Single nucleotide polymorphism (SNP) in the 5′-noncoding region of the bovine growth hormone receptor gene and its association with dairy production traits in Polish Black-and-White cattle

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ABSTRACT: The effects of cow's genotype for growth hormone receptor (*GHR*) were determined on milk production traits of the Polish Black-and-White (BW) cattle. It was shown that *GHR* genotypes significantly influenced most of the dairy traits studied. Cows of the RFLP-*Nsi*I –/– genotype of *GHR* produced more milk with higher content of milk components, including fat, protein, and lactose than those with +/+ genotype. The heterozygous +/– genotype at RFLP-*Acc*I appeared superior with respect to two milk composition parameters – gross energy and total solids. The combined *GHR* genotypes (CGGs) 2213 and 1113 were clearly favourable for most traits under study. Cows carrying the 2213 genotype combination produced daily more fat corrected milk, fat, protein and lactose than other genotypes, and the milk of 2213 and 1113 cows contained significantly more total solids, protein, and fat.

Keywords: cattle; *GHR*; gene; polymorphism; dairy traits

In dairy cattle populations QTL influencing milk production traits are increasingly elucidated (Boichard, 1998; Grisart *et al*., 2004). Recently, single nucleotide polymorphisms (SNPs) have gained high popularity in genetic studies due to their high accuracy and reproducibility. Moreover, they are indispensable for the generation of positional candidate genes (Vignal *et al*., 2002). Studying candidate gene polymorphisms in exons or other important regions, such as promoters has to be done using SNP approach. Because of their abundance, SNPs have a high potential for application in association studies. Hormones, growth factors, and other regulatory proteins associated with so called "somatotropic axis" are candidate markers for quantitative traits in farm animals (Parmentier *et al*., 1999).

The *GHR* gene is encoded in cattle, by a single gene located on chromosome 20 (Moody *et al*., 1995). The gene coding for bovine *GHR* consists of 9 exons (from 2 to 10) in the translated part and of a long 5′-noncoding region that includes 9 untranslated exons – 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I (Jiang and Lucy, 2001). These exons are spliced alternatively producing transcripts with different 5′-UTR regions. A LINE-1 element 1,206 bp-long, from the family of retrotransposons, was found upstream from promoter P1 preceding exon 1A (Lucy *et al*., 1998).

Several polymorphic sequences have been identified in the bovine *GHR* gene (Falaki *et al*., 1996; Moisio *et al*., 1998; Chrenek *et al*., 1998; Aggrey *et al*., 1999; Ge *et al*., 1999; Hale *et al*., 2000). We found a new RFLP in the bovine *GHR* gene at *Fnu*4HI/*Tse*I site (Maj and Zwierzchowski, 2002). The C/T transition was determined by sequencing at the position –1104. Two alleles and three genotypes were

Supported by State Committee for Scientific Research (KBN), Grants PBZ-KBN-036/P06/12, 3 P06D 025 25 and by IGAB PAS, Projects S.I.-2.2 and S.V.-I.

identified within the analysed populations of dairy and beef cattle. It was shown that the newly found RFLP-*Fnu*4HI/*Tse*I polymorphic site and previously identified polymorphic sites for restriction enzymes *Alu*I and *Acc*I are located within the 1,206 bp LINE-1 element. Sequencing of the 5′-noncoding region of the *GHR* gene (Maj and Zwierzchowski, GenBank accession No. AY249137) enabled the identification of additional nucleotide substitutions. All of them were located within the P1 promoter for exon 1A : G/C at position –475, C/T (–262), and C/T (–344). The two C/T substitutions were previously reported by Hale *et al*. (2000) as characteristic of *B. indicus* species.

From previous publications it is known that some productive traits of cattle, e.g. milk yield and composition, are correlated with polymorphism of *GHR* (Falaki *et al.,* 1996; Aggrey *et al*., 1999). Significant differences in the ligand binding parameters to the liver *GHR* were found between dairy and beef breeds (Grochowska *et al*., 2002); B_{max} (receptor binding capacity) was greater in Friesians as compared to beef breeds, while K_d (ligand dissociation constant) revealed the lowest figure in the dairy breed. A difference was also found in the K_d value between RFLP-*Alu*I and –*Acc*I genotypes within the 5′-flanking region of bovine *GHR* gene in Polish Friesians.

Test-day (TD) models, in which records from individual test days are used to determine lactation production, have recently gained considerable interest because they are flexible in handling records from different recording schemes (Swalve, 2000).

The objective of this study was to examine the effect of polymorphism in the 5′-noncoding region of the bovine *GHR* gene on the traits related to milk production in cattle. Four different single nucleotide polymorphisms (SNPs) were analysed, including one recently identified by the authors – the RFLP-*Fnu*4HI. In addition, effects of the combined *GHR* genotypes were estimated.

MATERIAL AND METHODS

Animals

One hundred forty-three Polish Black-and-White cows, with more than 80% of Holstein-Friesian (HF) gene pool, were used for GHR genotyping. The cows were maintained at the Polish Academy of Sciences Experimental Farm, Jastrzębiec. Cows were daughters of 48 bulls.

The cows were kept in loose barn and fed *ad libitum* with total mixed ration based on maize silage, wilted grass silage, and concentrates supplemented with minerals and vitamins. Cows were milked twice a day. Milk from both milkings was measured for every cow and milk samples were collected once a month. Average yield in the herd rose from 7 142 kg milk with 4.34% fat and 3.51% protein per lactation in the first year to 7 862, 3.98 and 3.41 in the third year, respectively. Milk data was collected throughout three consecutive lactations for every cow. The dataset had 2 217 test-day results for 129 cows, daughters of 46 sires.

All procedures carried out with the use of animals were approved by the Local Ethics Commission, Permission No. 67/2001.

DNA isolation from whole blood

An authorized veterinarian collected blood for isolation of DNA from the jugular vein. Blood was collected on K_2-EDTA and stored at –25°C for a few weeks or at –75°C up to several months. The isolation of DNA from whole blood was done using a rapid method described by Kanai *et al.* (1994).

Determination of GHR polymorphism

The RFLPs at *Alu*I and *Acc*I sites were estimated as previously described (Grochowska *et al*., 2002). To genotype *GHR* gene at *Fnu*4HI site the following primers were used to amplify the 836-bp fragment (–1866 to –1031 nt): forward – 5′-TGCGTGCACAGCAGCTCAACC-3′; reverse – 5′-AGCAACCCCACTG-CTGGGCAT-3′. The PCR was performed in a volume of 12 µl using 1.4 µl (approx. 100 ng) template DNA, 0.25 µM primers, PCR buffer (50 mM KCl; 10 mM Tris-HCl, pH 8.0; 1.5 mM $MgCl_2$), 2.5 mM dNTPs, and 1 unit of *Taq* polymerase (InGen, Sieradz, Poland). Amplification was carried out for 39 cycles: 95°C for 30 s, 69°C for 45 s, and 72°C for 120 s. The amplified DNA was digested for 3 hours at 37°C with 5 units of *Fnu*4HI restriction nuclease (BioLabs, New England, USA). The digested DNA fragments were then separated by electrophoresis in 2% agarose (Gibco, BRL, England) in 1 × TBE buffer (0.09 M Tris-boric acid, 0.002 M EDTA) with 0.5 mg/ml ethidium bromide (Et-Br) added to the gels, visualized under UV light, and scanned in FX Molecular Imager apparatus (Bio-Rad).

Based on the available sequences of the bovine *GHR* gene (GenBank; AF126288 and U15731), and using the Primer3 software (www.genome.wi.mit.edu), primers for the analysis of RFLP polymorphism at *Nsi*I site were designed: forward – 5′-CTGGCGTATGGTCTTTGTCA-3′; reverse – 5′-TGGTCTTGCTGCTTTC-CTAT-3′. The composition of PCR-mix was the same as for genotyping of *Fnu*4HI. Amplification was carried out for 35 cycles: 95°C for 20 s, 66°C for 30 s, and 72°C for 40 s. A 318-bp DNA fragment was amplified, position –319 to –2 in the *GHR* gene promoter P1, and digested with *Nsi*I nuclease. The restriction products were analysed electrophoretically.

The following symbols of alleles are used in this paper: RFPL-*Alu*I – (–) or (+) for the allele non-cut or cut by the enzyme, with T or A at pos. –1177, respectively; RFPL-*Acc*I – (–) or (+) for the non-cut or cut allele, with nucleotide T or C at pos. –887; RFPL-*Fnu*4HI – (–) or (+) for the non-cut or cut allele, with nucleotide T or C at pos. –1104; RFPL-*Nsi*I – (–) or (+) for the non-cut or cut allele, with nucleotide A or G at pos. –154.

Analysis of milk composition

The fat, protein and lactose content in milk samples was estimated in fresh milk using Milko Scan 104A/B, and somatic cells (SCC) were counted with Fossomatic apparatus. Percentage of total solids in each milk sample, expressed as a sum of percentage fat, total protein, lactose and minerals and the non-fat solids, were calculated as total solids minus fat. The concentration of minerals was calculated according to the following equation elaborated by Sherman: $P = 0.1 \times$ percent of total protein +0.38.

Statistical analysis

Data were analysed by univariate linear repeatability mixed models. Effect of the test-day, lactation number and stage of lactation were found to be significant in preliminary analyses. As an interaction between lactation number and lactation stage was proved to be significant, in final analyses lactation curves for considered traits were fitted within parity.

The model used for all traits and all kinds of considered polymorphism was:

$$y_{ijklmn} = Gen_i + parity_j + \sum_{r=1}^{ur} b_r LP_r + t\text{-}day_k + \text{sire}_l + cow_m + cow \times parity_{mj} + e_{ijklmn}$$

where: y = the individual measure of considered trait on given test-day

The fixed effects in the model were:

Gen – the considered polymorphism (with either 3 or 9 levels for single polymorphism or combined genotype, respectively) and 3 *parity* subclasses.

Table 1. Frequency of alleles and genotypes of growth hormone receptor gene 5′-region in a studied group of Polish Black-and-White dairy cattle

Polymorphism	Genotype	Number of animals (genotype frequency)	Allele frequency
	+/+	54 (0.38)	(+) 0.60
RFLP-*Alu*I	+/–	65 (0.45)	(–) 0.40
	–/–	24 (0.17)	
	+/+	85 (0.59)	(+) 0.77
RFLP-*Acc*I	+/–	51 (0.36)	(–) 0.23
	–/–	7 (0.05)	
	+/+	107 (0.75)	(+) 0.87
RFLP-*Fnu*4HI	+/–	34 (0.24)	(–) 0.13
	–/–	2 (0.01)	
	+/+	30 (0.21)	(+) 0.39
RFLP-*Nsi*I	+/–	51 (0.36)	(–) 0.61
	–/–	62 (0.43)	

Table 2. Least squares means (LSM) and their standard errors (SE) for daily milk yield, milk energy and per cent of major milk constituents as referred to RFLP-*Nsi*I, -*Fnu*4HI, -*Alu*I, and -*Acc*I genotype

Variation sources	*n**	Milk (kg)		Fat-corrected milk (kg)		Gross energy (MJ/l)		Total solids (%)		Solids-non-fat (%)		Fat (%)		Protein (%)		Lactose (%)	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
RFLP-*Nsi*I genotype																	
+/+ – 28	412	18.44	1.04	18.63^{A}	1.00	3.25^{A}	0.05	13.21^{A}	0.15	8.99^{A}	0.06	4.22^{A}	0.11	3.46^{A}	0.04	4.79^{A}	0.03
+/– – 43	763	18.75	1.00	19.31^{AB}	0.97	3.33^{AB}	0.04	13.49^{AB}	0.13	9.15^{B}	0.05	4.35^{AB}	0.09	3.52^{AB}	0.04	4.89^{B}	0.02
–/– – 58	1 042	18.83	0.99	19.61^{B}	0.95	3.35^{B}	0.04	13.56^{B}	0.12	9.16^{B}	0.05	4.40^{B}	0.09	3.55^{B}	0.03	4.86^{B}	0.02
RFLP-*Fnu*4HI genotype																	
+/+ – 96	1 614	18.84	0.97	19.41	0.94	3.32	0.04	13.45	0.11	9.12	0.04	4.34	0.08	3.52	0.03	4.86	0.02
+/– – 30	554	18.52	1.20	19.12	0.99	3.32	0.05	13.45	0.15	9.10	0.06	4.35	0.11	3.51	0.04	4.85	0.03
RFLP-*Alu*I genotype																	
+/+ – 47	841	18.38	1.00	19.18	0.96	3.34	0.04	13.53	0.13	9.16	0.05	4.38	0.09	3.55	0.04	4.86	0.02
+/– – 59	991	19.03	0.99	19.56	0.96	3.33	0.04	13.46	0.12	9.10	0.05	4.37	0.09	3.51	0.03	4.85	0.02
–/– – 23	385	18.69	1.06	19.02	1.02	3.28	0.05	13.33	0.17	9.12	0.07	4.23	0.12	3.51	0.05	4.87	0.03
RFLP-*Acc*I genotype																	
+/+ – 77	1 329	18.59	0.98	19.09	0.95	3.32^{AB}	0.04	13.44^{AB}	0.11	9.11	0.04	4.33	0.08	3.52	0.03	4.86	0.02
+/– – 45	788	18.84	1.00	19.64	0.97	3.35^{A}	0.04	13.56^{A}	0.13	9.16	0.05	4.41	0.09	3.54	0.04	4.87	0.02
–/– – 7	100	19.43	1.26	19.64	1.21	3.20^{B}	0.09	13.05^{B}	0.27	8.98	0.11	4.09	0.18	3.46	0.08	4.79	0.05

*number of milk samples analysed

^{aA} means within columns and variation sources bearing different letters are significantly different at: small letters – $P \leq 0.05$; capitals – $P \leq 0.01$

Table 3. Least square means (LSM) and their standard errors (SE) for daily yield of major milk constituents as referred to RFLP-*Nsi*I, -*Fnu*4HI, -*Alu*I, and -*Acc*I genotype

Variation source	*n**	VCM (kg)**		Solids-non-fat (g)		Total solids (g)		Fat (g)		Protein (g)		Lactose (g)	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
RFLP-*Nsi*I genotype													
+/+ – 28	412	22.16^{A}	1.25	1 652	93	2 402^{A}	132	749^{A}	41	638	37	879	49
+/– – 43	763	22.91^{AB}	1.21	1 704	90	2 493^{AB}	127	788^{AB}	39	655	36	913	47
–/– – 58	1 042	23.31^{B}	1.19	1 714	89	2 520^{B}	126	805^{B}	38	665	36	912	46
RFLP-*Fnu*4HI genotype													
+/+ – 96	1 614	23.07	1.18	1 709	88	2 501	124	791	38	660	35	912	45
+/– – 30	554	22.69	1.23	1 677	92	4 459	131	780	40	648	37	894	48
RFLP-*Alu*I genotype													
+/+ – 47	841	22.77	1.20	1 674	90	2 463	127	787	39	649	36	890	47
+/– – 59	991	23.17	1.20	1 722	89	2 519	126	796	39	663	36	920	46
–/– – 23	385	22.77	1.26	1 695	94	2 465	134	769	42	656	37	903	50
RFLP-*Acc*I genotype													
+/+ – 77	1 329	22.74	1.19	1 687	88	2 464	125	776	38	652	35	899	46
+/– – 45	788	22.23	1.21	1 711	90	2 519	127	807	39	661	36	912	47
–/– – 7	100	23.32	1.47	1 745	111	2 536	157	790	51	671	43	933	60

*number of milk samples analysed

**VCM – 0.05 × daily milk (kg) + 8.66 × fat (kg) + 25.98 x protein (kg), Arbel *et al.* (2001)

^{Aa}means within columns and variation sources bearing different letters are significantly different at: small letters – $P \leq 0.05$; capitals – $P \leq 0.01$

The *LPs* are Legendre polynomials of standardised days-in-milk (days in lactation), which were fitted as fixed covariates within each of *parity* subclasses, in order to represent changes in considered traits due to the stage of lactation. Fixed regressions were fitted up to the 5th power of Legendre polynomials ($r = 1, \ldots, 5$). Legendre polynomials are commonly used for test-day models and were shown to be better than other polynomials such as logarithmic polynomials (e.g. Kettunen *et al.*, 2000).

The effect of date of test day (*t-day*), with 50 levels, was considered as random. Animal effects were *cow*, cow-by-parity (*cow* × *parity*) as specific effect of *n*th cow in its *j*th lactation, and *sire* of a cow. The size of the dataset was too small to consider genetic variance in analyses, thus genetic relationships between animals were ignored. All random effects were assumed to be uncorrelated and to follow a normal distribution.

For combined *GHR* genotype (CGG) only most frequent genotypes (carried by more than 5 animals) were considered. As a consequence, the analyses were done based on 1 467 records from 70 cows, daughters of 32 sires. Procedure MIXED in SAS (SAS Institute Inc., 1999) was used for computations.

The expected frequencies of *GHR* genotypes and their combinations were calculated by simple allele counting (Falconer and Mackey, 1996).

RESULTS

Frequencies of genotypes and alleles of growth hormone receptor (*GHR*) gene in the studied group of Polish Black-and-White cattle are shown in Table 1.

The individual effects of *GHR* genotypes on the milk yield and composition are shown in Tables 2 and 3. Cows of the RFLP-*Nsi*I –/– and +/– genotype of *GHR* produced significantly more fat corrected milk (FCM) and gross energy than +/+ homozygotes, and their milks contained more solids, fat, and lactose. Moreover, the daily yield of all milk constituents tested – VCM, total solids, and fat were higher ($P \leq 0.01$) in milk of cows with –/– genotype than of those with +/+ genotype (Table 3). No effects of RFLP-*Fnu*4HI and RFLP-*Alu*I genotypes were shown on milk yield or composition

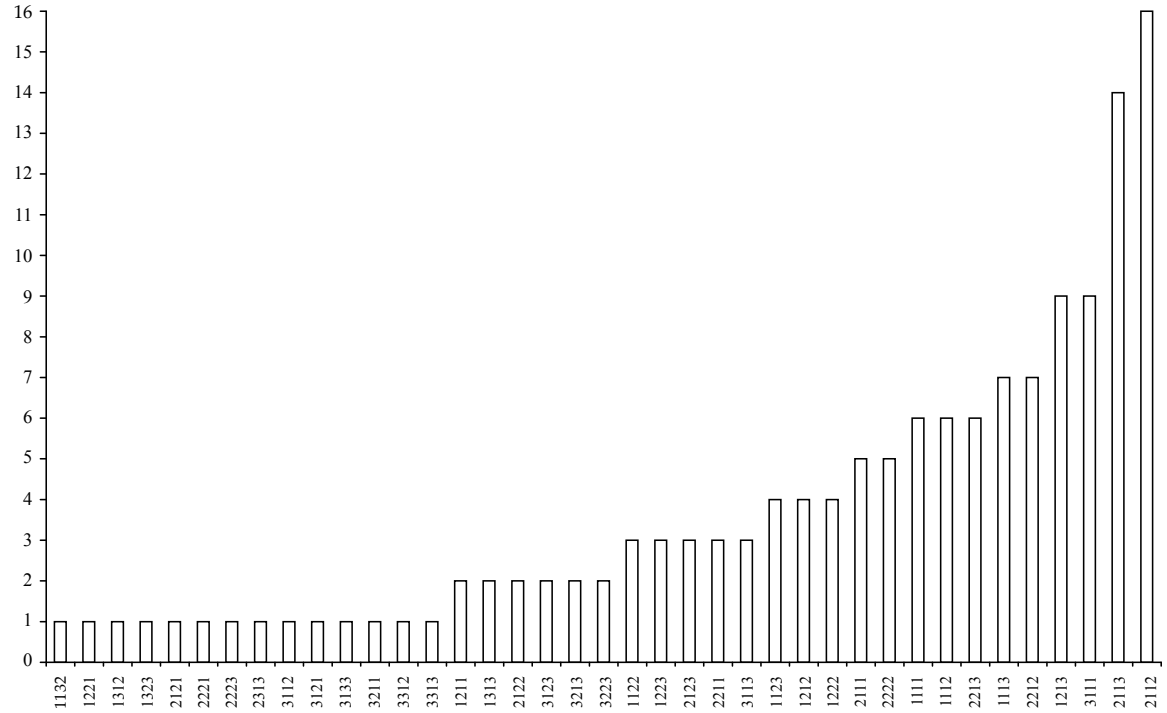

Figure 1. Frequency of combined genotypes within bovine *GHR* gene 5′ region. Out of 81 theoretically possible combinations of 4 individual genotypes (3^4), only 39 were identified in the studied group of animals. For combined genotype symbols see Table 4

Table 4. Observed and expected numbers of the combined genotypes (haplotypes) within bovine *GHR* gene 5′-region

Combined *GHR* genotype*	Expected frequency	Number of animals		Combined *GHR* genotype*	Expected frequency	Number of animals	
		Observed	Expected			Observed	Expected
1111	0.0239	6	3.4	2123	0.0241	3	3.4
1112	0.0765	6	10.9	2211	0.0189	3	2.7
1113	0.0589	7	8.4	2212	0.0605	7	8.7
1122	0.0234	3	3.3	2213	0.0466	6	6.7
1123	0.0181	4	2.6	2221	0.0058	1	0.8
1132	0.0020	1	0.3	2222	0.0185	5	2.6
1211	0.0142	2	2.0	2223	0.0143	1	2.0
1212	0.0454	4	6.5	2313	0.0067	1	1.0
1213	0.0350	9	5.0	3111	0.0106	9	1.5
1221	0.0043	1	0.6	3112	0.0340	1	4.9
1222	0.0139	4	1.9	3113	0.0262	3	3.7
1223	0.0107	3	1.5	3121	0.0033	1	0.5
1312	0.0065	1	0.9	3123	0.0080	2	1.1
1313	0.0050	2	0.7	3133	0.0007	1	0.1
1323	0.0015	1	0.2	3211	0.0063	1	0.9
2111	0.0319	5	4.6	3213	0.0155	2	2.2
2112	0.1020	16	14.6	3223	0.0048	2	0.7
2113	0.0786	14	11.2	3312	0.0029	1	0.4
2121	0.0098	1	1.4	3313	0.0022	1	0.3
2122	0.0313	2	4.5				

*The following numerical symbols were used for the GHR genotypes in Tables 4, 5, 6 and in Figure 1: 1 – homozygote +/+ (cut with the respective restriction nuclease); 2 – heterozygote +/– (one allele cut with the respective restriction nuclease); 3 – homozygote –/– (non-cut with the respective restriction nuclease). Consecutive numbers in the haplotype symbol indicate genotypes at GHR/*Alu*I, *Acc*I, *Fnu*4HI, and *Nsi*I restriction sites, respectively. For example, the combined genotype (haplotype) 1232 symbolises RFPL-*Alu*I +/+ with A at pos. –1 177; RFPL-*Acc*I +/– with nucleotides T/C at pos. –887; RFPL-*Fnu*4HI –/– with nucleotide T at pos. –1 104; RFPL-*Nsi*I +/– with nucleotides A/G at pos. –154

(Tables 2 and 3). The heterozygotes carrying +/– genotype at RFLP-*Acc*I site appeared better than homozygotes with respect to two milk composition parameters (Table 2). They produced daily more gross energy, and their milk contained more total solids than milks of cows with homozygous +/+ and –/– genotypes.

Associations were studied between the combined *GHR* genotypes (CGG) and milk production traits. For the practical reasons, the numerical symbols were used to identify combined genotypes in the bovine *GHR* gene 5′-region. Out of 81 theoretically possible combinations of 4 individual genotypes (3^4), only 39 were identified in the studied group of animals (Table 4 and Figure 1). More than a half of possible genotype combinations was missing and most of the found CGGs clearly escaped the Hardy-Weinberg equilibrium (Table 4). In most cases the actual numbers of animals were higher than expected (e.g. CGGs 1111, 1323, 3111, and 3133). The present results suggest the existence of preferable intragenic haplotypes within the bovine *GHR locus*. Out of the nine combined genotypes used in calculations eight appeared to have statistically significant effects on the traits under study (Tables 5 and 6). In particular, the CGGs 2213 (*Alu*I, +/–; *Acc*I, +/–; *Fnu*4HI, +/+; *Nsi*I, –/–) and 1113 (*Alu*I, +/+; *Acc*I, +/+; *Fnu*4HI, +/+; *Nsi*I, –/–) clearly appeared favourable for most traits under study. For example, cows carrying combined

Table 5. Least squares means (LSM) and their standard errors (SE) for daily milk yield, milk energy and per cent of major milk constituents as referred to combined *GHR* genotype (CGG)

Variation sources		*n**	Milk (kg)		Fat-corrected milk (kg)		Gross energy (MJ/l)		Total solids (%)		Solids-non-fat (%)		Fat (%)		Protein (%)		Lactose (%)	
			LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Combined GHR genotype (CGG)	CGG-1111	80	18.05	1.30	18.44^{A}	1.30	3.33	0.09	13.45	0.27	9.10	0.11	4.35	0.19	3.56	0.08	4.79^{A}	0.05
	CGG-1112	73	19.26	1.49	19.56	1.39	3.31	0.10	13.44	0.30	9.18	0.13	4.27	0.21	3.56	0.09	4.89	0.05
	CGG-1113	113	18.58	1.44	20.30	1.33	3.51^{A}	0.10	14.01^{A}	0.30	9.27	0.12	4.75^{AC}	0.21	3.62^{ac}	0.09	4.90	0.05
	CGG-1213	173	19.45	1.23	20.12^{AB}	1.14	3.36	0.07	13.57	0.21	9.14	0.09	4.45	0.15	3.49	0.06	4.91^{BC}	0.04
	CGG-2112	261	19.25	1.14	19.74	1.06	3.34	0.06	13.49	0.17	9.11	0.07	4.39	0.12	3.49	0.05	4.88	0.03
	CGG-2113	212	18.62	1.17	19.06^{AC}	1.09	3.34	0.06	13.50	0.19	9.10	0.08	4.41	0.13	3.53	0.05	4.83^{A}	0.03
	CGG-2212	74	19.37	1.50	19.02^{A}	1.38	3.18^{B}	0.10	13.09^{B}	0.31	9.11	0.13	4.00^{B}	0.22	3.41^{bc}	0.09	4.97^{B}	0.06
	CGG-2213	99	20.56	1.36	21.53^{B}	1.27	3.46^{A}	0.08	13.87^{C}	0.26	9.24	0.11	4.64^{C}	0.19	3.63^{a}	0.08	4.86	0.05
	CGG-3111	97	18.29	1.36	18.00^{C}	1.27	3.19^{B}	0.08	13.05^{B}	0.25	8.98	0.11	4.08^{B}	0.18	3.40^{b}	0.07	4.84^{AC}	0.05

*number of milk samples analysed

^{aA}means within columns and variation sources bearing different letters are significantly different at: small letters – $P \leq 0.05$; capitals – $P \leq 0.01$

Table 6. Least square means (LSM) and their standard errors (SE) for daily yield of major milk constituents as referred to combined GHP genotype

Variation source		n*	VCM (kg)**		Solids-non-fat (g)		Total solids (g)		Fat (g)		Protein (g)		Lactose (g)	
			LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Combined GHR genotype (CGG)	CGG-1111	80	22.31^{A}	1.56	1 637^{a}	122	2 384^{A}	170	746^{BC}	53	645^{a}	47	860^{a}	67
	CGG-1112	73	23.78	1.65	1 767	130	2 560	181	789	57	689	49	937	71
	CGG-1113	113	23.09	1.59	1 718	125	2 576	174	856^{AD}	54	674	48	907	69
	CGG-1213	173	23.62	1.40	1 761	108	2 586	151	823^{ACE}	46	672	42	948	58
	CGG-2112	261	23.30^{A}	1.31	1 743	101	2 547	141	802^{CD}	42	666^{a}	39	937	54
	CGG-2113	212	22.75^{A}	1.34	1 685^{a}	103	2 460^{A}	144	772^{BCD}	44	653^{a}	40	895	55
	CGG-2212	74	22.58^{A}	1.65	1 748	130	2 500	180	748^{BC}	56	656	49	953	71
	CGG-2213	99	25.60^{B}	1.52	1 875^{b}	119	2 762^{B}	166	883^{A}	51	729^{b}	46	994^{b}	65
	CGG-3111	97	21.63^{A}	1.52	1 640^{a}	119	2 354^{A}	166	707^{BE}	52	629^{a}	46	878	65

*number of milk samples analysed; **VCM – 0.05 × daily milk (kg) + 8.66 × fat (kg) + 25.98 × protein (kg), (Arbel *et al.*, 2001)

^{aA}means within columns and variation sources bearing different letters are significantly different at: small letters – $P \leq 0.05$; capitals – $P \leq 0.01$

GHR genotype 2213 produced daily 3.53 kg fat corrected milk, 235 g non-fat solids, 408 g total solids, 176 g fat, 100 g protein more than the "worst" 3111 combined genotype animals. They also produced on average 134 g lactose daily more than cows with CGG 1111. On the other hand, the CGG 1113 appeared superior for gross energy in the milk and for % of total solids, fat, and lactose in milk (Table 5).

DISCUSSION

Hormones, growth factors, and other regulatory proteins associated with so called "somatotropic axis" are candidate markers for quantitative traits in farm animals. Genes encoding the growth hormone (GH), GH receptor (*GHR*), transcription factor *Pit*-I (activating expression of GH and prolactin genes in the anterior pituitary), insulin-like growth factor-I (*IGF*-I), and perhaps genes coding for GH signal transduction pathways, could contribute to the progress in genetic selection of farm animals (Parmentier *et al*., 1999). However, only few authors reported the effects of *GHR* gene polymorphism on the production traits in cattle. Hale *et al*. (2000) showed a correlation between a microsatellite marker in the P1 promoter of the bovine growth hormone receptor gene and growth rates in young cattle. According to their results the TG_{11} GH receptor allele decreased growth rates in Angus steers. Falaki *et al*. (1996) reported the effect of RFLP-*Taq*I polymorphism in the *GHR* gene 3′ end. The effect of this polymorphism on breeding value for milk protein was highly significant in Italian Holstein-Friesian bulls. Aggrey *et al*. (1999) showed an association between the polymorphism at *Alu*I site in the 5′-noncoding region of the *GHR* gene with milk production traits. Holstein bulls with *Alu*I (+/+) genotype of the *GHR* gene had a higher breeding value for milk fat than bulls with (–/–) genotype. Finally, Blott *et al.* (2003) provided strong evidence that a chromosome segment including the gene coding for the growth hormone receptor accounted for at least a part of the chromosome 20 QTL effect. By sequencing individuals with known QTL genotype, they identified an F to Y substitution in the transmembrane domain of the growth hormone receptor gene that is associated with a strong effect on milk yield and composition in the general population.

In the present study the polymorphism was investigated at four different sites within the 5′-noncoding region of the bovine *GHR* gene, recognised with different restriction nucleases. All of them were single nucleotide polymorphisms (SNPs) and three (*Alu*I, *Acc*I, and *Fnu*4HI) were located within the 1,206-bp LINE-1 element, a retrotransposon of the viral origin. The fourth polymorphic site – RFLP-*Nsi*I – was located within the promoter P1 for the exon 1A. The SNPs have gained high popularity in genetic studies due to their high accuracy and reproducibility. Moreover, they are indispensable for generation of positional candidate genes (Vignal *et al.*, 2002). These genes are usually less polymorphic than non-coding repetitive sequences, but the advantage of analysing polymorphism of structural, protein coding genes is that they may be directly involved in determining important traits in farm animals.

Summarizing the effects of the *GHR* gene polymorphism on the traits studied the following preliminary conclusion could be drawn: (1) *GHR* genotypes significantly influenced most of the dairy traits studied. (2) Cows of the RFLP-*Nsi*I –/– genotype of *GHR* produced more milk (FCM) with higher content of most milk components, including fat, protein, and lactose than those with +/+ genotype. These results might indicate either a favourable influence of the allele (–) or a disadvantageous effect of the allele (+) on the traits tested. (3) The RFLP-*Fnu*4HI and RFLP-*Alu*I had no effect on milk yield and composition traits. (4) The RFLP-*Acc*I heterozygotes +/– appeared superior with respect to two milk yield and composition parameters – gross energy and percentage of total solids. (5) The combined *GHR* genotypes (CGG) 2213 and 1113 are clearly superior for most traits under study. Cows carrying the 2213 genotype combination produced daily more fat corrected milk, and both CGGs appeared favourable for fat, protein, and lactose content in the milk than other genotypes.

It should be stressed that in this study a cattle population was used without family structure. Thus, there was no possibility of studying the segregation of alleles and their effects on traits within a family. For the same reason, identification of genuine haplotypes was not possible, and instead the combined genotypes were used to estimate associations with meat production traits. Therefore, the presented result can be interpreted only as a correlation between marker and production in this time and this population.

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Received: 04–03–23
Accepted after corrections: 04–09–10

ABSTRAKT

Jednoduchý nukleotidový polymorfismus (SNP) v 5'-nekódovací oblasti genu receptoru růstového hormonu u skotu a jeho asociace s vlastnostmi produkce mléka u polského černobílého skotu

U polského černobílého (BW) skotu byly stanoveny efekty genotypu dojnice pro receptor růstového hormonu (*GHR*) na vlastnosti produkce mléka. Bylo prokázáno, že genotypy *GHR* mají významný vliv na většinu sledovaných vlastností mléka. Dojnice genotypů *GHR* RFLP-*Nsi*I–/– vyprodukovaly více mléka s vyšším obsahem jednotlivých mléčných složek včetně tuku, bílkovin a laktózy než dojnice s genotypem +/+. Heterozygotní genotyp +/– na RFLP-*Acc*I se ukázal jako lepší s ohledem na dva ukazatele složení mléka – hrubou energii a celkovou sušinu. Kombinované genotypy *GHR* (CGG) 2213 a 1113 byly z hlediska většiny sledovaných vlastností evidentně příznivé. Dojnice nesoucí genotypovou kombinaci 2213 denně vyprodukovaly více mléka upraveného na 4% tučnost, tuku, bílkovin a laktózy než ostatní genotypy; mléko dojnic 2213 a 1113 mělo významně vyšší obsah celkové sušiny, bílkovin a tuku.

Klíčová slova: skot; *GHR*; gen; polymorfismus; mléčné vlastnosti

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