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The relationship between the T945M single nucleotide polymorphism in the leptin receptor gene (*LEPR*) and milk production traits in Jersey cows*

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Investigation was conducted on 219 Jersey cows. *LEPR* genotypes were established with the PCR-RFLP using the *Taq*I restriction endonuclease. The frequencies of the threonine encoding *C* allele and the methionine encoding *T* allele were 0.79 and 0.21, respectively. The across-families analysis did not revealed any significant effect of the *LEPR* T945M polymorphism on milk, fat and protein yields. The significant relations were, however, found for fat and protein contents. Animals with the *TT* genotype were characterized by the lowest values of both traits. The within-family analysis, showed the significant effect only on milk fat content in one half-sibs group.

KEY WORDS: cattle / gene polymorphism / leptin receptor / milk traits

Leptin is a protein hormone secreted primarily from white adipose tissue [Zhang *et al*. 1994], and also by placenta [Masuzaki *et al*. 1997], skeletal muscle [Wang *et al*. 1998] and mammary gland [Chelikani *et al*. 2003]. The well-known function of the leptin includes the regulation of food intake and energy expenditure [Zhang *et al*. 1994, Houseknecht *et al*. 1998]. However, the hormone appears to be also implicated in much more physiological functions including the modulation of reproduction, hormone secretion by several endocrine glands, immune and stress responses, blood pressure, as well as cell differentiation and proliferation [Houseknecht *et al*. 1998, Fruhbeck 2001].

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In cattle, polymorphism in the leptin gene (*LEP*) was shown to be associated with feed intake, growth, carcass composition, milk-related traits and calving interval [van der Lende *et al*. 2005]. Since leptin exerts its effect by interacting with receptors located in most bovine tissues [Silva *et al*. 2002], leptin receptor gene (*LEPR*) can also be considered as a candidate gene affecting productive traits.

Leptin receptor gene produces, by alternative splicing, at least five membrane and one soluble leptin receptor isoforms. They are characterized by the identical extracellular domains, whereas differences between them are due to changes in the length of the intracellular domains. The long form of the receptor (*LEPR*-b) has the complete cytoplasmic domain and is responsible for most of the physiological effects of leptin [Tartaglia 1997].

In cattle, *LEPR* gene is located in chromosome 3 [Pfister-Genskow *et al*. 1997]. It has only been partly sequenced, and, until now, only one mutation in it has been reported [Liefers *et al.* 2004]. The C → T SNP (single nucleotide polymorphism) is located in exon 20 at position 115, and causes the threonine → methionine amino acid substitution in the intracellular domain of the *LEPR*-b isoform, at residue 945.

The aim of this study was to determine whether the *LEPR* T945M polymorphism influences milk yield and composition in Jersey cattle.

Material and methods

The study included 219 Jersey cows, born between year 1997 and 2002, maintained on the Siedlec farm belonging to Horse Stud in Iwno, and sired by 21 bulls. Mean number of daughters per sire was 10.4, ranging from 1 to 51.

Genomic DNA was isolated from peripheral blood using the standard phenol method. Genotypes were determined using the PCR-RFLP technique. Primers for PCR amplification (IBB PAS, Poland) were designed using the Primer3 software (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) on the basis of gene sequence available in the GenBank data base (accession number AJ580801) as follows:

F: 5'- GCAACTACAGATGCTCTACTTTTGT* -3' and

R: 5'- CAGGGAAATTTCCCTCAAGTTTCAA -3'.

The forward primer included a purposeful mismatch (marked with an asterisk) that incorporated a *Taq*I restriction site to the sequence.

The PCR reaction volume of 10 μl contained approximately 50 ng of genomic DNA, 0.5 unit of Taq DNA polymerase (FERMENTAS, Lithuania), 1 × PCR buffer with $(NH_4)_2SO_4$ (FERMENTAS, Lithuania), 2 mM $MgCl_2$, 5% DMSO, 1 μM of each primer, and 200 μM of each dNTP. Thermal cycling conditions included an initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 40 s, followed by the final extension at 72°C for 5 min. The PCR reactions were carried out using a TGradient thermocycler (BIOMETRA, Germany). The amplified fragments were digested overnight at 65°C with 5 units of *Taq*I restriction

endonuclease (FERMENTAS, Lithuania), and next subjected to electrophoretic separation in 2.5% ethidium bromide-stained agarose gel.

Effect of genotypes on milk traits was tested using the GLM procedure of the SAS package [SAS, 1999]. The statistical model included effects of sire, *LEPR* genotypes, herd-year-season of calving and lactation number (parity). The additional analysis was performed within two families sired by non-segregating bulls with the largest number of daughters. In the within-family test, the differences between cows receiving either *T* or *C* allele from their dams were estimated using a model that included effects of *LEPR* genotypes, herd-year-season of calving and lactation number (parity). The records of milk, fat and protein yields as well as fat and protein contents were obtained from the official milk recording system.

Results and discussion

The PCR amplification yielded a 400 bp long *LEPR* fragment. After digestion with the *Taq*I restriction enzyme, the *C* allele was cleaved into two fragments of 375 bp and 25 bp, while allele *T* remained uncut at 400 bp (Fig. 1).

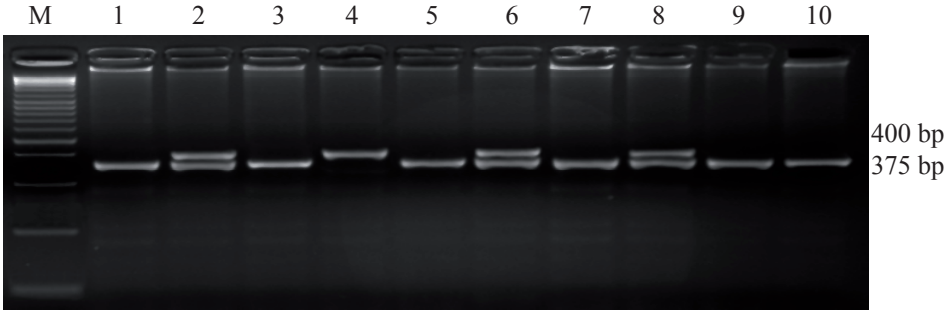

Fig. 1. LEPR genotyping by PCR-RFLP method. M – DNA marker: GeneRuler™ DNA Ladder Mix (Fermentas); lanes 1, 3, 5, 7, 9 and 10 – *CC* genotype (25 bp band not seen on the gel); lanes 2, 6 and 8 – *CT* genotype (25 bp band not seen on the gel); lane 4 – *TT* genoptype.

Among the 219 cows examined, the *TT* genotype was identified in 12, *CT* in 68, and *CC* in 139 cows, which gives the allele frequencies of 0.21 (*T*) and 0.79 (*C*). The genotypes were distributed according to the Hardy-Weinberg equilibrium.

Effects of the *LEPR* genotypes on milk production traits in all Jersey cows examined are presented in Table 1. The analysis did not reveal any significant effect of the T945M polymorphism on milk, fat and protein yields. The significant associations were, however, found for fat and protein contents. Animals with the *TT* genotype were characterized by the lowest values of both traits. Results of the within-family analysis are shown in Table 2. The significant effect was found only for milk fat content in the group of daughters sired by bull no 2.

Table 1. Means and their standard deviations (SD) for milk production traits in Jersey cows with different T945M *LEPR* genotypes

Trait		Genotype *TT* (n=12)	Genotype *CT* (n=68)	Genotype *CC* (n=139)	Probability (P≤)
Milk yield (kg)	mean	4669	4571	4560	0.219
	SD	803	840	782	
Fat yield (kg)	mean	245	257	259	0.413
	SD	51	50	45	
Fat content (%)	mean	5.15^{AB}	5.57^{Aa}	5.69^{aB}	0.013
	SD	0.64	0.57	0.52	
Protein yield (kg)	mean	176	179	180	0.192
	SD	32	31	31	
Protein content (%)	mean	3.69^{ab}	3.87^{a}	3.94^{b}	0.044
	SD	0.16	0.23	0.25	

$^{aA...}$Means within rows bearing the same superscripts differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

Table 2. Means and their standard deviations (SD) for milk production traits in cows with different T945M *LEPR* genotypes in two half-sib families sired by non-segregating bulls with the largest daughter numbers

Trait		Family 1 (no. of daughters = 51) genotype *CT*	Family 1 genotype *CC*	Family 1 P≤	Family 2 (no. of daughters = 48) genotype *CT*	Family 2 genotype *CC*	Family 2 P≤
Milk yield (kg)	mean	4424	4484	0.273	4544	4454	0.108
	SD	533	712		533	653	
Fat yield (kg)	mean	258	256	0.546	245	243	0.179
	SD	41	51		47	37	
Fat content (%)	mean	5.80	5.72	0.193	5.33^{a}	5.49^{a}	0.048
	SD	0.49	0.44		0.38	0.45	
Protein yield (kg)	mean	179	180	0.331	172	169	0.287
	SD	26	33		20	25	
Protein content (%)	mean	4.05	3.99	0.389	3.76	3.75	0.161
	SD	0.22	0.21		0.19	0.19	

^{a}Means within rows bearing the same superscripts differ significantly at P≤0.05.

Since their discovery in 1994 and 1995, leptin and its receptor have gained much interest, initially as factors regulating the energy balance, and later as mediators of many other physiological functions. Mutations in the *LEPR* gene were found to result in obesity in mice [Chen *et al*. 1966], rat [Chua *et al*. 1996] and humans [Clement *et al.* 1998]. Among farm animals, impact of *LEPR* polymorphism on production traits

was most frequently examined in pigs. No evident effect has been identified, although the associations were suggested with the feed efficiency and certain carcass traits [Chen *et al*. 2004, Maćkowski *et al*. 2005].

In cattle, the only known *LEPR* mutation was reported by Liefers *et al*. [2004]. The T945M polymorphism is present in the intracellular domain of the leptin receptor long isoform (LEPR-b), detectable in most bovine tissues [Chelikani *et al*. 2003]. It might be responsible for the efficiency of the signaling pathway in cells and, as a consequence, for the modulation of the leptin effects. Liefers *et al*. [2004] found the association between T945M SNP and the circulating leptin concentration in Holstein-Friesian (HF) cattle. As the relationship exists between leptin, energy balance and lactation [Block *et al*. 2001], the *LEPR* polymorphism might be associated with the milk production in cows. However, the mutation effect on the leptin concentration was observed by Liefers *et al*. [2004] only in the late pregnancy, but not in lactation. Moreover, no association was revealed with milk-related traits (Liefers, unpublished data).

In the present study, the impact of T945M SNP on milk yield and composition was analysed in Jersey cows. The obtained *T* allele frequency (0.21) was higher than 0.08 [Komisarek and Dorynek 2005] and 0.04 [Liefers *et al*. 2004] being reported earlier for HF cattle. Therefore, the *T* allele seems to be more favoured by selection, which in Jersey breed puts most pressure on the milk composition. In the family no 2, daughters that received the *T* allele from their dams were characterized by a significantly higher milk fat content than those receiving the allele *C*. However, the across-families analysis revealed the contrary results, namely animals of the *TT* genotype produced milk with the lowest fat and protein content.

In conclusion, this study suggests the possible impact of the T945M polymorphism in the *LEPR* gene on milk composition in Jersey cattle, mainly on milk fat content. The association should, however, be confirmed in the further studies including the larger number of animals. That would increase the power of the statistical test and the reliability of the results obtained.

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Zależność między polimorfizmem T945M w genie receptora leptyny (*LEPR*) a cechami produkcji mleka krów rasy jersey.

S t r e s z c z e n i e

Badaniem objęto 219 krów. Genotypy zwierząt ustalono metodą PCR-RFLP, stosując enzym restrykcyjny *Taq*I. Frekwencje allelu *C*, kodującego treoninę oraz allelu *T*, kodującego metioninę, wyniosły odpowiednio 0,79 i 0,21. Analiza przeprowadzona w całej populacji nie wykazała istotnego wpływu polimorfizmu T945M w *LEPR* na wydajność mleka, białka i tłuszczu. Stwierdzono jednak istotną zależność dla zawartości (%) tłuszczu oraz białka. Osobniki o genotypie *TT* charakteryzowały się najniższą wartością obu tych cech. W analizie przeprowadzonej w rodzinach, istotny efekt udowodniono tylko dla zawartości tłuszczu w mleku w jednej grupie półrodzeństwa.