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The effects of polymorphisms in *DGAT1*, *GH* and *GHR* genes on reproduction and production traits in Jersey cows

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The aim of the study was to examine the impact of four single nucleotide polymorphisms in *DGAT1*, *GH* and *GHR* genes on reproduction, production and udder health in Jersey cattle. The study was conducted on 209 cows from the Polish active dairy population and genotypes were identified using the PCR-RFLP technique. The significant effects on certain of analysed traits were revealed of GH-L127V and GHR-F279Y polymorphisms. Replacement of the phenylalanine encoding T allele by the tyrosine encoding A variant at GHR-F279Y *locus* led to decrease in milk, fat and protein yields. The *GH*-L127V-CC genotype was found to be significantly associated with the shorter calving interval and shorter interval from calving to conception than the other two *GH* genotypes. No relations were found between *DGAT1*-K232A mutation and health or reproductive traits of cows.

KEY WORDS: cattle / *DGAT1* / *GH* / *GHR* / gene polymorphism / milk production traits / reproduction traits

During last decades, the genetic selection aiming solely at increased milk yield has led to decreased reproduction efficiency as well as greater incidence of *mastitis* and other diseases in dairy cattle. The declined health and fertility are thought to be associated with the negative energy balance (NEB) resulting from insufficient, for demands of high milk production, dietary energy intake [Reist *et al.* 2003, Wathes *et al.* 2007]. It seems justified to presume that genes affecting milk yield and composition

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may also alter the calorific demand for milk production and influence the severity and duration of NEB in early lactation. Thus, their polymorphism may be associated with the reproductive traits variation. The acyl-CoA:diacylglycerol acyltransferase (*DGAT1*) and growth hormone receptor (*GHR*) are examples of such genes.

DGAT1 encodes an enzyme playing the key role in synthesis of triacylglycerols, the major milk lipids [Farese *et al.* 2000]. An ApA to GpC dinucleotide substitution located in exon 8 of *DGAT1* that replaces positively charged lysine by neutral alanine at the 232th residue of the encoding protein (K232A polymorphism) has been proved to have a pronounced effect on milk- related traits in cattle, especially on production of fat, the most energetically expensive to synthesize component of milk [Grisart *et al.* 2002, Thaller *et al.* 2003, Sanders *et al.* 2006, Kaupe *et al.* 2007, Näslund *et al.* 2008]. An F279Y polymorphism (a nonconservative T to A replacement resulting in the phenylalanine to tyrosine change at position 279) in exon 8 of bovine *GHR* gene was also found to be significantly associated with milk yield and composition [Blott *et al.* 2003].

Growth hormone receptor largely determines the biological activity of growth hormone, the major regulator of mammalian metabolism, affecting growth rate, body composition and milk production [Etherton 2004]. Some effects of growth hormone are mediated by the insulin-like growth factor 1 (IGF1) derived from the liver in response to GH. IGF1 is a member of a major growth-promoting signalling system important for reproduction. In cattle, its concentration of blood was shown to be associated with age at puberty, twin ovulations, embryo development and *post-partum* ovarian activity [Velasquez *et al.* 2008].

The aim of this study was to examine an impact of four single nucleotide polymorphisms (SNPs) in *DGAT1*, *GH* and *GHR* genes, earlier reported to influence either milk production traits or serum IGF1 concentration, on reproductive, productive and udder health traits in Jersey cows. The summarized information on these SNPs is given in Table 1.

Table 1. Description of the single nucleotide polymorphisms in Jersey cows considered in this study

Gene	Chromosome	SNP code	Sequence polymorphism	Gene region	Reference
DGAT1	BTA14	K232A	AA/GC	exon 8	Grisart *et al.* [2002]
GH	BTA19	L127V	C/G	exon 5	Seavey *et al.* [1971]
GHR	BTA20	A(-154)G	A/G	promoter	Ge *et al.* [1999]
		F279Y	T/A	exon 8	Blott *et al.* [2003]

Material and methods

The study was performed on 209 Jersey cows from the active Polish dairy population, born between 2000 and 2003 and kept on the Siedlec farm belonging to

the Horse Stud Farm at Iwno, Poland. The animals considered were the progeny of 18 sires, the mean number of daughters by sire was 11.6, ranging from 1 to 61. The mean milk yield per cow per 305-day lactation in years 2003-2006 was 4717 kg with 5.4% and 3.9% of fat and protein, respectively.

DNA for molecular analyses was extracted from peripheral blood using the standard phenol chloroform procedure. Genotypes were determined with the PCR-RFLP method. Primers for PCR (Tab. 2) were established based on gene sequences available in the GenBank database (accession numbers: *DGAT1* – AY065621, *GH* – M57764, *GHR* – U15731 and X70041). Primer *GHR*-F279Y-R was earlier used by Blott *et al.* [2003]. The PCR reaction volume of 10 µl contained approximately 20-50 ng of genomic DNA, 0.5 units of Taq DNA polymerase (FERMENTAS, Lithuania), 1×PCR buffer with $(NH_4)_2SO_4$ (FERMENTAS, Lithuania), 2 mM $MgCl_2$, 5% DMSO (*DGAT1* – 7% DMSO), 1 µM of each primer (IBB PAS, Poland), and 200 µM of each dNTP (FERMENTAS, Lithuania). Thermal cycling conditions were as follows: 5 min at 94°C, 30 cycles of 94°C for 30 s, annealing temperature (Tab. 2) for 30 s, and 72°C for 40 s, followed by a final step of 72°C for 5 min. Amplified fragments were digested overnight with 5 units of respective (Tab. 2) restriction enzyme (FERMENTAS, Lithuania), and next subjected to electrophoretic separation in 3% ethidium bromide-stained agarose gel (Basica LE GQT, Prona, Spain).

Nucleotide sequence around polymorphism located in the promoter region of the GHR gene was analysed for the presence of putative transcription factor (TF) binding sites. A search for consensus matches was

Table 2. Selected PCR-RFLP conditions for the polymorphisms analysed in Jersey cows considered in this study

SNP	Primers (5'-3')	Annealing temp. (°C)	PCR product size (bp)	Restriction endonuclease	Digestion product size (bp)
DGAT1-K232A	F – TGCCGCTTGCTCGTAGCTTTGGCC* R – ACCTGGAGCTGGGTGAGGAACAGC	58.5	378	*Bgl*I	AA (K) – 282, 96 GC (A) – 254, 96, 28
GH-L127V	F – TAGGGGAGGGTGGAAAATGGA R – GACACCTACTCAGACAATGCG	57.0	404	*Alu*I	C (L) – 236, 132, 36 G (V) – 185, 132, 51, 36
GHR-A(-154)G	R - CTGGCGTATGGTCTTTGTCA R – CGTCATGTATGGAAGCAGAC	57.5	434	*Mph*1103I	A – 434 G – 269, 165
GHR-F279Y	F – ACTTGGGCTAGCAGTGACATTAA* R – ACTGGGTTGATGAAACACTTCACTC	57.5	180	*Vsp*I	T (F) – 180 A (Y) – 160, 20

*A mismatch incorporating the restriction site to a sequence intentional.

performed with the use of the Genomatix MatInspector software (www.genomatix.de).

Effects of four analysed SNPs on reproductive traits and udder health were assessed with the GLM procedure of the SAS software (SAS Institute Inc. 2002-2005). The statistical model included effects of sire, SNP (*DGAT1*, *GH* or *GHR*) genotype as well as year and season of calving. Additionally, the allele substitution effects were estimated by regressing the number of copies of SNP allele against the analysed trait value.

Data on milk yield (MY, kg), fat yield (FY, kg), fat percentage (FP, %), protein yield (PY, kg), protein percentage (PP, %), age at first calving (AFI, days), number of inseminations per conception (INC, inseminations), calving interval (CI, days), interval from calving to conception (ICC, days), gestation length (GL, days) and somatic cell count (SCC, cells per microl) were withdrawn from the farm records. Since SCC does not present the normal distribution, the trait was transformed according to the formula: SCS=log2 (SCC/100) + 3, where: SCS – somatic cell score after transformation, and SCC – somatic cell count from direct measurement.

Out of 209 cows considered in the study, 128 possessed phenotype records for at least three calvings, whereas data from two or only one calving were available for the remaining 56 and 25 animals, respectively.

All investigations followed the requirements of ethics and were approved by the Local Ethics Commission for Investigation on Animals (permission No. 25/2008).

Results and discussion

Frequencies of genotypes and alleles obtained in this study (listed in Table 3) were similar to those previously reported for the *DGAT1* [Spelman *et al.* 2002, Winter *et al.* 2002, Suchocki *et al.* 2010], *GH* [Lucy *et al.* 1993, Sørensen *et al.* 2002] and *GHR* [Blott *et al.* 2003] gene polymorphisms in Jersey cattle. For each *locus*, all three possible genotypes were identified. However, the considerable discrepancy in *DGAT1*-K232A and *GHR*-F279Y allele frequencies resulted in a very low number of GC/GC (10 cows) and A/A (8 cows) homozygotes, respectively. These genotypes were excluded from the association analysis. Genotypes in all analysed *loci* were distributed according to the Hardy-Weinberg equilibrium.

Table 3. Genotype and allele frequencies for *DGAT1*, *GH* and *GHR* in Jersey cows considered in this study

SNP	Genotype frequencies			Allele frequencies	
DGAT1-K232A	AA/AA – 0.62	AA/GC – 0.33	GC/GC – 0.05	AA – 0.79	GC – 0.21
GH-L127V	CC – 0.31	CG – 0.44	GG – 0.25	C – 0.53	G – 0.47
GHR-A(-154)G	AA – 0.12	AG – 0.50	GG – 0.38	A – 0.37	G – 0.63
GHR-F279Y	TT – 0.74	TA – 0.23	AA – 0.04	T – 0.85	A – 0.15

A computer analysis of the bovine *GHR* gene promoter sequence fragment with the MatInspector programme has not shown any potential transcription factor binding sites at position of the A(-154)G polymorphism. Thus, the A(-154)G is unlikely to be a causative mutation underlying phenotype variation.

Significant effects of investigated SNPs on production and functional traits in Jersey cows are presented in Table 4. Summarizing, the additive effects of *GH* and *GHR* genes on milk-related traits were significant only for the *GHR*-F279Y polymorphism. Replacement of the phenylalanine encoding T allele by the tyrosine encoding A variant led to decrease in milk, fat and protein yields. Effects previously estimated by Blott *et al.* [2003] and Viitala *et al.* [2006] for the same mutation in Holstein-Friesian (HF), Jersey and Ayrshire cattle were a bit different. In their research, F279Y most pronouncedly influenced protein and fat percentages, whereas yields of these components were only hardly affected. Moreover, the TT (FF) genotype was characterized by the lowest milk production compared to both TA (FY) and AA (YY) genotypes. Dissimilarities in results obtained might arise from the low number of animals analysed in this study and from excluding the very small AA genotype group from the association test.

Table 4. Least squares means (± SE) for productive and non-productive traits in Jersey cows considered in this study of different *GHR* or *GH* genotypes and regression coefficients for the number of copies of the *GHR*-F279Y^{T} or *GH*-L127V^{C} allele representing half of the allele substitution effects (± SE)

Trait	Genotype effects			Allele substitution effects
	GHR-F279Y			
	TT n=154	TA n=47		α/2
MY	4424.14^{a} (±703.85)	4135.48^{a} (±643.83)		-211.35 (±86.61)*
FY	244.32^{a} (±39.48)	232.67^{a} (±34.43)		-10.28 (±4.96)*
PY	169.88^{a} (±25.78)	161.29^{a} (±20.05)		-7.54 (±3.17)*
	GH-L127V			
	CC n=65	CG n=92	GG n=52	α/2
CI	366.12^{aA} (±42.35)	378.52^{a} (±55.30)	373.25^{A} (±49.11)	22.46 (±6.85)**
ICC	85.08^{aA} (±41.26)	100.47^{a} (±56.62)	99.10^{A} (±56.49)	23.34 (±6.79)**

MY – milk yield, FY – fat yield, PY – protein yield, CI – calving interval, ICC– interval from calving to conception

$^{aA...}$Means bearing the same superscript letter differ at: small letters – P≤0.05; capitals – P≤0.01.

*Allele substitution effects significant at P≤0.05.

**Allele substitution effects significant at P≤0.01.

No relations were found between *GHR* gene polymorphisms and non-productive traits. However, the *GH*-L127V-CC genotype was found to be significantly associated with the shorter calving interval (CI) and shorter interval from calving to conception (ICC) compared to two other *GH* genotypes. The leucine encoding C allele was earlier reported to increase the plasma concentration of IGF1 significantly [Grochowska *et al.* 2001, Katoh *et al.* 2008], that plays an important role in fertility. IGF1 has been

demonstrated to influence the ovarian follicular growth [Diskin *et al.* 2003]. It was also found to be required for the estradiol-induced release of gonadotropins in brain [Etgen *et al.* 2006] as well as to mediate the estradiol-induced uterine epithelial cell proliferation [Zhu and Pollard 2007].

The positive effect of L127V-C allele on CI and ICC might, therefore, be due to the more efficient binding of growth hormone leucine variant to the hepatic GH receptors, resulting in higher IGF1 release. On the other hand, certain reports do not support the association between L127V and reproductive traits in cattle. No effect was found of the interval from calving to first ovulation [Balogh *et al.* 2009] as well as for age at first calving and calving interval [Kovács *et al.* 2006].

Effects of *DGAT1* on milk production traits in Jersey cows population were estimated earlier by authors of the present study [Komisarek *et al.* 2004, Suchocki *et al.* 2010]. The lysine encoding AA allele was found to increase fat yield as well as fat and protein contents significantly, simultaneously reducing milk and protein yields, that was in accordance with results obtained in most other dairy cattle breeds and populations [Grisart *et al.* 2002, Spelman *et al.* 2002, Kaupe *et al.* 2007, Näslund *et al.* 2008]. Recently, some reports suggesting the relation between *DGAT1*-K232A and fertility traits have been published [Kaupe *et al.* 2007, Komisarek and Michalak 2008, Oikonomou *et al.* 2009]. The unfavourable impact of the AA allele on non-return rate, number of inseminations per conception, conception rate, and presence of reproductive problems was identified in German, Polish and Greek Holstein-Friesians. AA variant, proved to improve the milk fat production, might negatively affect fertility through influencing the degree of NEB during early lactation. Some unfavourable correlations between the *DGAT1*-AA allele and total body energy content, BCS condition, blood glucose and NEFA concentrations in Holstein-Friesian cows found by Oikonomou *et al.* [2009] support this hypothesis. In Jerseys, impact of *DGAT1*-K232A polymorphism on reproduction was not earlier tested, whereas its effect on fat yield was shown to be less pronounced than in Holstein-Friesian cattle [Spelman *et al.* 2002]. In the present study, no K232A associations with fertility traits were revealed.

The results reported in this paper suggest that polymorphism *GHR*-F279Y in Jersey cattle may affect the variation of milk yield as well as milk fat and protein yields. Moreover, *GH*-L127V mutation may be associated with lengths of calving interval and interval from calving to conception.

Results of this study also indicate that polymorphism of *DGAT1* gene seems to have no influence on functional traits in Jersey cows.

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