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Association between the growth hormone combined genotypes and dairy traits in Polish Black-and-White cows

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Associations were analysed between the bovine growth hormone combined genotypes (*GH/Alu*I-*Msp*I) and milk production traits in a total of 900 Polish Black-and-White (Polish Friesian) cows. PCR-RFLP method was used for genotyping. The following were frequencies of *GH/Alu*I-*Msp*I: 0.463 *LL/*++, 0.276 *LV/*++, 0.157 *LL/*+-, 0.059 *LV/*+-, 0.025 *VV/*++, 0.019 *LL/*-- and 0.001 (only 1 observation) of *VV/*+-. Significant differences were found in analysed dairy traits between cows of different *GH/Alu*I-*Msp*I combined genotypes. It is difficult, however, to trace a defined trend in all lactations, what to some extent complicates the inference. It is concluded that introducing the information on *GH/Alu*I-*Msp*I into dairy cattle marker-assisted selection (MAS) programmes would be risky.

KEY WORDS: cattle / dairy traits / genotyping / growth hormone / PCR-RFLP

Molecular markers that reveal polymorphism at the DNA level are now key players in animal genetics. Recently, a number of potential candidate genes have been recognized. Allelic variation in the regulatory and structural regions of candidate genes may influence diversification of milk yield and composition. Polymorphism of nucleotide sequences in these regions may affect the gene expression or the amino acid sequence

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of a product. Variations in introns or flanking sequences are potentially useful as genetic markers [Beckmann and Soller 1983].

Bovine growth hormone is a single peptide of about 22-kDa molecular weight [Wallis 1973]. It is composed of 190 or 191 amino acids, containing Ala or Phe at the N terminus, due to alternative processing of bGH precursors [Lingappa *et al.* 1977, Wood *et al.* 1989]. Bovine growth hormone gene *(GH)* is located in chromosome 19 [Hediger *et al.* 1990], and consists of five exons separated by four introns [Woychick et al. 1982; Gordon *et al*. 1983]. Several polymorphisms were identified in the *GH* gene. Cowan *et al*. [1989] and Hilbert *et al.* [1989] found a polymorphic site for *Msp*I restriction endonuclease, while Zhang *et al*. [1993] localized the polymorphism in intron 3 of the gene *GH*, at position 1547. According to Yao *et al*. [1996] molecular basis of this polymorphism is transition C→T. However, Hoj *et al*. [1993] revealed that the molecular basis of this polymorphism was insertion of T at position +837 and transversion C→G at position +838 of the gene *GH*. Moreover, Leu or Val amino acid substitutions at residue 127 exist due to the allelic polymorphism [Seavey *et al.* 1971], molecular basis of which is transversion C→G in the exon 5 (at position 2141) of the *GH* [Lucy *et al.* 1991].

The studies on the influence of the *GH-Msp*I polymorphism on production traits are quite advanced, but the results obtained by various authors are not always corresponding. Hoj *et al*. [1993] and Lee *et al*. [1993] showed that GH^{Msp-} allele was more frequent in cows of lines selected for high fat content of milk. Similar results were reported by Falaki *et al*. [1996], who demonstrated a non-significant association between GH^{Msp-} allele and increased fat content of milk in Holstein-Friesian cows. Lagziel *et al*. [1996, 1999] reported that heterozygous cows produced milk containing more protein than did $Msp\mathrm{I}^{+/+}$ individuals. Different results were published by Yao *et al*. [1996], showing that milk, fat and protein yields were positively influenced by allele GH^{Msp+}.

Although the studies on an effect of Leu/Val polymorphism on production traits of cattle are quite advanced, the results published by various authors are not always corresponding. In the case of milk production traits it has been shown that the Holstein-Friesian cows homozygous for Leu-127 of GH produced more milk than *LV* animals [Lucy *et al*. 1993]. Lee *et al*. [1996] found that genetic merit for estimated breeding values for milk (EBV-milk) as well as average yield deviations for milk (AYD-milk) were reduced in the presence of Val-127 allele of the gene *GH*. In contrary, Sabour *et al*. [1997] showed that there was a higher frequency of *LV* genotypes among the bulls with the top estimated transmitting abilities (ETA) than among those from a bottom ETA group, suggesting that the allele *V* is favourable for milk, fat and protein yield. Zwierzchowski *et al*. [2002] demonstrated that cows carrying allele *V* of the *GH* show better performance in daily milk yield and milk composition.

The aim of this study was to determine the allelic frequencies of the bovine growth hormone combined genotypes (RFLP/*Msp*I-*Alu*I) and to investigate the relationship between *GH* genotypes and milk production traits of Black-and-White cows.

Material and methods

Genotyped were 900 Black-and-White cows, with different share of Holstein-Friesian (HF) breed, kept in five herds in the West Pomerania region of Poland (Tab. 1). Only cows with complete lactation were included in the statistical evaluation (900 cows with lactation I, 593 with lactations I and II, and 366 cows with lactations I, II and III).

The *GH/Msp*I-*Alu*I genotypes were analysed using the PCR-RFLP technique. Crude DNA was isolated from blood samples using MasterPure™ kit (EPICENTRE TECHNOLOGIES). The yields were approximately 60-70 μg of DNA/ml blood. A 329 base pair (bp) fragment of the gene *GH* gene was amplified by polymerase chain reaction (PCR) using the forward (5'-CCCACGGGCAAGAATGAGGC-3') and reverse (5'-TGAGGAACTGCAGGGGCCCA-3') primers [Mitra *et al*. 1995]. The following cycles were applied: denaturation at 94°C/5 min, followed by 30 cycles: 94°C/40 s, 60°C/40 s, and 72°C/ 30 s. Amplified DNA was digested with *Msp*I (MBI FERMENTAS). The digestion products were separated with horizontal electrophoresis (90 volts, 50 min) in 2% agarose gels (GIBCO BRL) in 1 × TBE and 1.0 μM ethidium bromide.

A 223-bp fragment of the *GH* was PCR-amplified using forward (5'-GCTGCTCCTGAGGGCCCTTCG-3') and reverse (5'-GCGGCGGCACTTCATGAC-

Table 1. Characteristics of the cows examined

Herd	Number of cows	Number of cows with a given share of H-F breed		Average milk yield in the 305-day lactation I (kg)
		0-50%	50.1-100%	
1	116	8 (6.90%)	108 (93.10%)	6233
2	209	40 (19.14%)	169 (80.86%)	4668
3	126	4 (3.17%)	122 (96,83%)	7797
4	140	50 (35.71%)	90 (64.29%)	5382
5	309	72 (23.30%)	237 (76.70%)	4782

CCT-3') primers [Schlee *et al*. 1994]. Amplification was carried out in 35 cycles: 94°C/40 s, 60°C/ 40 s, and 72°C/30 s, using a DNA thermal cycler (PERKIN ELMER CETUS Corp.). Amplified DNA was digested with *Alu*I enzyme (MBI FERMENTAS) and separated by horizontal electrophoresis (90 volts, 50 min) in 2.5% agarose gel (GIBCO BRL) in 1 × TBE and 1.25 μM ethidium bromide.

The data for 305-day milk production in lactations I, II and III were obtained from the farm records. Statistical evaluations were performed using procedures of SAS®. The effects of *GH/Msp*I-*Alu*I genotypes on milk production traits were analysed using General Linear Model (GLM) procedure. Type III ANOVA was used to evaluate the differences

between means with Duncan multiple range test. The followig model was used:

$$Y_{ijklmno} = \mu + G_i + S_j + HF_k + YS_l + H_m + b_1 (x_1 - DD)_n + e_{ijklmno}$$

where:

$Y_{ijklmno}$ – 305-day production trait (milk yield, milk fat and milk protein yield and per cent) in lactation I, II and III of cow *o*;
μ – overall mean;
G_i – fixed effect of *GH* combined genotype (i = 1..7);
S_j – fixed effect of the sire;
HF_k – per cent of H-F blood;
YS_l – fixed effect of year-season of calving class;
H_m – fixed effect of the herd;
DD – mean number of days in milk;
b_1 – linenar regression coefficient of days in milk;
x_1 – days in milk of cow *n*;
$e_{ijklmno}$ – random error.

Results and discussion

The *GH/Alu*I-*Msp*I frequencies (PCR-RFLP) in cows are presented in Table 2, while in Table 3 given are means for their milk production traits as referred to genotypes.

Seven *GH/Alu*I-*Msp*I combined genotypes in lactation I and II and six in lactation III were found. In Table 2 the values marked with asterisk (*) were excluded from the

Table 2. Frequencies of the growth hormone genotypes (*AluI-MspI*)

GH/AluI-MspI	Lactation I		Lactation II		Lactation III	
	n	%	n	%	n	%
LLA++	417	46.33	262	44.18	161	44.85
LLA+-	141	15.67	94	15.85	58	16.15
LL+-	17	1.89	14	2.36	7*	1.95
LVA++	248	27.56	164	27.66	98	27.30
LVA+-	53	5.89	39	6.58	26	7.24
LV+-	–	-	–	–	–	–
VVA++	23	2.55	19	3.20	9*	2.51
VVA+-	1*	0.11	1*	0.17	-	
VV+-	–	–	–	–	-	–
Total	900	100	593	100	359	100

*Excluded from the statistical analysis.

Table 3 Means and their standard errors (SE) for milk production traits of cows carrying different *GH* combined genotypes

Lactation	*GH* genotype	Number of cows	Milk yield (kg)	Milk fat		Milk protein		F×P (%)
				kg	%	kg	%	
I	*LL*/++	417	5409^{abc} (69.7)	221.9^{abc} (3.15)	4.10^{a} (0.03)	170.9^{ab} (2.35)	3.15 (0.01)	7.25^{abc} (0.12)
	LL/+–	141	5077^{d} (109.6)	212.2^{d} (4.83)	4.20^{d} (0.03)	160.7^{cd} (3.60)	3.16 (0.02)	7.36^{d} (0.12)
	LL/–	17	5281 (279.5)	213.8 (11.86)	4.07 (0.12)	165.1^{a} (9.28)	3.12 (0.05)	7.19 (0.17)
	LP/++	348	5150^{bc} (85.0)	213.9^{d} (3.87)	4.14^{d} (0.03)	162.9^{c} (2.90)	3.15 (0.01)	7.29^{d} (0.12)
	LP/+–	53	4700^{ef} (179.3)	201.5^{ef} (8.12)	4.19 (0.05)	150.2^{abcd} (5.70)	3.15 (0.03)	7.34^{b} (0.14)
	PP/++	23	5103 (294.2)	219.8 (12.73)	4.31 (0.08)	165.2 (8.95)	3.24 (0.05)	7.55^{a} (0.17)
II	*LL*/++	362	5816 (85.2)	240.1 (4.02)	4.12^{b} (0.03)	183.1^{ef} (18.50)	3.23 (0.01)	7.35 (0.04)
	LL/+–	94	5704 (127.9)	240.8 (6.15)	4.22^{d} (0.05)	190.9^{f} (18.50)	3.18 (0.02)	7.40 (0.06)
	LL/–	14	5640 (324.0)	229.9 (15.79)	4.07 (0.12)	180.2 (20.93)	3.16 (0.06)	7.23 (0.16)
	LP/++	164	5695 (100.9)	239.2 (5.30)	4.18 (0.04)	182.0^{b} (18.60)	3.23 (0.02)	7.41 (0.05)
	LP/+–	39	5455 (171.3)	232.2 (7.61)	4.28^{bc} (0.08)	194.0^{ab} (19.05)	3.21 (0.03)	7.49 (0.10)
	PP/++	19	5518 (277.5)	238.6 (14.20)	4.30 (0.12)	176.9 (19.39)	3.27 (0.06)	7.57 (0.14)
III	*LL*/++	161	6151 (331.6)	260.9 (5.63)	4.02^{abcd} (0.04)	197.4 (3.77)	3.19 (0.02)	7.21^{abcd} (0.05)
	LL/+–	58	6113 (349.7)	258.1 (6.36)	4.24^{d} (0.07)	192.5 (6.61)	3.18 (0.02)	7.42^{d} (0.07)
	LP/++	98	6346 (338.2)	264.5 (7.00)	4.19^{d} (0.06)	196.8 (4.43)	3.21 (0.02)	7.36^{d} (0.07)
	LP/+–	26	6489 (448.5)	265.2 (11.83)	4.23^{d} (0.11)	185.0 (7.46)	3.20 (0.03)	7.43^{b} (0.13)

$^{a-f}$ Within each lactation, frequencies in columns bearing the same superscripts are significantly different: small letters = $P \leq 0.05$, capitals = $P \leq 0.01$

F×P = sum of the fat and protein contents (%)

statistical evaluation due to the small number of observations.

Milk yield (kg)

In lactation I, the cows of the *LL*/++ genotype yielded more milk (by 332, 259

and 649 kg) than the *LL*/+-, *LV*/++ and *LV*/+- individuals, respectively (P≤0.01), and *LV*/++ yielded more milk (by 390 kg) than *LV*/+- cows (P≤0.05). The *VV*/+- cow (only one observation) was excluded from the statistical evaluation. In lactation II and III no significant differences between the cows of different *GH*/*Alu*I-*Msp*I combined genotypes were found (*LL*/-- and *VV*/++ cows were excluded from the statistical evaluation).

Yao *et al*. [1996] found that $GH^{Msp(+)}$ allele increased milk yield by 300 kg. Sabour *et al*. [1997] examined an influence of *GH/Msp*I polymorphism on the genetic value of Holstein-Friesian bulls (for milk yield), and found no significant differences between animals of different *GH* genotypes. However, significantly higher frequency of $MspI^{+/-}$ genotype was found in bulls with the lowest breeding value for milk yield.

In the case of RFLP-*Alu*I in the bovine *GH* gene (Leu/Val) Lucy *et al*. [1993] showed that Holstein-Friesian cows homozygous for Leu-127 of *bGH* (*LL*) yielded more milk than *LV* cows. On the contrary, according to Lee *et al*. [1996] the genetic merit (for EBV-milk and AYD-milk) was decreased in the presence of Val-127 allele of the *GH* gene. Injections of recombinant form of bGH led to a greater increase in milk yield when cows were treated with $valine_{127}$-bGH than with $leucine_{127}$-bGH [Eppard *et al*. 1992]. Those results may indicate that $valine_{127}$ allele is associated with an increase in milk yield in dairy cows. However, in contemporary Holstein cows, the $valine_{127}$ allele was found to be associated with a reduced genetic merit for milk yield [Lucy *et al.* 1993]. Zwierzchowski *et al*. [2002] reported that cows carrying allele *V* of the *GH* showed better performance in daily milk yield and milk composition. Cows of the *VV* genotype produced more milk daily than those of other genotypes. According to Abdel-Meguid *et al*. [1987] amino acid 127 of bGH is localized at the end of the third α-helix. Aston *et al*. [1991] revealed that the fragment of somatotropin between amino acid 120 and 140 is both lactogenic and somatogenic, and although the region did not participate in binding of growth hormone to its receptors [De Vos *et al.* 1993], the interaction between the four α-helixes could affect the structure of somatotropin [Chou and Zheng 1992].

Fat and protein yield (kg)

In lactation I the cows of the *LL*/++ genotype yielded more milk fat (by 9.6, 8.0, and 20.7 kg) than the *LL*/+-, *LV*/++ and *LV*/+- individuals, respectively (P≤0.01), and *LL*/+- yielded more fat (by 11.1 kg) than *LV*/+- cows (P≤0.05).

In lactation I the *LL*/++ cows yielded more milk protein (by 20.7 and 10.2 kg) than *LV*/+- and *LL*/+- individuals, respectively (P≤0.01 and P≤0.05), and those of *LL*/--, *LV*/++ and *LL*/+- combined genotypes yielded more than did *LV*/+- cows. On the other hand, in lactation II the *LV*/+- cows yielded more milk protein (by 10.9 and 12.0 kg) than *LL*/++ and *LV*/++ animals, respectively (P≤0.01 and P≤0.05). *LL*/+- cows yielded more milk protein than *LL*/++ individuals (P≤0.05).

Yao *et al*. [1996] demonstrated an elevated milk fat and protein yields in the animals of $MspI^{+/+}$ genotype. Different results were obtained by Lee *et al*. [1993] who showed that GH^{Msp-} allele was more frequent in cows of lines selected for high milk fat yield.

Falaki *et al.* [1996] and Lagziel *et al*. [1999] reported that *GH/Msp*I$^{+/-}$ cows produced more milk protein than those of genotype *GH/Msp*I$^{+/+}$. In the case of RFLP-*Alu*I of the *GH* a higher frequency of *LV* genotypes among the top group than among the bottom group of ETA bulls was observed by Sabour *et al*. [1997]. Similar tendencies were described by Zwierzchowski *et al*. [2002] who showed that cows carrying allele *V* of the *GH* showed better milk performance.

Fat and protein content (%)

In all three lactations, differences were found between individuals of different *GH*/*Alu*I-*Msp*I genotypes (P≤0.01 and P≤0.05). In lactations I, II and III, the milk of cows of the *LL*/+/+ genotype contained less fat than those of other *GH*/*Alu*I-*Msp*I genotypes. In lactation I the *LL*/++ cows produced milk with lower fat content (by -0.10 and -0.06 per cent points – pp) than *LL*/+- and *LV*/++ individuals (P≤0.01 and P≤0.05, respectively). Similarly, in lactation II the milk of *LL*/++ cows contained by -0.16 and -0.10 pp less fat than milks of *LV*/+- and *LL*/+- individuals (P≤0.05). In lactation III the *LL*/++ cows produced milk with lower fat content (by -0.22, -0.21 and -0.13 pp) than *LL*/+-, *LV*/+- and *LV*/++ animals, respectively. Hoj *et al*. [1993] and Falaki *et al.* [1996] reported that *GH*$^{Msp-}$ allele was more frequent in the lines of cows with high milk fat content and increased milk fat yield.

Significant differences in milk protein content were not found between the cows of different *GH* genotypes. This is in contradiction to Lagziel *et al*. [1996, 1999], who reported homozygous *Msp*I$^{+/+}$cows to produce milk lower in protein than that of heterozygotes.

As far as the pool of fat and protein per cent of milk (F+P) is concerned, differences between the cows of different *GH*/*Alu*I-*Msp*I genotypes were mostly due to higher milk fat content. In lactations I and II the highest F+P (7.55 and 7.57%, respectively) appeared in *VV*/++. In lactation I the difference between *VV*/++ and most frequent *LL*/++ genotype reached 0,3% pp of F+P in favour of the former. Zwierzchowski *et al*. [2002] demonstrated that the cows carrying allele *V* of the *GH* had better performance in milk composition.

Based on the results presented here it is difficult to indicate the most desirable *GH/Alu*I-*Msp*I genotype. In relation to milk composition, *VV*/++ cows produced milk with the highest F+P (fat and protein per cent pooled) content. However, the small sample size (only 23 *VV*/++ cows) does not allow drawing definite conclusions. It is concluded that introducing the information on *GH/Alu*I-*Msp*I combined genotype into dairy cattle marker-assisted selection (MAS) programmes would probably be risky.

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Zależności między kombinowanymi genotypami hormonu wzrostu a cechami użytkowości mlecznej krów rasy cb

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

Analizowano zależności między „kombinowanymi" genotypami (*combined genotypes*) hormonu wzrostu (GH/*Alu*I-*Msp*I) a cechami użytkowości mlecznej krów rasy cb. Badaniami objęto 900 krów, o różnym udziale genów rasy hf. Dla ustalenia genotypów krów zastosowano metodę PCR-RFLP. Stwierdzono następujące frekwencje genotypów: 0,463 *LL/*++, 0,276 *LV/*++, 0,157 *LL/*+-, 0,059 *LV/*+-, 0,025 *VV/*++, 0,019 *LL/*-- i 0,001 *VV/*+-. Wykazano istotne różnice w poziomie badanych cech zależnie od „kombinowanego" genotypu GH/*Alu*I-*Msp*I. Trudno jednak o wskazanie jednakowego trendu we wszystkich laktacjach, co znacznie komplikuje wnioskowanie. Wprowadzanie do programów selekcyjnych bydła mlecznego informacji o genotypie GH/*Alu*I-*Msp*I wydaje się ryzykowne.