Livestock Science 217 (2018) 157–161

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

# 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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ARTICLE INFO

*Key words:*
Pig
Leptin
Leptin receptor
Polymorphism
Fat deposition
Genetic marker

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.

## 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 (Pena et al., 2016; Tyra et al., 2013) 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; Pérez-Montarelo et al., 2012) with some of them being associated with carcass weight, daily weight gain, subcutaneous fat content and composition (Bauer et al., 2006; De Oliveira Peixoto et al., 2006; Kennes et al., 2001). 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 (Galve et al., 2012; Ovilo

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https://doi.org/10.1016/j.livsci.2018.09.015
Received 17 April 2018; Received in revised form 19 September 2018; Accepted 22 September 2018
1871-1413/ 

et al., 2000,2005; Varona et al., 2002). 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 (Chorev et al., 2012; Kennes et al., 2001;) 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 (Amills et al., 2008; Kuehn et al., 2009;Uemoto et al., 2012; Mackowski et al., 2005). 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 was demonstrated that all the animals used in the present study had a CC genotype, e.g. the mutant allele variant was absent.

### 2.2. Analyses of backfat thickness and average daily weight gain

Backfat thickness was measured by a portable digital Renco Lean-Meter device (Renco Corporation, USA) in the following three locations: (i) at the 10th rib; (ii) at the 6th-7th rib and (iii) at sacrum (Getya et al., 2006).

An average daily weight gain was calculated based on the data obtained from the birth and over the whole period of animal life. The age of animals at 100 kg of live weight was recorded.

### 2.3. DNA isolation, amplification and genotyping

Blood samples (1 ml) were obtained from the jugular vein in the morning before feeding. The blood samples were mixed with 0.05 M EDTA as an anticoagulant and stored up to seven days at +4 °C until used for DNA isolation. Genomic DNA was isolated by a sorbent method using DiatomTM DNA Prep100 kit (Isogen, Moscow, Russia) following the manufacturer instructions with guanidine thiocyanate as a lysis reagent.

Genotyping of the *LEP* and *LEPR* polymorphisms was carried out using the polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLF) assay with primer pairs described in Table 1. PCR reactions were performed in 25 µl (final volume) of the mixture containing genomic DNA, 200 nM of forward and reverse primers, 2.5 mM $MgCl_2$, 0.25 mM of each of the dNTPs and one unit of the recombinant Taq DNA Polymerase (Fermentas, Vilnius, Lithuania). *LEP* was genotyped on the SNPs g.2845A > T (intron 2, rs344615147) and g.3996T > C (3′UTR, rs337366389); *LEPR* was genotyped on the SNPs c.232A > T (exon 4, AF092422), c.915C > T (exon 8, NM_001024587), and c.2856 C > T (exon 20, AF092422). The PCR amplification conditions are given in Table 1.

### 2.4. Statistical analysis

The allele frequencies, genotype frequencies, polymorphic information content (PIC), and levels of heterozygosity (observed heterozygosity $H_o$, and expected heterozygosity $H_e$) were calculated using GenAlEx 6.0 software (Peakall, 2006). Analysis of associations between genotypes and backfat thickness and average daily weight gain were conducted by One Way ANOVA.

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

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

where $\bar{X}_{11}$, $\bar{X}_{12}$, $\bar{X}_{22}$ are arithmetic mean values of productivity traits for the genotypes “11″ (homozygote for the first allele), “12″ (heterozygote) and “22″ (homozygote for the second allele).