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EFFECT OF LEPTIN AND LEPTIN RECEPTOR GENES ON MEAT PRODUCTION TRAITS OF SLOVAK LARGE WHITE AND LANDRACE PIGS

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ABSTRACT

The polymorphisms in fatness related leptin (LEP) and leptin receptor (LEPR) genes have been characterized and their effect on economically important meat production traits was evaluated in the population of Slovak Large White (SLW) and Landrace pig breeds. We tested HinfI polymorphism of the LEP gene, HpaII and RsaI polymorphisms of the LEPR gene. The data obtained by PCR-RFLP genotyping of 140 animals of SLW breed show the frequencies of 0.707 and 0.293 for LEP-HinfI alleles T and C, respectively. The frequencies of LEPR-HpaII alleles were 0.214 (allele A) and 0.786 (allele B). The LEPR-RsaI locus showed a low frequency of 0.00357 for allele A in tested population. The genotyping data obtained for Landrace breed (62 animals) showed a very low polymorphism of all three markers in tested population. In case of SLW breed, results of GLM analysis demonstrate the LEP gene effect (P≤0.05) on average daily gain (ADG) and the LEPR gene shows small effect (P≤0.05) both on average backfat thickness (ABF) and lean meat (LM). A highly significant difference (P≤0.01) was observed for association of LEP genotypes TT and CC with ADG showing an increased ADG in CC homozygous sows. A significant difference (P≤0.05) was also found in the association of LEPR genotypes with ABF and LM, demonstrating a lower ABF in BB homozygotes. It seems to be probable that linkage disequilibrium with another mutation (s) could explain observed association of tested markers with meat production traits.

Key words: genetic markers; meat production traits; *S. scrofa;* leptin; leptin receptor

INTRODUCTION

Numerous efforts have focused recently on understanding the role of leptin (LEP) and its receptor (LEPR) in regulating growth, fat deposition and reproduction in rodents, humans and domestic animals. LEP and LEPR genes are considered to be promising candidate genes related to meat quality and fatness traits in farm animals. Several DNA polymorphisms have been detected in the pig leptin gene (Stratil *et al.,* 1997; Jiang and Gibson, 1999). Kulig *et al.* (2001) have investigated the effect of the LEP-HinfI locus on growth intensity and carcass quality in Landrace breed. Significant differences between genotypes were found for lean meat content and average daily gain. Similarly, Urban *et al.* (2002) described an association of the LEP-HinfI allele C with higher average daily gain, higher percentage of lean meat and lower backfat thickness in Duroc breed. In contrast, Kennes *et al*. (2001) reported a higher average daily weight gain associated with T allele in the Landrace breed.

The leptin receptor is related to the control of feed intake and the regulation of energy balance since it modulates the leptin effect. There are six splicing variants of the leptin receptor which are expressed in multiple organs and tissues (Ahima and Flier, 2000). Several restriction fragment length polymorphisms in the porcine LEPR gene have been identified (Vincent *et al*., 1997; Stratil *et al*., 1998). Hardge *et al*. (2000) have found an association between HpaII restriction site polymorphism

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in third intron of the LEPR gene and fatness related traits in a porcine resource family. Óvilo *et al.* (2002) have also analyzed the effect of the LEPR HpaII polymorphism on backfat, intramuscular fat and eye muscle area in F_2 cross between Iberian and Landrace pig.

The aim of this study was to investigate the effect of the HinfI polymorphism at position 3469 of the leptin gene and HpaII and RsaI polymorphisms in the leptin receptor gene on fatness-related traits of Slovak Large White and Landrace pig breeds. We also measured leptin mRNA levels in adipose tissue collected from pigs of different LEP-HinfI genotypes using a reverse transcription-quantitative PCR (RT-qPCR).

MATERIALS AND METHODS

The animals (135 sows, 5 boars of Slovak Large White and 62 sows of Landrace) were randomly distributed into full-sib and half-sib groups of different size. The pigs were fed with commercial mixed fodder *ad libitum* within the test period. Records were taken for average daily gains since birth (ADG; g). An ultrasound measurement of backfat thickness was carried out according to standard procedure (STN 466164) and was used for the calculation of average backfat thickness (ABT; mm). The percentage of lean meat (LM) was calculated from the measurement performed by the PIGLOG 105 instrument. All data were corrected for the uniformity of body weight at 100 kg.

DNA was extracted from hair roots by the silica matrix method according to Bauerova *et al.* (1999). White adipose tissue (approx. 100 mg) was collected from the middle layer of subcutaneous neck fat by the biopsy (10 animals of each LEP-HinfI genotype) and RNA was extracted using Trizol (Invitrogen, USA) following the manufacturer´s instructions. A PCR-RFLP genotyping of the HinfI polymorphism in LEP locus was performed as previously described by Jiang and Gibson (1999). The analysis of HpaII and RsaI polymorphisms in the LEPR gene was carried out according to Stratil *et al.* (1998).

cDNA used for reverse transcription-qPCR (RT-qPCR) was synthesized using oligo dT primer and 1µg of total RNA from each sample in 20µl reactions consisting of 1x PCR buffer, 5 mM $MgCl_2$, 4 mM dNTP, 2.5 µM oligo $(dT)_{16}$, 20 U RNase inhibitor (Applied Biosystems, USA) and 50 U MuLV reverse transcriptase (Applied Biosystems, USA). The reaction was performed at 25°C for 5 min, 42°C for 15 min, 99°C for 5 min and 4°C for 5 min. A qPCR was performed using leptin primers (leprtF 5´-ACGTTGAAGCCGTGCCCATCTG-3´; leprtR 5´-AAGGTCCCGGAGGTTCTCCAG-3´), primers for GAPDH as a reference gene (Duvigneau *et al*., 2002), Rotor-Gene 6000 real-time PCR system (Corbett Research, Australia) and ABsolute qPCR SYBR Green Mix (ABgene Ltd., UK). A relative quantification of the leptin mRNA was carried out in two parallels of 20 µl qPCR reactions with LEP and GAPDH primers which contained 1 x ABsolute qPCR SYBR Green Mix, 300 nM primers and 2 µl of template cDNA in each reaction. The cycling conditions consisted of 15 min incubation at 95°C followed by 40 cycles of 95°C for 15s, 57°C for 15s and 72°C for 20 s. A melting curve analysis was performed at 72°C-95°C. Standard curves were built using serial dilutions of template cDNA for both LEP and GAPDH primers.

The genetic equilibrium of analysed population was evaluated by χ^2 test. Analysis of variance (Statistica 4.3) was applied to test an association of the LEP Hinf I polymorphism with leptin mRNA levels. The differences between the genotypes were evaluated by Scheffe test. Associations of genotypes with the meat production traits were analysed by GLM procedure (SAS, 2000) using a model equation with fixed and random effects:

$$y_{ijk} = \mu + LEP_i + LEPRH_j + LEPR_k + b.W_{ijk} + e_{ijk},$$

where y_{ijk} = ijk observation, μ = mean of population, LEP_i = effect of i^{th} genotype of LEP (i = 1, 2, 3), $LEPRH_j$ = effect of j^{th} genotype of LEPR-HpaII (j = 1, 2, 3), $LEPR_k$ = effect of k^{th} genotype of LEPR-RsaI (k = 1, 2, 3), $b.W_{ijk}$ = regression of ijk^{th} observation on standard weight, e_{ijk} = residual effect. The observed associations of genotypes with the meat production traits were evaluated for individual genotype using LSM ± SE (least squares means ± standard error) and significance of difference at $P \leq 0.05$; $P \leq 0.01$; and $P \leq 0.001$.

RESULTS AND DISCUSSION

We tested 140 pigs of Slovak Large White (SLW) and 62 pigs of Landrace for the Hinf I polymorphism at position 3469 in the LEP gene and HpaII and RsaI polymorphisms in the LEPR gene by PCR-RFLP. The allele and genotype frequencies were calculated and summarized in Table 1. We found no evidence for a significant deviation from the Hardy-Weinberg equilibrium ($P \leq 0.05$) for all three polymorphic loci. Our data regarding LEP-HinfI polymorphism showed the frequency 0.293 of the alelle C in SLW but very low frequency (0.04) in Landrace breed. Similarly, Jiang and Gibson (1999) have found the frequency of 0.28 for the allele C in the population of Large White and 0.07 in Landrace breed. Kennes *et al*. (2001) have reported low frequency of the allele C in Landrace (0.06) but also in Duroc (0.09) and Yorkshire (0.15) breeds. On the contrary, genotyping data obtained by Urban *et al.* (2002), analyzing Czech Duroc population, have shown much higher frequency (0.35) of the allele C. Recently, Szydlowski *et al.* (2004) have reported the frequencies

Table 1: Allele and genotype frequencies of the LEP and LEPR genes in tested population of Slovak Large White (SLW) and Landrace pigs.

Breed	Polymorphism	Allele frequency		Genotype frequency		
SLW (n=140)	LEP-HinfI	T 0.707	C 0.293	TT 0.5	TC 0.414	CC 0.086
	LEPR-HpaII	A 0.214	B 0.786	AA 0.0357	AB 0.357	BB 0.607
	LEPR-RsaI	A 0.00357	B 0.99643	AA 0.0	AB 0.071	BB 0.9929
L (n=62)	LEP-HinfI	T 0.96	C 0.04	TT 0.9193	TC 0.0645	CC 0.016
	LEPR-HpaII	A 0.0484	B 0.9516	AA 0.0322	AB 0.0645	BB 0.9032
	LEPR-RsaI	A 0.008	B 0.992	AA 0.0	AB 0.016	BB 0.984

Table 2: GLM analysis - determination coeficients (R^2) and significant values (P) of the effects for Slovak Large White breed.

		Model			
Parameter	n	R^2 (%)	LEP-HinfI (P)	LEPR-HpaII (P)	W
ADG	140	51.8	0.034	0.109	< 0.0001
ABF	140	44.2	0.310	0.045	0.0113
LM	140	40.9	0.540	0.047	0.0231

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

0.11, 0.10, and 0.11 for the allele C in Polish Large White, Polish Landrace and Polish synthetic line 990, respectively.

Regarding the genotyping of the LEPR-HpaII locus, our results correlate with the data reported previously (Hardge *et al*., 2000; Óvilo *et al*., 2002). The frequencies 0.214 and 0.786 were detected for LEPR-HpaII alleles A and B, respectively in SLW. In case of Landrace breed the frequency of the alelle A was much lower (0.0484) than in SLW. A LEPR-RsaI locus generally exhibited low polymorphism in both tested breeds, with the frequency of the alelle A only at 0.00357 (SLW) or 0.008 (Landrace).

Since all three tested markers had shown a low polymorphism in the population of Landrace, we had to entirely exclude this breed from the GLM analysis together with LEPR-RsaI marker in SLW. Further, we tested the association of LEP-HinfI and LEPR-HpaII polymorphisms in SLW with average daily gain (ADG), average backfat thickness (ABF) and lean meat percentage (LM) using GLM procedure (Table 2). Our results demonstrate that the LEP gene contributes to ADG ($P \leq 0.04$) and the LEPR gene shows an effect on both ABF ($P \leq 0.05$) and LM ($P \leq 0.05$) in SLW breed.

Observed associations of individual genotypes of both the LEP and the LEPR genes with phenotypic parameters are shown in Table 3. A highly significant difference ($P \leq 0.01$) was observed for the association of LEP genotypes TT and CC with ADG showing an increased ADG in CC homozygotes. A significant difference ($P \leq 0.05$) was also found in association of LEPR genotypes with ABF and LM demonstrating decrease in ABF and higher LM in BB homozygotes.

Previous results of association of the LEP-HinfI polymorphism at position 3469 with porcine production traits are not conclusive and various effects of this locus have been observed in pig breeds. Jiang and Gibson (1999) have found the association of the allele C with lower ABF in Large White pigs. Kulig *et al.* (2001) have investigated the effect of the LEP-HinfI locus in Landrace breed, and significant differences were found for LM and ADG. But Kennes *et al*. (2001) have reported only higher

Table 3: Associations of LEP and LEPR genotypes with selected parameters of Slovak Large White breed (LSM ± SE).

Polymorphism	Parameter	Genotype		
LEP-HinfI		TT	TC	CC
	ADG	[a] 540.61 ± 10.62	542.92 ± 11.35	[a] 584.43 ± 14.21
	ABF	11.20 ± 0.52	11.64 ± 0.72	10.98 ± 0.62
	LM	59.40 ± 0.42	58.63 ± 0.39	59.48 ± 0.64
LEPR-HpaII		BB	AB	
	ADG	545.76 ± 12.62	543.68 ± 13.44	
	ABF	[b] 10.50 ± 0.52	[b] 11.40 ± 0.61	
	LM	[b] 59.96 ± 0.43	[b] 58.71 ± 0.39	

ADG = average daily gain (g); ABF = average backfat thickness (mm); LM = lean meat (%). Values with the same superscripts show significant differences : [a] = $P \leq 0.01$; [b] = $P \leq 0.05$.

ADG associated with the T allele in Landrace. Our results are partially consistent with the observation of Urban *et al*. (2002). They have described an association of the allele C with higher ADG, lower ABF and higher LM in Duroc breed.

Regarding the association of the LEPR-HpaII polymorphism with the meat production traits in SLW our results demonstrate the effect of allele B on the lower ABF, as well as on the higher LM. But Hardge *et al*. (2000) have found favourable effect of the allele A both on the lower ABF and intramuscular fat in the porcine resource family. Later, Óvilo *et al.* (2002) have confirmed their results analyzing the effect of the LEPR HpaII polymorphism on ABF, intramuscular fat and eye muscle area in F_2 cross between Iberian and Landrace pigs using an animal model. But this effect was not observed using the QTL regression analysis. Our data, demonstrating the association of the LEPR BB genotype with the lower ABF and the higher LM in tested population of SLW, might be influenced by a limited number of analysed pigs in our study. Other explanation might be a linkage disequilibrium of tested polymorphisms with different alleles of the causative mutations or different genetic background of analysed populations.

To test whether the effect of the LEP-HinfI polymorphism on ADG might be due to the changes in leptin expression we analyzed the leptin mRNA levels in white adipose tissue collected from 10 SLW pigs of each genotype. Although HinfI mutation in the second exon of the LEP gene is silent, its effect on transcription and/or transcript stability cannot be ruled out. A quantitative real-time RT-PCR method was used to investigate the relative leptin mRNA level in pig adipose tissue (data not shown). The RT-qPCR data confirmed our previous results based on less precise semi-quantitative RT-PCR (Bauer *et al*., 2006) and demonstrated that the leptin mRNA level in adipose tissue was not significantly altered by the LEP-HinfI genotype. Therefore, it seems to be probable, that linkage disequilibrium with another mutation(s) explains the observed association of LEP with ADG.

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