fermentas, Vilnius,
145 Lithuania). *LEP* was genotyped on the SNPs g.2845A>T (intron 2, rs344615147) and
146 g.3996T>C (3'UTR, rs337366389); *LEPR* was genotyped on the SNPs c.232A>T (exon 4,

147 AF092422), c.915C>T (exon 8, NM_001024587), and c.2856 C>T (exon 20, AF092422). The

148 PCR amplification conditions are given in Table 1.

149

150 **Table 1**

151 Primer sequences, PCR conditions, and PCR-RFLF patterns of different *LEP* and *LEPR* alleles

152 in Ukrainian Large White pig breed.

Genes and SNPs	Primer sequence	PCR conditions		
		Product length (bp)	Annealing temp (°C)	Restriction enzymes and PCR-RFLP patterns of different alleles
LEP g. 2845 A>T	Forward TTGGCGAGCCTGGAGCAGT Reverse GCAGCCTCCATCCCTAAGTGGG	242	55	(XbalI): allele g.2845A, 242 bp allele g.2845T,170+72 bp (Kennes et al., 2001)
LEP g. 3996 T>C	Forward GCAGCCTCCATCCCTAAGTGGG Reverse ACCCTGCTTGATGGTCGAAAGGCT	192	55	(BglII): allele g.3996T, 192 bp allele g.3996C,107+85 bp (Kennes et al., 2001)
LEPR c.232A>T	Forward TGCCTGCTGGAATCTCAAAG Reverse TTCCCTGCAATGTTGTCTGC	184	56	(*Tas*I): allele c.232A, 71+13 bp allele c.232T, 184 bp (Mackowski et al., 2005)
LEPR c. 915C>T	Forward GCTGATGAGATCGTCTCAG Reverse CTTGTGTGGTAAAAGTAAAGG	145	55	(*Msp*I): allele c.915T, 145 bp allele c.915C, 50+95 bp (Li et al., 2010)
LEPR c. 2856C>T	Forward CCCTCTTCTTTTGGAGCCTGA Reverse GAGAAGCTTCTGGAATGAACTTA GACG	886	64	*(AvaII)*: allele c.2856T, 796 bp allele c.2856C,502+293bp (Li et al., 2010)

153

154

155 *2.4. Statistical analysis*

156 The allele frequencies, genotype frequencies, polymorphic information content (PIC),

157 and levels of heterozygosity (observed heterozygosity H_o, and expected heterozygosity H_e) were

158 calculated using GenAlEx 6.0 software (Peakall, 2006). Analysis of associations between
159 genotypes and backfat thickness and average daily weight gain were conducted by One Way
160 ANOVA.

161 Significance of differences between the mean values was determined by two-tailed *t*-test
162 using JMP12 (SAS Inst. Inc., Cary, NC). A $P\ value \leq 0.05$ was considered significant.
163 Calculations of the additive (A) and dominance (D) components were carried out using the
164 following equations:

165 $$A = \overline{X}_{22} - \overline{X}_{11}; \qquad D = \overline{X}_{12} - \frac{\overline{X}_{11} + \overline{X}_{22}}{2}$$

166 where $\overline{X}_{11}, \overline{X}_{12}, \overline{X}_{22}$ are arithmetic mean values of productivity traits for the genotypes "11"
167 (homozygote for the first allele), "12" (heterozygote) and "22" (homozygote for the second
168 allele).
169
170 Effects of the alleles 1 and 2 (a_1 and a_2 respectively) were determined using the following
171 equations $\begin{matrix} \alpha_1 = m_1 - \overline{X} \\ \alpha_2 = m_2 - \overline{X} \end{matrix}$ where:

172 $$\begin{matrix} m_1 = p \cdot \overline{X}_{11} + q \cdot \overline{X}_{12} \\ m_2 = p \cdot \overline{X}_{12} + q \cdot \overline{X}_{22} \end{matrix}$$

173 p and q are the frequencies of the alleles 1 and 2 respectively; $\overline{X}$ is the arithmetic mean value for
174 each trait.

175 Allelic substitution effects $\frac{\alpha}{2}(1 \to 2)$ were calculated using the equation

176 $$\frac{\alpha}{2}(1 \to 2) = \frac{\alpha_2 - \alpha_1}{2}$$

177
178
179 ## 3. Results and discussion

180 ### *3.1. LEP and LEPR allele frequencies and heterozygosity*

181 *LEP* gene encodes for a hormone leptin that is produced in adipose tissue and circulates in
182 blood either in a free or bound forms (Mantzoros et al., 2001; Margetic et al., 2002; Park et al.,

183 2014). Activity of leptin is regulated by Leptin Receptors which belong to the superfamily of
184 class I cytokine receptors (Clément et al., 1998).

185 Results presented in Table 2 show segregation of the *LEP* SNP g.2845A>T with almost equal
186 frequencies of the alternative alleles in Ukrainian Large White breed. These results are consistent
187 with data of the literature reporting the presence of the *LEP* SNP g.2845A>T in Duroc and
188 Landrace breeds although the g.2845T allele frequency in these breeds was low (Kennes et al.,
189 2001). At the same time, Kennes et al. (2001) did not observe the g.2845T allele in Yorkshire
190 pigs which suggest a breed-specific character of the allele distribution.

191 Using the Hardy-Weinberg equation, we established a statistically significant deviation (χ^2 =
192 20.8) of the genotype distribution from an equilibrium with an increased proportion of
193 heterozygote animals**.** The H_o value was significantly higher than the H_e value (0.70 and 0.489
194 respectively). We have also observed a high PIC value (0.37) which was close to the maximum
195 value for diallelic genetic systems. According to Hao et al. (2011), this level of informativity is
196 the most favourable for undertaking associative studies.

197 In our study the *LEP* polymorphism g.3996T>C which has been reported for some breeds
198 was absent and all the Ukrainian Large White pigs had g.3996 CC genotype. These results are
199 consistent with the data for Yorkshire pig breed (Kennes et al., 2001).

200 In the present study we observed segregation of *LEPR* SNP c.2856 C>T and an almost equal
201 frequency of the alternative alleles in Ukrainian Large White pigs. The H_o and the PIC values
202 were 0.509 and 0.370 respectively which provided justification for undertaking associative
203 studies. According to Hardy-Weinberg distribution, there was a balanced representation of all the
204 mentioned above *LEPR* genotypes. This is in contrast to the genotype distribution for the *LEP*
205 SNP g.2845A>T when one of the homozygote variants was present only in eight animals.
206 According to the literature, the *LEP* SNP c.2856C>T was found to be polymorphic in Yorkshire
207 and Korean local pig breed (Liu et al., 2010), as well as in some commercial cross-breeds (Zang
208 et al., 2014).

The present study demonstrated segregation of the *LEPR* SNP c.915C>T in Ukrainian Large White pigs with c.915C allele frequency being dominant. At the same time, according to Liu et al. (2010) this SNP was monomorphic in a population of Yorkshire and local Korean breeds with only c.915C *LEPR* allele present. In our study, the level of *LEPR* heterozygosity and the PIC value were low. However, since all the above *LEPR* genotypes were present in the Ukrainian Large White pigs, we viewed this as a sufficient justification for undertaking association studies.

Table 2

LEP and *LEPR* genotypes, allele frequencies and heterozygosity in Ukrainian Large White pig breed.

Gene	Genotype	n[a]	Genotype frequency	Allele frequency		H_o[b]	H_e[c]	PIC[d]
				g.2845A	g.2845T			
LEP	g.2845AA	24	0.22			0.704	0.489	0.370
	g.2845AT	76	0.70	0.57	0.43			
	g.2845TT	8	0.08					
				g.3996C	g.3996T			
LEP	g.3996CC	108	1,00					
	g.3996CT	-	0.00	1	0.00	0.000	0.000	0.000
	g.3996TT	-	0.00					
				c.2856C	c.2856T			
LEPR	c.2856CC	21	0.19					
	c.2856CT	55	0.51	0.45	0.55	0.509	0.495	0.370
	c.2856TT	32	0.30					
				c.915C	c.915T			
LEPR	c.915CC	81	0.75					
	c.915CT	17	0.16	0.83	0.17	0.157	0.284	0.240
	c.915TT	8	0.09					
				c.232A	c.232T			
LEPR	c.232AA	17	0.16					
	c.232AT	21	0.19	0.25	0.75	0.185	0.375	0.300
	c.232TT	71	0.66					

[a] Number of animals.

[b] Observed heterozygosity.

[c] Expected heterozygosity.

[d] Polymorphic Information Content.

In the present study, the *LEPR* SNP c.232A>T in Ukrainian Large White breed was polymorphic with a frequency of the alternative alleles c.232A and c.232T being 0.25 and 0.75 respectively. The H_o value (0.185) was substantially lower than the H_e value (0.375) (Table 2). There was a statistically significant deviation from an equilibrium in the distribution of the genotypes towards an increased proportion of c.232 TT (χ^2= 27.7). Undertaking associative studies was justified by the specifics of the *LEPR* c.232A>T genotype distribution and a relatively high, as for diallelic polymorphisms, PIC value (0.300). Our results are consistent with the data reported for Landrace, Duroc, Berkshire, and Large White breeds where the *LEPR* c.232 allele was also shown to be predominant (Mackowsky et al., 2005; Uemoto, 2012).

Taken together, results of our study demonstrated that Ukrainian Large White pigs are genetically different from other breeds reported in the literature in terms of the *LEP* SNP g.2845 A>T and *LEPR* SNP c.915C>T. The study also established that the *LEP* SNP g.3996 T>C in Ukrainian Large White pigs is monomorphic. These results informed the selection of the following SNPs for associative studies: *LEP* g.2845A>T, *LEPR* c.232A>T, *LEPR* c.915C>T and *LEPR* c.2856C>T.

3.2. Analysis of associations between LEP and LEPR SNPs, backfat thickness and daily weight gain

Our study showed that *LEP* SNP g.2845 A>T was not associated with any of the traits analysed in Ukrainian Large White pigs (Table 3). This is in contrast to findings by Kennes et al. (2001) who demonstrated association of *LEP* g.2845 A>T polymorphism with food intake and growth rate in Landrace pigs. This discrepancy can be related to breed-specific presence and frequency of this SNP (Kennes et al., 2001).

Similar situation was observed for the *LEPR* SNPs c.*232A*>T and c. 915C>T. No relationship was found between these polymorphisms and backfat thickness and daily weight

gain in Ukrainian Large White pigs. This is not in agreement with the data obtained on Polish Landrace whether significant relationship was found between *LEPR* SNP c.232A>T and backfat thickness (Mackowski et al., 2005).

Our study established association between the *LEPR* SNP c. 2856C>T and backfat thickness in two areas: at the level of 6-7th ribs and at the10th rib (Table 3). A lower backfat thickness in these areas was observed in animals with c.2856TT genotype when compared to c.2856CC and c.2856CT genotypes.

In the present study we found association between *LEPR* c.2856C>T and average daily weight gain with this parameter being lower in pigs with c.2856TT genotype when compared to c.2856CC and c.2856CT (by 13.1 and 24.8 g respectively).

The association between *LEPR* SNP c. 2856C>T and expected selection index highlighting an importance of this SNP in control of main productivity traits in Ukrainian Large White pigs.

Table 3

Effect of *LEP* and *LEPR* polymorphisms on productivity traits in Ukrainian Large White pigs.

Traits	Genotypes			*P*
	LEP g.2845 A>T			
	AA (n^{a} = 24)	AT (n = 76)	TT (n = 8)	
Age (days) of gaining of 100 kg of weight	194.55 ± 3.70	198.37 ± 2.15	198.75 ± 4.97	0.66
Backfat at 10th rib (mm)	18.12 ± 0.66	19.44 ± 0.49	18.99 ± 0.68	0.36
Backfat at 6th-7th rib (mm)	23.38 ± 0.83	24.31 ± 0.57	23.07 ± 1.19	0.59
Backfat at sacrum (mm)	19.69 ± 0.64	19.98 ± 0.52	19.31 ± 1.17	0.89
Average daily weight gain (g)	518.20 ± 9.63	508.51 ± 5.45	505.44 ± 13.14	0.65
	LEPR c.232A>T			
	AA (n = 17)	AT (n = 21)	TT (n = 71)	
Age (days) of gaining 100 kg of weight	199.91 ± 3.73	194.99 ± 4.15	197.71 ± 2.24	0.71

Backfat at 10th rib (mm)	18.89 ± 0.57	18.75 ± 0.76	19.26 ± 0.52	0.85
Backfat at 6th-7th rib (mm)	24.69 ± 0.86	23.60 ± 0.85	23.96 ± 0.62	0.78
Backfat at sacrum (mm)	20.15 ± 0.83	19.84 ± 0.88	19.81 ± 0.52	0.95
Average daily weight gain (g)	503.01 ± 9.35	517.21 ± 10.82	510.31 ± 5.71	0.66
	LEPR c. 915C>T			
	CC (n = 81)	CT (n = 17)	TT (n = 8)	
Age (days) of gaining 100 kg of weight	195.94 ± 1.91	201.64 ± 4.79	203.68 ± 7.34	0.27
Backfat at 10th rib (mm)	19.37 ± 0.87	18.73 ± 1.02	17.62 ± 1.19	0.38
Backfat at 6th-7th rib (mm)	24.13 ± 0.51	24.38 ± 1.22	22.38 ± 0.74	0.51
Backfat at sacrum (mm)	20.03 ± 0.44	20.01 ± 1.14	18.28 ± 0.46	0.45
Average daily weight gain (g)	514.21 ± 4.95	500.45 ± 11.99	496.84 ± 18.17	0.34
	LEPR c. 2856C>T			
	CC (n = 21)	CT (n = 55)	TT (n = 32)	
Age (days) of gaining 100 kg of weight	198.36 ± 4.05	193.96 ± 2.51	203.20 ± 2.91	0.07
Backfat at 10th rib (mm)	19.40 ± 0.79	19.85 ± 0.51	17.65 ± 0.73	0.04
Backfat at 6th-7th rib (mm)	24.86 ± 1.05	24.89 ± 0.61	21.95 ± 0.77	0.01
Backfat at sacrum (mm)	20.84 ± 0.97	20.21 ± 0.56	18.63 ± 0.67	0.11
Average daily weight gain (g)	508.39 ± 10.48	520.04 ± 6.43	495.28 ± 7.16	0.05

[a] Number of animals.

The data are presented as Least Square Means ± SEM.

Parameters of additive-dominant model for an impact of the *LEPR* 2856C> T on backfat thickness and average daily weight gain were calculated. The study established the prevalence of the dominant component with respect to the backfat thickness at 10th rib and average daily weight gain (Table 4). In both cases, the c.2856C allele was dominant and associated with a

greater backfat thickness and a higher average daily weight gain. In contrast to the results for the 10th rib, the backfat thickness at the 6-7th ribs was determined by an interaction between the additive and dominant components. These results suggest that (i) the fat deposition in different carcass areas of Ukrainian Large White pigs might be controlled by different sets of genes and (ii) effect of *LEPR* SNPs on fat deposition depends on the genetic environment.

Table 4

Effect of the *LEPR* c.2856C>T polymorphisms on backfat thickness and average daily weight gain calculated using the Additive Dominant Model.

Traits	Additive Dominant Model				
	A[a]	D[b]	ac[c]	a_T[d]	$\frac{\alpha}{2}(L \rightarrow V)$[e]
Backfat at 10th rib (mm)	-0.874	1.326	0.537	-0.472	-0.505
Backfat at 6th-7th rib (mm)	-1.454	1.483	0.863	-0.742	-0.802
Average daily gain (g)	-6.559	18.204	4,372	-4.041	-4.207

[a] Additive component.

[b] Dominant component.

[c] Effect of allele C.

[d] Effect of allele D.

[e] Allelic substitution effect.

Previously, Li et al. (2010) and Zhang et al. (2014) demonstrated association between *LEPR* c. 2856C>T and meat quality traits in cross-breed pigs. The traits investigated included intramuscular fat content, cholesterol level, taste, moisture content, and tenderness. These data, taken together with the results of our study, suggest that *LEPR* c. 2856C>T polymorphism plays a significant role in regulation of productivity and meat quality traits in pigs.

It is known that the *LEPR* c.2856C>T mutation is synonymous e.g. it does not lead to a change in the encoded amino acid (Asp) (Li et al., 2010). Therefore, the effect of this polymorphism could be mediated via the two possible mechanisms: (i) a replacement of the third

nucleotide in the codon for Asp (C>T) might affect the rate of mRNA translation which, in turn, can influence the strength of the codon-anticodon interaction between tRNA and the codons for the same amino acid; (ii) the c. 2856C>T mutation might be located near a causative mutation and therefore could genetically mark the causative as the results of the linkage disequilibrium. In the latter case, the c. 2856C>T SNP could be considered as a linkage disequilibrium genetic marker.

4. Conclusions

Results of the present study suggest that the *LEPR* SNP c.2856C>T can be considered as a genetic marker for subcutaneous fat deposition and average daily gain in Ukrainian Large White pigs. Therefore results of this study will have an impact on genetic markers-assisted selection aiming to improve meat quality and carcass composition in the breed which is widely used for commercial crossbreeding. Furthermore, result of association studies between *LEP* and *LEPR* polymorphisms and fat distribution could be used for optimization of carcass-processing technologies in meat plants.

We recognize that a relatively low number of animals is a limitation of this study and further evaluation of the *LEPR* SNPc.2856C>T polymorphism on a larger scale and in different breeds is needed.

Conflict of interest statement

There are no conflict of interests.

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