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Associations between *Leu/Val* polymorphism of growth hormone gene and milk production traits in Black-and-White cattle

Summary

Associations between polymorphism of the bovine *growth hormone (GH)* gene (*Leu/Val*) and milk production traits of Black-and-White cattle with different proportional share of Holstein Friesian genes were analysed. A total of 1086 cows were included in the study. PCR-RFLP method was used for genotyping. The frequencies of genotypes and alleles were as follows: 0.653 for *LL* genotype, 0.324 for *LV* and 0.023 for *VV*, and 0.815 for *GH^L* and 0.185 for *GH^V*. Associations between *Leu/Val* polymorphism and milk production traits of cows were found only in first lactation. Cows with *LL* genotype had higher milk, fat and protein yield compared to *LV* individuals ($P \leq 0.01$).

Key Words: growth hormone gene, PCR-RFLP, dairy cattle, milk production traits

Zusammenfassung

Titel der Arbeit: **Zusammenhänge zwischen dem Polymorphismus des Wachstumshormogens (*Leu/Val*) und der Milchleistung schwarzbunter Rinder**

Zusammenhänge zwischen dem Polymorphismus des Wachstumshormogens (*Leu/Val*) und den Merkmalen der Milchleistung von Rindern der Rasse Schwarzbunt wurden ausgewertet. Die Untersuchungen erfolgten an 1086 Kühen mit unterschiedlichem Holstein-Friesian Genanteil. Zur Genotypbestimmung wurde die Methode PCR-RFLP eingesetzt. Folgende Genotyp- und Allelfrequenz wurden ermittelt: 0,653 - Genotyp *LL*, 0,324 - *LV* und 0,023 - *VV* sowie 0,815 - *GH^L* und 0,185 - *GH^V*. Signifikante Unterschiede wurden zwischen den Genotypen *GH* und den Milchleistungsmerkmalen nachgewiesen. In der ersten Laktation zeichneten sich die Kühe mit dem Genotyp *LL* durch größere Milch-, Fett- und Eiweißleistung als die Tiere mit dem Genotyp *LV* (bei $P \leq 0,01$) aus.

Schlüsselwörter: Wachstumshormongen, PCR-RFLP, Milchrind, Milchleistungsmerkmale

Introduction

Selection of dairy cattle is focused primarily on the improvement of yield and composition of milk. Recently, a number of potential candidate genes have been recognised. Candidate genes are selected on the basis of known relationships between physiological or biochemical processes and the trait. Occurrence of allelic variation in the regulatory and structural regions of those genes may influence the diversification of quantity and composition of milk. Polymorphism of nucleotide sequences in these regions may influence the gene expression or a sequence of the product. Variations in introns or flanking sequences have potential usefulness as genetic markers (BECKMANN and SOLLER, 1983).

Bovine growth hormone is a single peptide of molecular weight about 22-kDa (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 (WOOD et al., 1989). Additionally, Leu or Val amino acid substitutions at residue 127 exist due to the allelic polymorphism (SEAVEY et al., 1971).

Bovine growth hormone gene is localised in chromosome 19 (HEDIGER et al., 1990), and consists of five exons separated by interval introns (GORDON et al., 1983). Six sites of variable nucleotides were identified in the 5'-flanking region of the gene and one in the intron I (HETCH and GELDERMANN, 1996). Moreover, RODRIGUES et al. (1998) identified an AAG deletion in the promoter region of the *GH* gene, localised 9 nucleotides upstream from the TATAAA sequence. LAGZIEL et al., (1996) found and sequenced (LAGZIEL et al., 1999) 14 different haplotypes for the entire *bGH* gene using SSCP techniques. COWAN et al., (1989) and HILBERT et al., (1989) detected a polymorphic site for *MspI* restriction endonuclease. This polymorphism was localised in the intron III of the *GH* gene in position 1547 (ZHANG et al., 1993a). LUCY et al. (1993) detected a polymorphic site located in the exon V of the *GH* gene (transversion C→G in position 2141 of the gene) and consequently the substitution of Leu by Val in a protein product. YAO et al. (1996) also reported the polymorphism in the exon V of the *GH* gene, in position 2241 (transversion A→C). The additional polymorphic site in the somatotropin gene (point mutation C→T in the codon 172) was found in the Japanese cattle and consequently threonine was changed to methionine in a protein chain (CHIKUNI et al., 1994). Furthermore, UNANIAN et al. (1994) detected a polymorphic site in the 3'-region of the *GH* gene, probably in position 2637.

The studies on the influence of the Leu/Val polymorphism on production traits are quite advanced but the results obtained by various researchers are not always corresponding. LUCY et al. (1993) showed that Holstein-Friesian cows homozygous for Leu-127 of *bGH* produced more milk than *LV* animals. LEE et al. (1996) determined that genetic merit (for EBV-milk and AYD-milk) was decreased in the presence Val-127 allele of the *GH* gene. SABOUR et al. (1997) determined that the effects of L/V locus on milk, fat and protein EBVs in Holstein bulls were not significant, however the genotypic proportion of *LL* and *LV* genotypes differed among the top, middle and bottom groups of bulls in ETAs of milk ($P = 0.06$), fat ($P = 0.04$) and protein ($P = 0.01$). There was a higher frequency of *LV* genotypes among the top ETA bulls than among the bottom group, suggesting that the V allele is favourable for milk, fat and protein ETAs.

The aim of this study was to estimate the allelic frequencies at the Leu/Val locus of the bovine GH gene and to investigate the relationship of this polymorphism and milk production traits of black and white cows.

Materials and methods

A total of 1086 Black-and-White (BW) cows, with different proportional share of HF genes, were genotyped. The cows were kept in the five herds in West Pomerania region of Poland (Table 1). Only cows with complete lactation were included in the statistical analysis (900 cows with I lactation, 600 cows with first and second lactation and 366 cows with first, second and third lactation).

The Leu/Val genotypes were analysed using the PCR-RFLP method. Crude DNA was isolated from blood samples using MasterPure™ kit (Epicentre Technologies). The procedure developed by SCHLEE ET AL., (1994a) was used to determine the polymorphism in exon V of the *GH* gene (*GH-AluI*). PCR products were digested with

AluI (MBI Fermentas) and separated by horizontal electrophoresis through 2.5% agarose gel (Gibco BRL) in 1 x TBE and 1.25 μ M ethidium bromide.

Table 1

Characterisation of the investigated population of dairy cows (Charakteristik der untersuchten Milchkuhpopulationen)

Herd	n	Number of cows related with proportional share of HF genes		Average milk yield in the first/305-day of lactation (kg)
		0-50%	50.1-100%	
Nowielice	124	9 (7.25%)	115 (92.75%)	6233
Trzebusz	234	45 (19.23%)	189 (80.77%)	4668
Smardzewo	170	5 (2.94%)	165 (97.06%)	7797
Kosierzewo	185	65 (35.13%)	120 (64.87%)	5382
Ostrowiec	373	86 (23.06%)	287 (76.94%)	4782

Data for 305-day milk production in the first, second and third lactation, including production of milk, milk fat and milk protein and proportions of milk fat and milk protein were obtained from the farm documentation. Statistical calculations were performed using procedures of SAS[®]. Distribution frequencies of the two alleles within the herds were compared by Chi-square test. The effect of Leu/Val genotypes on the milk production traits of dairy cows were analysed using GLM procedure. The model used was as follows:

$$Y_{ijklmnop} = \mu + G_i + S_j + HF_k + b_1 (x_1 - A)_l + YS_m + H_n + b_2 (x_2 - DD)_o + E_{ijklmnop}$$

where: $Y_{ijklmnop}$ – 305-day milk production record at first, second and third lactation of cow p , μ – the overall mean, G_i – the fixed effect of GH genotype ($i = 1, \dots, 3$), S_j – the fixed effect of sire, HF_k – percentage of HF genes, A_l – the calving age, x_1 – the calving age in days of cow l , b_1 – the linear regression coefficient of age of calving, YS_m – the fixed effect of year-season of calving class, H_n – the fixed effect of the herd, DD_o – days of milk, b_2 – the linear regression coefficient of days of milk, x_2 – days of milk of cow o , $E_{ijklmnop}$ – the random error

Results and discussion

The following DNA restriction fragments were obtained for the GH -*AluI* polymorphism: 171 and 52 bp for the LL genotype, 223, 171 and 52 bp for the LV and 223 bp (no digestion) for the VV (Fig.).

The LL genotype was the most frequent in all the herds under study (0.595-0.735) and it was followed by the heterozygotic LV (0.259-0.378) whereas the least frequent was the VV (0.006-0.057). The frequency of the GH^L was ranged from 0.784 to 0.865 (Table 2).

The highest frequency of the GH^L (0.865) was observed in Smardzewo herd, where was the highest average proportional share of Holstein-Friesian genes.

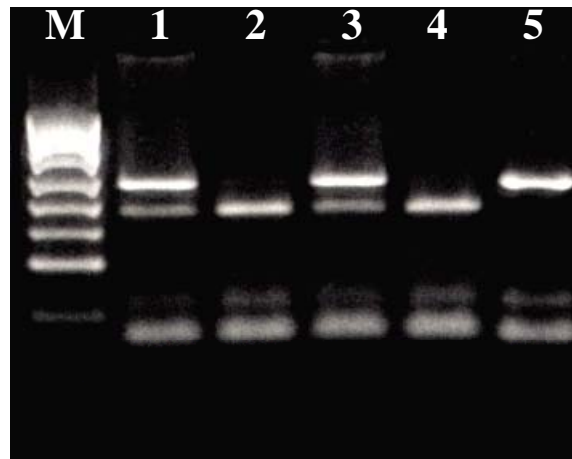


Figure: Representative results of *GH-AluI* analysis detected by agarose gel electrophoresis (Elektrophoretische Trennung von Restriktionsfragmenten *GH-AluI*)

M – DNA marker (pUC19/*MspI*); lanes 1,3 – *LV* genotype, lanes 2, 4 – *LL* genotype, lane 5 – *VV* genotype. The small 52 bp fragments were invisible in the gel.

Table 2

Frequencies of genotypes and alleles of the *GH* gene (Genotyp und Allelfrequenz von *GH*-Gens)

Herd	n	Genotypes			Alleles	
		<i>LL</i>	<i>LV</i>	<i>VV</i>	<i>GH^L</i>	<i>GH^V</i>
Kosierzewo	185	0.595 ^A	0.378 ^a	0.027	0.784 ^A	0.216 ^A
Nowielice	124	0.645	0.298	0.057	0.794 ^b	0.206 ^b
Smardzewo	170	0.735 ^{Ab}	0.259 ^{ab}	0.006	0.865 ^{Abc}	0.135 ^{Abc}
Trzebusz	234	0.620 ^b	0.359 ^b	0.021	0.799 ^c	0.201 ^c
Ostrowiec	373	0.670	0.311	0.019	0.826	0.174
Total	1086	0.653	0.324	0.023	0.815	0.185

Frequencies in columns with the same letter differ significantly; capitals - $P \leq 0.01$, small letters - $P \leq 0.05$

Frequencies of *Leu/Val* alleles obtained in this study were similar to the frequencies obtained earlier for the Black and White cattle. Higher frequency of the *GH^L* (0.86-0.93) was observed in the Holstein breed (LEE et al., 1996; LUCY et al., 1993; SABOUR et al., 1997; VUKASINOVIC et al., 1999; ZHANG et al., 1993b). Similar frequency of the L allele of *GH* gene, ranged from 0.80 to 0.83 (in German Black&White cattle) was observed in studies carried out by ČITEK et al. (1998) and SCHLEE et al. (1994b). Somewhat smaller frequency of the *GH^L* (0.78 and 0.72) was observed in studies of CHIKUNI et al. (1991) and CHRENEK et al. (1998). Considerably smaller frequencies of *Leu₁₂₇* allele of *GH* gene (0.58-0.62) were observed in Polish Black and White cattle (ZWIERZCHOWSKI et al., 1995; ZWIERZCHOWSKI et al., 1998; KLAUZIŃSKA et al., 2000). The genetic equilibrium in the population under study was not disturbed and the size of certain genotypes of the *GH* was not statistically different from the theoretically calculated size.

Table 3 shows associations of the *Leu/Val* polymorphism of the *GH* gene on milk production traits in the BW cows.

The statistically significant differences between individuals of different genotypes were found in milk, fat and protein yield. In the first 305-day lactation, the cows of the *LL* genotype produced more milk (+225 kg), fat (+7 kg) and protein (+7 kg) than the *LV* individuals ($P \leq 0,01$). In the case of milk yield, similar tendencies were observed in the studies by LUCY et al. (1993) and LEE et al. (1996). However, SABOUR et al. (1997) observed a higher frequency of *LV* genotypes among the top ETA bulls than among the bottom group, suggesting that the *V* allele is favourable for milk, fat and

protein ETAs. Injections of recombinant form of bGH produced a greater increase in milk yield when cows were treated with valine₁₂₇-bGH than when treated with leucine₁₂₇-bGH (EPPARD et al., 1992). Those results may indicate that valine₁₂₇ allele is associated with increase of milk yield in dairy cows. However, in contemporary Holstein cows, the valine₁₂₇ allele was associated with a reduced genetic merit for milk yield (LUCY et al., 1993). Amino acid 127 of bGH is localised at the end of the third α -helix (ABDEL-MEGUID et al., 1987). In another study, ASTON et al. (1991) determined that the fragment of somatotropin between 120 and 140 amino acid had lactogenic and somatogenic action, and although that region did not take part in binding of growth hormone with its receptors, the interaction between the four α -helixes could influence the structure of somatotropin (CHOU and ZHENG, 1992). It is supposed that bovine GH with Ala at the N terminus (191 aa) and Leu₁₂₇ stimulate the release of IGF-I more than another variants of bGH (VANDERKOOI et al., 1995). SCHAMS et al. (1991) determined that higher concentration of GH in blood was linked to smaller concentration of IGF-I and higher milk yield.

Table 3

Means and standard deviations of milk production traits in cows carrying different *Leu/Val* genotypes (Mittelwerte und Standardabweichungen der Milchleistungsmerkmale bei Kühen mit verschiedenen Genotypen *Leu/Val*)

Lactation	Genotype	n	Milk yield		Fat		Protein	
			(kg)	kg	%	kg	%	
I	LL	577	5308 ^A (1411.8)	218.7 ^B (63.1)	4.126 (0.449)	167.7 ^D (47.4)	3.152 (0.189)	
	LV	300	5083 ^A (1329.9)	211.7 ^B (60.7)	4.151 (0.420)	160.7 ^D (45.2)	3.154 (0.199)	
	VV	23	5115 (1539.6)	218.6 (69.3)	4.254 (0.415)	164.9 (50.9)	3.213 (0.247)	
	Total	900	5228	216.4	4.138	165.3	3.154	
	II	LL	379	5680 (1458.6)	235.2 (68.8)	4.121 (0.531)	183.3 (50.5)	3.212 (0.206)
II	LV	203	5621 (1295.7)	236.7 (64.9)	4.196 (0.524)	182.5 (45.4)	3.227 (0.244)	
	VV	18	5706 (1195.6)	245.6 (62.4)	4.287 (0.541)	184.3 (39.2)	3.224 (0.246)	
	Total	600	5661	236.0	4.151	183.1	3.217	
III	LL	233	6024 (1563.8)	247.1 (76.6)	4.067 (0.522)	192.2 (53.2)	3.179 (0.196)	
	LV	126	5954 (1418.9)	248.6 (71.9)	4.162 (0.579)	191.2 (46.7)	3.196 (0.195)	
	VV	7	6127 (1517.6)	274.8 (96.5)	4.306 (0.521)	203.4 (59.1)	3.291 (0.193)	
	Total	366	6002	248.2	4.104	192.1	3.187	

Frequencies in columns with the same letter differ significantly; capitals - $P \leq 0.01$, small letters - $P \leq 0.05$.

There were no statistically significant differences between the cows of different *Leu/Val* genotypes and milk production traits in the second and third lactation. The observed differences could have resulted from another source of variation (effect of herd, effect of sire).

The influence of Val₁₂₇ allele of the *GH* gene on the milk fat and milk protein content should be verified in further studies.

Summarising, it appears that the results received in this study didn't unequivocally indicate which allele of *GH* gene should be promoted in improvement of milk production traits.

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