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ASSOCIATION OF HinfI POLYMORPHISM IN THE LEPTIN GENE WITH PRODUCTION TRAITS IN WHITE IMPROVED PIG BREED

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ABSTRACT

The HinfI polymorphism in fatness related leptin gene (LEP) has been characterized and evaluated for its association with economically important meat production traits in the population of White Improved pig breed. The data obtained from the PCR-RFLP genotyping of 96 sows indicate the frequencies of 0.71 and 0.29 for LEP-HinfI allele T and C, respectively. The results of GLM analysis demonstrate, that the LEP gene contributes to average daily gain (ADG) but no significant effect was shown in case of backfat thickness (ABF) and lean meat (LM). Highly significant differences ($P \leq 0.01$) were found between LEP genotypes and ADG, where allele C is associated with increased daily gain.The statistical analysis of leptin mRNA levels in adipose tissue of selected animals demonstrates that leptin mRNA level is not altered by LEP-HinfI genotype.

Key words: pig, genetic markers, S. scrofa, meat production traits, leptin, PCR-RFLP

INTRODUCTION

Much effort has been provided on understanding the role of leptin (LEP) and its receptor (LEPR) in growth regulation, fat deposition and reproduction in rodents, humans and domestic animals. LEP and LEPR genes are considered as candidate genes related with meat quality and fatness traits in farm animals. Leptin is a small, 16 kDa protein secreted mainly by adipose tissue and acts as a satiety signal on the hypothalamus, thereby regulating body weight and energy expenditure (Campfield et al., 1995). LEP gene was mapped to the chromosomal region 18q13-q21 in pig (Cepica et al., 1999). Leptin mRNA levels are higher in adipose tissue from obese pigs than lean pigs (Robert et al., 1998). Furthermore, the injection of recombinant porcine leptin reduces the feed intake and increases the growth hormone secretion in swine (Barb et al., 1998).

Several DNA polymorphisms have been detected in pig leptin gene. Stratil et al. (1997) described HinfI polymorphism in pig LEP gene which is characterized by the substitution of $T^{3469} \rightarrow C$ in the second exon. Jiang and Gibson (1999) have found a possible association between allele C of this polymorphism and lower backfat thickness in the Large White pigs. Kulig et al. (2001) investigated the effect of LEP-HinfI locus on growth intensity and parameters of carcass quality in Landrace breed. Significant differences between genotypes were found for lean meat content and average daily gains. Similarly, Urban et al. (2002) described the association of allele C with higher average daily gain, higher percentage of lean meat and lower backfat thickness in Duroc breed. In contrary, Kennes et al. (2001) reported a higher average daily weight gain associated with T allele in the Landrace breed.

In this study we investigated the effect of HinfI polymorphism in leptin gene on fatness related traits in White Improved pig breed. Although the mutation $T^{3469} \rightarrow C$ in the second exon of LEP gene is silent, its effect on transcription and / or transcript stability can not be ruled out. Therefore, we also measured leptin mRNA levels in adipose tissue collected from the pigs with different LEP-HinfI genotypes.

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MATERIAL AND METHODS

The animals (96 sows of White Improved breed) used in the study were from the pig farm Agrokombinat Velke Bierovce. The gilts were sampled randomly, the sample consisted of full- and half- sib groups of different size.

Records were taken for average daily gain since birth (ADG; g) and adjusted to the weight of 100 kg. Ultrasound measurement of backfat thickness was carried out according to standard procedure (STN 466164) and used for the calculation of average backfat thickness (ABT; mm). The calculated value was corrected to uniform body weight. The percentage of lean meat (LM; %) was calculated on the basis of obtained data using the apparatus PIGLOG 105 with the correction to 100 kg.

DNA was extracted from plugged hairs by immersion of hair roots into 50 µl of PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl) supplemented with proteinase K, 0.5 mg/ml. Samples were incubated at 55°C for 3 hours, heated at 96°C for 10 min to inactivate proteinase K and centrifuged at 13 000 rpm (15 200 x g) for 10 min in the table-top centrifuge.

White adipose tissue (approx. 100 mg) was collected from the middle layer of subcutaneous neck fat by biopsy. The samples from 7 animals of each LEP genotype were taken for the analysis. RNA was extracted using TRIZOL (Invitrogen, USA) following the manufacturer´s protocol. Total RNA was quantified by using a GeneQuant Pro spectrophotometer (Amersham Pharmacia).

Genotyping of the Hinf I polymorphism of LEP gene was performed as previously described (Jiang and Gibson, 1999).

The GeneAmp RNA PCR kit (Applied Biosystems, USA) was used for cDNA preparation.

Equal amounts (2 µl) of each cDNA samples were used for the leptin as well as for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH, housekeeping gene) amplification in parallel PCR reactions. The primers used for amplification of 270 bp fragment of leptin cDNA were LEP-RT forward: 5´ - ACGTTGAAGCCGTGCCCATCTGG - 3´ and LEP-RT reverse: 5´- AAGGTCCCGGAGGTTCTCCAGG - 3´ while the primers used for amplification of 318 bp fragment of GAPDH cDNA were as described by Duvigneau et al. (2002).

PCR was performed as follows: first denaturation at 94°C for 2 min, the cycling temperature consisted of 30 cycles of denaturation at 94°C for 40s, annealing at 57°C for 30s, extension at 72°C for 30s (LEP) or 27 cycles of denaturation at 94°C for 40s, annealing at 55°C for 30s and extension at 72°C for 30s (GAPDH) with a final extension at 72°C for 7 min. After PCR analysis, corresponding LEP and GAPDH reactions were mixed and separated in 2 % TAE agarose gel, stained with ethidium bromide.

To quantify the leptin expression, the gels were scanned using a GDS 8000 scanner and the density of LEP and GAPDH bands was determined using the GelWorks 1D Software (UVP Inc., USA). Genetic equilibrium of analysed population was evaluated on the basis of χ^2 test was used to evaluate the genetic equilibrium of the population under study. The analysis of variance (Statistics 4.3) was performed to find an association of the LEP Hinf I polymorphism with leptin mRNA levels. The differences between the genotypes were evaluated by Scheffe test. Associations of candidate gene genotypes with phenotypic parameters of production traits were analysed by means of the GLM procedure (SAS, 2000) using a the model equation with fixed and random effects:

$$y_i = \mu + LEP_i + b.W_i + e_i$$

where y_i = i observation; μ = mean of population;
LEP_i = effect of i^{th} genotype of LEP; (i = 1, 2, 3);
$b.W_i$ = regression of i^{th} observation on standard weight;
e_i = residual effect.

The observed associations of candidate gene genotypes with meat and fat production traits were evaluated for individual genotypes using LSM ± SE (least squares means ± standard error) and significance of their differences at $P \leq 0.05$; $P \leq 0.01$; and $P \leq 0.001$.

RESULTS AND DISCUSSION

We tested 96 pigs of the White Improved breed for HinfI polymorphism in LEP gene by PCR-RFLP (Fig. 1).

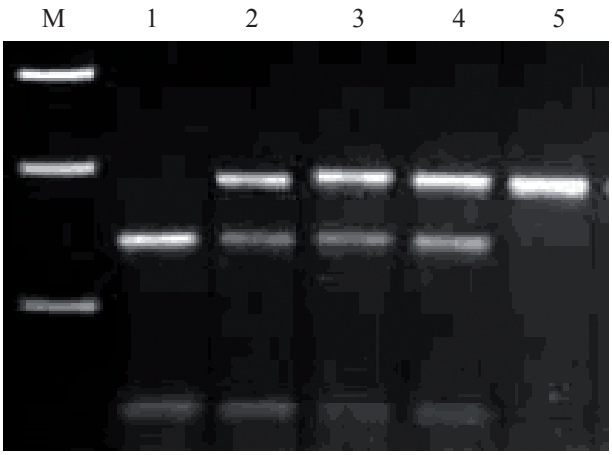

Fig. 1. Agarose gel electrophoresis of PCR products following digestion with Hinf I. **Lane M- 250 bp ladder; lane 1- 347 bp+ 118 bp (homozygote CC); lanes 2, 3, 4 - 465 bp + 347 bp + 118 bp (heterozygotes TC); lane 5- 465 bp (homozygote TT)**

Table 1: Allele- and genotype frequencies of candidate genes in the test population of White Improved pigs

Gene	Allelic frequencies		Genotype frequencies		
LEP	T	C	TT	TC	CC
	0.71	0.29	0.5	0.427	0.073

Table 2: GLM analysis - determination coeficients (χ^2) and significant values (P) of the effects

Meat parameter	n	Model		
		R^2 (%)	LEP (P)	W (P)
ADG	96	45.1	0.036	< 0.0001
ABF	96	10.2	0.313	0.2113
LM	95	23.4	0.543	0.0131

ADG = average daily gain (g); ABF = average backfat thickness (mm); LM = lean meat (%); W = regression on standard weight

The allele- and genotype frequencies were calculated and summarized in Table 1. We found no evidence for a significant deviation of this polymorpfic locus from the Hardy-Weinberg equilibrium ($P \leq 0.05$). The association of LEP-HinfI polymorphism with selected parameters of meat production like average daily gain (ADG), average backfat thickness (ABF) and lean meat percentage (LM) was analysed using GLM procedure. The results of GLM analysis are presented in Table 2. Our results demonstrated that LEP gene contributed to ADG ($P \leq 0.05$). The observed significant associations of individual genotypes of LEP gene with phenotypic parameters are shown in Table 3. Highly significant differences ($P \leq 0.01$) were found between LEP genotypes and ADG (TT = 540.61 ± 10.62 g/day; CC = 584.43± 12.21 g/day) where allele C is associated with increased daily gain.

To test whether the LEP-HinfI polymorphism has an effect on ADG is due to the changes in leptin expression we analyzed leptin mRNA levels in white adipose tissue collected from seven pig genotypes. For investigating a relative leptin mRNA level in the pig adipose tissue a semiquantitative RT-PCR was developed using GAPDH

Table 3: Association of the LEP-HinfI genotypes with meat parameters (LSM ± SE)

Gene	Parameter	Genotypes		
LEP-Hinfl		TT	TC	CC
	ADG	a540.61 ± 10.62	542.92 ± 11.35	a584.43 ± 12.21
	ABF	11.1 ± 0.62	11.4 ± 0.77	10.9 ± 0.60
	LM	59.41 ± 0.47	58.73 ± 0.39	59.25 ± 0.42

ADG = average daily gain (g); ABF = average backfat thickness (mm); LM = lean meat (%);
Values with the same exponents show significant differences: ^{a} = $P \leq 0.01$

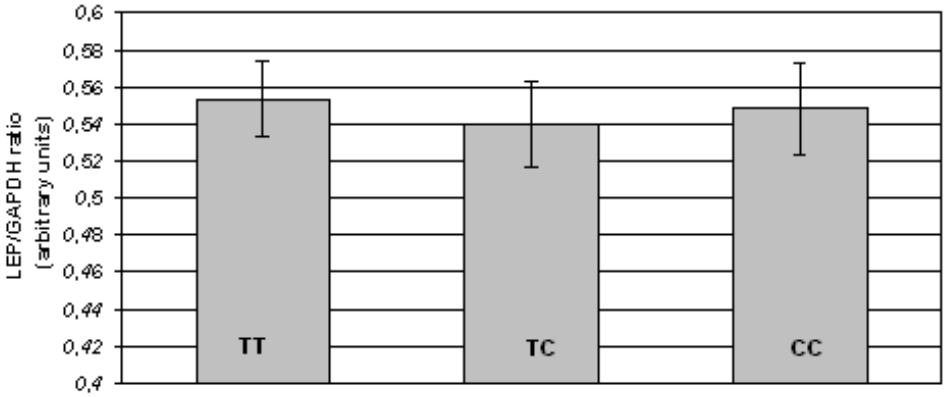

Fig. 2: Optical density of the ratio of LEP to GAPDH RT-PCR products from selected animals with different LEP-HinfI genotype. Analysis of variance has not shown a significant ($P \leq 0.05$) difference between genotypes (TT-TC: 0.014, P = 0.4050, TC-CC: -0.008, P = 0.7945; TT-CC: 0.006, P = 0.8989)

as a housekeeping gene. To avoid competition among amplicons we amplified LEP and GAPDH fragments in separate reactions. A linear accumulation of PCR products (data not shown) occured within the cycles 29-31 (LEP) and 26-28 (GAPDH). Therefore, we decided to use 30 cycles of amplification for LEP and only 27 cycles in case of GAPDH. The statistical analysis of densitometry data are presented in Fig. 2. These data clearly demonstrate that the leptin mRNA levels in adipose tissues of White Improved pigs were not significantly altered by LEP-HinfI genotype.

The data obtained from the PCR-RFLP genotyping of 96 White Improved pigs showed the frequencies 0.71 and 0.29 of LEP-HinfI allele T and C, respectively. Jiang and Gibson (1999) have found a similar frequency of 0.28 for allele C in population of Large White and a very low frequency (0.07) in Landrace breed. Kennes et al. (2001) have reported the frequency of allele C for three pig breeds, Landrace (0.06), Duroc (0.09) and Yorkshire (0.15). The genotyping data recently obtained by Urban et al. (2002) showed much higher frequency (0.35) of allele C in Duroc breed. Szydlowski et al. (2004) have reported the frequencies 0.11, 0.10, and 0.11 for C allele in the Polish Large White, Polish Landrace and Polish synthetic line 990.

Further, we tested the association of this candidate gene with the meat production traits. The results of GLM analysis demonstrated that LEP gene contributes to ADG. Highly significant differences ($P \leq 0.01$) were observed between LEP genotypes and ADG, where allele C is associated with increased daily gain. The results from previous analysis of LEP-HinfI polymorphism involving 3469 and porcine productive traits are not conclusive. Jiang and Gibson (1999) reported an association between allele C of LEP-HinfI polymorphism and lower backfat thickness in the Large White pigs. Kulig et al. (2001) investigated the effect of LEP-HinfI locus on growth intensity and parameters of carcass quality in Landrace breed. Significant differences between the genotypes were recorded for lean meat content and average daily gains. Similarly, Urban et al. (2002) described association of allele C with higher average daily gain, higher percentage of lean meat and lower backfat thickness in Duroc breed. In contrary, Kennes et al. (2001) reported higher average daily weight gain associated with T allele in the Landrace breed. It was confirmed by semi-quantitative RT-PCR that the LEP-HinfI mutation is not only silent but it does not alter the leptin mRNA level in adipose tissue. It seems to be probable that linkage disequilibrium with another mutation (s) will explain the observed significant association of LEP with ADG.

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