

Effect of Leptin Gene Polymorphisms on Milk Production Traits of Jersey Cows

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Received: 05.10.2007

Abstract: A total of 181 Jersey cows were used to investigate how leptin gene polymorphisms affect milk production traits such as milk yield, fat and protein yield, and fat and protein content. Two single-nucleotide polymorphisms (SNPs) were genotyped, including the A59V in exon 3 and *Sau3AI* in intron 2. The genotype, allele, and haplotype frequencies were estimated. Statistical analysis revealed that the A59V polymorphism significantly affected the milk, protein, and fat yield ($P \leq 0.01$, $P \leq 0.05$, respectively) with *C* as a desirable allele. No associations were observed between the *Sau3AI* polymorphism and the traits analysed in this study. However, cows with the *CC/TT* haplotype were characterised by a significantly lower milk fat content compared with cows with other haplotypes. The results indicated that selection for the A59V *CC* and *CT* animals might contribute to increase in milk yield as well as fat and protein yields in Jersey cattle.

Key Words: Leptin, SNP, Jersey cows, milk production traits

Introduction

Leptin is a 16 kDa polypeptide hormone, produced primarily by fat cells (1). However, lower amounts of leptin are also synthesised in the mammary gland during lactation (2). The presence of leptin was demonstrated in the colostrum and/or milk of cattle and other species (2-4). Leptin is involved in maintaining the energy balance by controlling food intake and energy expenditure. It also regulates the functions of the reproduction as well as immune and endocrine systems (5). It is suggested that leptin may inform the hypothalamus about energy reserves sufficient to support the energy demands to guarantee the success of lactation (6).

The leptin-encoding gene was mapped in the bovine chromosome 4 (7) and it is proposed as a potential candidate gene for milk yield and quality in cattle. Associations between certain single nucleotide

polymorphisms (SNPs) in the leptin gene and milk traits in cattle have been reported by Buchanan et al. (8), Liefers et al. (9), and Zwierzchowski et al. (10). However, there are few reports on associations between leptin genotypes and those traits in Jersey cattle.

The aim of this study was to establish leptin allele and genotype frequencies and possible associations between leptin gene polymorphisms (A59V, *Sau3AI*, and their combination A59V/*Sau3AI*) and selected milk production traits in Jersey cows.

Materials and Methods

Materials

The study involved a total of 181 Jersey cows kept on a farm located in the Wielkopolska region in Poland. All the studied animals were born between 1990 – 1998 and

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came from 19 sires. The animals were kept in identical environmental conditions and were fed a standard diet. All the 181 cows completed the first lactation, 151 animals completed the first and second lactations, and 95 animals completed the first, second, and third lactations.

Genotyping

The blood from an external jugular vein was collected into tubes with K₃EDTA. DNA was isolated with MasterPure™ Genomic DNA Purification kit (Epicentre® Biotechnologies) according to the producer's instructions. Genotyping was performed using the PCR-RFLP method. The first fragment (331 bp long) of the leptin gene (A59V polymorphism) was amplified using a pair of primers with the following nucleotide sequences: 5'-GGG AAG GGC AGA AAG ATA G-3' and 5'-TGG CAG ACT GTT GAG GAT C-3' (11). Amplification of the next, 1820 bp long fragment of the leptin gene (*Sau3AI* polymorphism) was carried out with a pair of the following primers: 5'-GTC ACC AGG ATC AAT GAC AT-3' and 5'-AGC CCA GGA ATG AAG TCC AA-3' (7). The PCR was performed in a total volume of 20 µl containing 50-70 ng DNA, 2 mM MgCl₂, 10 pmol of each primer, 200 µM each dNTP, 0.5 U *Taq* DNA polymerase (MBI Fermentas Burlington, Ontario, Canada), 20 mM *Taq* polymerase buffer and deionised water up to 20 µl. The amplified fragments were digested with *HphI* (331 bp fragment) and *Sau3AI* (1820 bp fragment) restriction endonucleases. The restriction fragments obtained were separated on 2% agarose gels with ethidium bromide in the presence of appropriate DNA standard, and described using the software for photodocumentation of electrophoretic separation and image storage (Vilber Lourmat).

Statistical analysis

The allele and genotype frequencies were determined. It was verified by χ^2 test whether the distribution of the genotype frequencies was in the Hardy-Weinberg equilibrium. The next stage involved an analysis of associations between the leptin genotypes and the following milk production traits: milk yield (kg), protein yield (kg), fat yield (kg), milk protein content (%), and milk fat content (%). The study covered the first, second, and third lactations. The analysed traits were standardised for the lactation length (305 days). Statistical analysis of milk production traits in relation to leptin genotypes was carried out using the GLM (General Linear Model) multiple mixed model (12). The following linear model was applied:

$$y_{ijkl} = \mu + a_i + b_j + c_k + d_l + e_{ijkl}$$

where: y_{ijkl} – observed trait value in $ijkl$ -th animal; μ – mean trait value for herd; a_i – effect of genotype/haplotype ($i = 1, 2, 3$ for A59V and *Sau3AI*; $i = 1, 2, 3, 4, 5, 6$ for A59V/*Sau3AI*); b_j – effect of lactation parity ($j = 1, 2, 3$); c_k – effect of year/month of calving ($k = 1, 2, \dots, 87$); d_l – effect of sire ($l = 1, 2, \dots, 19$); e_{ijkl} – random error.

The differences between mean values of the traits were analysed with the Duncan multiple range test.

Results

The frequencies of the A59V genotypes and alleles were as follows: *CC* – 0.52, *CT* – 0.40, *TT* – 0.08, *C* – 0.72, *T* – 0.28. The frequencies of the *Sau3AI* genotypes and alleles were as follows: *CC* – 0.30, *CT* – 0.57, *TT* – 0.13, *C* – 0.59, *T* – 0.41. In the studied herd, no disruption in the genetic equilibrium was found between the observed and the theoretical genotype frequencies. In the present study, 8 out of 9 possible A59V/*Sau3AI* genotype combinations (haplotypes) were identified. The most frequent haplotype was the *CC/CT* haplotype (0.29). The frequencies of the other haplotypes were as follows: *CT/CT* – 0.27, *CT/CC* – 0.12, *CC/CC* – 0.11; *CC/TT* – 0.11, *TT/CC* – 0.07, *CT/TT* – 0.02, and *TT/CT* – 0.01.

The Table shows mean values for the milk production traits studied in the cows with different A59V genotypes and A59V/*Sau3AI* haplotypes. Due to the low number of the *TT/CT* haplotype (1 individual), this haplotype was not included in the statistical analysis. The study results show statistically significant differences between the mean values for milk yield as well as protein and fat yield in Jersey cows with different A59V leptin genotypes. The *TT* cows produced significantly ($P \leq 0.05$) less milk than the *CC* and *CT* cows, the difference between the *TT* and *CC* individuals was 173 kg in average. The difference between the *TT* and *CT* cows was slightly higher (177 kg). Similarly, milk protein yield in the *TT* cows was significantly ($P \leq 0.05$) lower than that produced by the *CC* and *CT* cows (by 7.6 and 7.8 kg, respectively). Moreover, the *TT* cows produced milk of a significantly ($P \leq 0.01$) lower fat yield compared with *CC* or *CT* cows, the differences being 10.6 and 15.3 kg, respectively. These results were confirmed by an analysis of total fat and protein yield. This trait was significantly lower in the *TT* cows compared with the *CC* and *CT* cows. In terms of

protein and fat content, the differences between the respective A59V genotypes were statistically non-significant.

Regarding the *Sau3AI* polymorphism, no significant differences were found between the genotypes and the analysed milk production traits in this study (data not shown). However, it was found that the *TT* genotype cows were characterised by the highest milk, protein, and fat yields. The highest protein and fat content was recorded in the *CC* genotype cows. Analysis of the haplotype data showed statistically significant differences between cows with different haplotypes in relation to total fat and protein yield as well as milk fat content (Table). Total fat and protein yield of the *TT/CC* cows was significantly lower compared with the *CC/TT* and *CT/CC* cows. Moreover, the *CT/CC* cows were characterised by a significantly higher fat content than the *CC/TT* ($P \leq 0.01$), *CT/CT* ($P \leq 0.05$), and *CC/CT* ($P \leq 0.05$) cows, with differences amounting to 0.49%, 0.38%, and 0.34%, respectively.

Discussion

The results obtained in this study demonstrate that there are associations between the leptin A59V polymorphism and milk production traits in Jersey cows.

The *CT* and *CC* cows produced significantly more milk, fat, and protein than the *TT* cows. In a previous study (13), associations between the A59V genotypes and milk, protein, and fat yields in Polish Holstein-Friesian (Black-and-White) cows were observed. These traits were significantly higher in the *CC* genotype cows compared with the *CT* and *TT* genotype ones. Associations between this polymorphism and milk production traits were also studied by other researchers in the Holstein-Friesian cattle. However, Liefers et al. (9) found no significant differences in milk, protein, and fat yields as well as in milk fat and protein percentage between cows with different A59V genotypes. On the other hand, Madeja et al. (14) reported associations between the A59V polymorphism and breeding value for milk and protein yields in Polish Holstein-Friesian bulls. The *TT* homozygous animals were found to have higher estimated breeding values for these traits.

No associations were found between the *Sau3AI* polymorphism and the milk traits analysed in the present study. Similarly, the Polish Holstein-Friesian cows analysed by Zwierzchowski et al. (10) showed no associations between the *Sau3AI* genotypes and milk yield, but the per cent content of some milk components depended on the genotype. On the other hand, no effect of this polymorphism on breeding value for milk

Table. Means and standard deviations of milk production traits in cows with different A59V and A59V/*Sau3AI* haplotypes (N – number of observations among the group in the first, second, and third lactations).

| Genotype | N | Trait | | | | | |
|---------------------|-----|--------------------------|----------------------------|----------------------------|----------------------------|-------------|----------------------------|
| | | Milk (kg) | Protein (kg) | Fat (kg) | Protein + Fat (kg) | Protein (%) | Fat (%) |
| A59V | | | | | | | |
| <i>CC</i> | 209 | 4585 ± 753 ^a | 180.6 ± 29.0 ^a | 257.1 ± 41.3 ^A | 417.6 ± 70.6 ^A | 3.96 ± 0.31 | 5.64 ± 0.55 |
| <i>CT</i> | 181 | 4589 ± 740 ^b | 180.8 ± 28.2 ^b | 261.8 ± 40.1 ^B | 425.8 ± 66.3 ^B | 3.94 ± 0.23 | 5.74 ± 0.57 |
| <i>TT</i> | 36 | 4412 ± 723 ^{ab} | 173.0 ± 25.2 ^{ab} | 246.5 ± 38.8 ^{AB} | 398.3 ± 64.6 ^{AB} | 3.94 ± 0.22 | 5.62 ± 0.53 |
| A59V/ <i>Sau3AI</i> | | | | | | | |
| <i>CC/CC</i> | 51 | 4496 ± 804 | 178.3 ± 29.2 | 256.9 ± 40.3 | 435.2 ± 66.3 | 3.97 ± 0.21 | 5.77 ± 0.63 |
| <i>CC/CT</i> | 120 | 4547 ± 698 | 180.2 ± 27.9 | 255.6 ± 42.0 | 435.8 ± 68.3 | 3.99 ± 0.36 | 5.64 ± 0.49 ^c |
| <i>CC/TT</i> | 38 | 4828 ± 818 | 185.2 ± 32.1 | 262.3 ± 41.0 | 447.5 ± 70.3 ^A | 3.83 ± 0.23 | 5.49 ± 0.58 ^A |
| <i>CT/CC</i> | 63 | 4474 ± 698 | 180.1 ± 29.8 | 267.7 ± 42.1 | 447.8 ± 69.4 ^{AB} | 4.00 ± 0.21 | 5.98 ± 0.55 ^{Abc} |
| <i>CT/CT</i> | 111 | 4659 ± 748 | 181.6 ± 26.7 | 258.4 ± 38.5 | 440.1 ± 62.7 | 3.91 ± 0.24 | 5.60 ± 0.55 ^b |
| <i>CT/TT</i> | 7 | 4530 ± 944 | 173.9 ± 39.1 | 263.5 ± 47.3 | 437.7 ± 86.0 | 3.83 ± 0.19 | 5.85 ± 0.33 |
| <i>TT/CC</i> | 34 | 4378 ± 703 | 171.5 ± 23.7 | 247.4 ± 39.3 | 406.4 ± 94.1 ^{AB} | 3.93 ± 0.22 | 5.68 ± 0.50 |

The means in columns marked with the same superscript letter differ significantly. Capital letters denote significance of difference at $P \leq 0.01$, whereas small letters denote significance of difference at $P \leq 0.05$.

production traits was found in Polish Holstein-Friesian bulls (14). Another study on Polish Holstein-Friesian cows showed associations between the *Sau3AI* genotypes and milk, protein, and fat yields (13); these traits were significantly higher in the *TT* genotype cows. All those studies covered a rare additional polymorphism recognised by *Sau3AI* restriction enzyme. On the other hand, the Holstein-Friesian cows analysed by Liefers et al. (9) showed a significantly higher daily milk yield for the *CT* genotype cows compared with the *CC* homozygote ones. In addition, the *CT* cows produced significantly more milk protein compared with the *CC* group.

Associations between the leptin *A59V/Sau3AI* haplotypes and milk performance were previously studied in Polish Holstein-Friesian cows by Kulig (15). The *CC/TT* genotype individuals were found to be characterised by statistically significantly higher milk, protein, and fat yields compared with cows with other genotypes. The fact that the results of our study differ from those reported by other authors may be explained by breed/population differences, the use of the rare additional *Sau3AI*

polymorphism in some studies or the presence of factors affecting milk production traits.

The present results suggest that the *A59V* genotypes may be used to improve milk, fat, and protein yield. Therefore, in the selection for the improvement of these traits, cows with allele *C* (*CT* or *CC* genotypes) should be preferred. Cows with the *A59V/Sau3AI CT/CC* haplotype may be helpful in improving milk fat content. Moreover, the results show that the *CC/TT* haplotype is associated with decreased milk fat content without any decrease in milk and protein yields. Thus, it seems reasonable to continue studies on associations between leptin genotypes and milk production traits in order to verify the above results before using them in dairy selection programmes.

Acknowledgment

This work was financially supported by the Westpomeranian University of Technology – project BW/IB/15/2004.

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