

ASSOCIATION BETWEEN LEU127VAL POLYMORPHISM OF GROWTH HORMONE GENE AND MILK YIELD OF POLISH RED CATTLE

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Abstract

Identification of Leu127Val (Alu I) polymorphism in growth hormone (GH) locus in Polish Red cattle was carried out using PCR-RFLP technique. The following distribution of genotypes was found: Leu/Leu 0.574, Leu/Val 0.408, Val/Val 0.018. Due to a very low frequency of Val/Val genotype this genotype was excluded from the analyses. The GH^L allele frequency was 0.774. The relationship between Leu127Val polymorphism and milk performance traits was studied based on the data obtained from the first lactation and data from the first three lactations was pooled. In the first lactation the cows with Leu/Leu genotype were characterized by 0.15% higher protein content in milk ($p \leq 0.05$) compared with cows with Leu/Val genotype. The genotype did not affect the yield of: milk, protein and fat as well as fat content in milk.

Key words: SHEEP, BLOOD GROUPS, POLYMORPHIC PROTEINS

Introduction

The growth hormone gene was mapped on the short arm of 19th cattle chromosome - 19q26 (Hediger et al., 1990). The studies determined that in cattle genome there is a one copy of somatotrophin gene of 1.8 kb. It consisted of five exons, separated by four introns (Gordon, 1983). In GH gene many polymorphic sites were localized. The substitution C → T at position 2141 within the exon V, in codon 127 of amino acid chain, was described with regard to a potential relationship with production traits in cattle (Seavey et al., 1971). Up to date, it was observed that the frequencies of the polymorphic genotypic forms of GH displays a high variation within particular cattle breeds. It was found that the frequency of GH^L allele is the highest in dairy breeds (Holstein Frisian, Brown Swiss), whereas GH^V allele is three times more frequent in beef breeds than in Holstein-Frisian (Zhang et al., 1993).

The aim of the study was to determine the frequency of alleles and genotypes in *GH locus* and to investigate an association between the polymorphic site (Leu127Val) and the chosen milk performance traits in Polish Red cows.

Material and methods

The studies were carried out on 115 Polish Red cows located in two herds of conservation breeding from the southern Poland: Szczyrzyc (n = 59) and Jodłownik (n = 56). The data of milk performance during the first three lactations were analyzed together.

DNA was isolated from the whole blood collected from jugular vein to sterile test-tubes (Wizard Genomic DNA Purification Kit, Promega). The 492 bp fragment was amplified in PTC-200 Engine (MJ Research, Watertown, MA, USA). The PCR was carried out in 20 μl of a mixture containing: 2mM MgCl₂ (Fermentas), 0.2mM dNTP Mix, DNA genome 150-200ng, 1.25U Taq Polymerase recombinant (MBI Fermentas), 0.2 μM each of primers (Lechniak et al., 2002). The

following PCR protocol was used: initial denaturation 95°C for 5 min; 36 cycles: 95°C– 30 s, 57°C – 30 s, 72°C – 30 s, final synthesis 72°C – 10 min. 10 µl of PCR products were digested with *AluI* restriction endonuclease (3U) at 37° C for 16 hours. The obtained fragments of DNA were subjected to electrophoresis in 3% agarose gel „NuSieve GTG agarose” (BioWhittaker Molecular Applications).

In aforementioned herds the frequencies of alleles and genotypes in the growth hormone *locus* were calculated. In order to estimate the effect of Leu127Val polymorphism in *GH locus* on milk performance traits [milk, protein and fat yield per lactation (kg), protein and fat content (%)] the analyses of variance using the least-square method were performed. Two calving seasons were assumed: winter - from 1. October to 31. March and summer - from 1. April to 30. September. The data on milk performance in the first lactation and first three lactations were analysed separately by the SAS GLM Procedure (SAS Institute Inc., 2001). The standard 305-day lactations were used for calculations. The lactations shorter/longer than 305 days were standardized according to the methodology of milk recording. In case of the lactations shorter than 305 days the regression on days in milk was applied. Because the effect of the lactation proved to be significant in the preliminary analysis, the milk performance data were adjusted for the successive lactation and the final model for the evaluation of individual effects was as follows:

$$Y_{ijkl} = \mu + g_i + r_j + g_k + bw_{ijk} + e_{ijkl} \text{ where:}$$

Y_{ijkl} – value of the trait, μ – general mean, g_i – effect of genotype ($i = \text{Leu/Leu, Leu/Val}$), r_j – effect of year-calving season ($j = 1, \dots, 18$), g_k – effect of the farm ($k = 1, 2$), bw_{ijk} – regression on days in milk, e_{ijkl} – random error

Results and Discussion

The performed analysis of the Leu/Val polymorphism in the population of 115 Polish Red cows showed a high frequency of Leu/Leu homozygote (0.57) vs. very low frequency of Val/Val homozygote (0.18) (Table 1). The distribution of genotypes corresponded with that observed by Citek et al. (2000) and Skinkyte et al. (2005) in Polish and Lithuanian cows.

Table 1

Distribution of genotypes and alleles for Leu127Val polymorphism in *GH locus* in Polish Red cows

Farm (number of animals)	Genotype			Allele	
	Leu/Leu (n)	Leu/Val (n)	Val/Val (n)	GH ^L	GH ^V
Szczyrzyc (59)	0.559 (33)	0.441 (26)	0.000	0.771	0.229
Jodłownik (56)	0.589 (33)	0.375 (21)	0.036 (2)	0.777	0.223
Total (115)	0.574 (66)	0.408 (47)	0.018 (2)	0.774	0.226

n — number of observations

Analysis of variance showed that the effect of Leu127Val polymorphism of growth hormone on milk performance traits in Polish Red cattle was generally not significant (Table 2). It was found that only milk protein content in the first lactation was significantly higher in the cows with Leu/Val genotype but that effect was not observed when the data from the first three lactations were used for calculations.

Also, there is no evidence in literature of the association between the polymorphism of growth hormone gene and milk performance traits in Polish Red cattle. However, the results obtained for other breeds indicate the existence of such relationships. Lucy et al. (1993) observed the positive effect of GH^L allele on milk yield of Holstein cows

Table 2

Least-square means (LSM) for the analysed traits of milk performance in the 1st and 1st – 3rd lactations depending on genotype

Trait	1 st lactation Genotype		p	1 st – 3 rd lactation Genotype		p
	Leu/Leu (n=54)	Leu/Val (n=43)		Leu/Leu (n=119)	Leu/Val n=92)	
Milk (kg)	2535,98	2552,22	0,279	2847,15	2787,74	0.320
±SE	577.36	677.73		659.01	737.64	
Protein (kg)	81.17	83.83	0.279	92.57	91.45	0.409
±SE	26.04	26.75		21.7	23.54	
Protein (%)	3.20*	3.35*	0.032	3.26	3.32	0.159
±SE	0.23	0.27		0.19	0.339	
Fat (kg)	110.42	107.84	0.884	120.22	116.04	0.645
±SE	40.88	34.29		46.60	32.36	
Fat (%)	4.21	4.24	0.835	4.38	4.44	0.135
±SE	0.39	0.41		0.374	0.413	

n — number of observations, SE- standard error

* — significant differences at $p \leq 0.05$

The analysis performed by Lee et al. (1996) proved that the HF cows of with Leu/Leu and Leu/Val genotypes were characterized by higher milk yield than the cows with Val/Val genotype. Grochowska and Zwierzchowski (2000) observed that the Leu/Leu homozygotes of Danish Holstein cattle produced more milk and fat but less protein compared with heterozygotes. Contrary to above statements, Sabour et al. (1997) noted a statistically significant effect of GH^V allele on protein content in milk. A higher frequency of Leu/Val genotype observed by this authors in the group of Holstein bulls of the highest breeding value may suggest that GH^V allele are more desirable for milk performance improvement. Chrenek et al. (1999) noted a higher fat and protein content, by 0.36% and 0.18 % - respectively, in milk of Brown Swiss cows with Leu/Leu compared with Val/Val genotype. Also, the Black-and-White cows of various *GH* genotypes differed significantly in milk performance. The highest yield was observed in cows with Leu/Leu and Leu/Val genotypes and the lowest in those with Val/Val genotype (Grochowska et al., 2001). In the same paper the relationship between *GH* genotype and milk traits in the Danish Jersey and Danish Red cattle was not found. The higher (by 225 kg) milk yield in the first lactation of Black-and-White cows with Leu/Leu genotype, as compared to the Leu/Val heterozygote, was observed also by Dybus (2002). On the other hand, Zwierzchowski et al. (2002) reported higher milk yield in Val/Val homozygote of Black-and-White cows, as compared to the cows with Leu/Leu and Leu/Val genotypes, respectively by 2.1 kg and 3.3 kg. Milk of Val/Val cows was characterized by a higher content of components (e.g. protein - by 0.3 %) than milk of Leu/Leu homozygotes.

The most probably growth hormone can affect the milk production, but certainly it is not the only one of factor influencing that process. The investigations carried out by Zwierzchowski et al. (2002) indicated that the highest effect on milk yield of Black-and-White cows had cow's age, stage of lactation and somatic cell count in milk (SCC). A considerable effects of *GH*, *PIT* (*Pit-1* transcription factor) and *LEP* (leptin) genotypes were also detected for *PIT* and *LEP* genotypes. They amounted to 25% of the total effects. Zwierzchowski et al. (2002) sequenced the influence of the studied effects as follows: cow's age (successive lactation) > lactation stage > SCC > PIT ≥ LEP > GH. However, Sørensen et al. (2002) claim that GH can be a physiological indicator of milk yield. The lines of dairy cows selected for high milk yield are characterized by a higher concentration of endogenous growth hormone in blood compared with the lines of low yielding cows (Klemetsdal et al., 1992).

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ВЗАЄМОЗВ'ЯЗОК МІЖ ПОЛІМОРФІЗМОМ ГЕНА ГОРМОНУ РОСТУ LEU127VAL І НАДОЄМ МОЛОКА ПОЛЬСЬКОЇ ЧЕРВОНОЇ ВЕЛИКОЇ РОГАТОЇ ХУДОБИ

Резюме

Идентифікація Leu127Val (Alu I) поліморфізму в локусі гормону росту (GH) у польської червоної породи ВРХ була здійснена, використовуючи техніку PCR-RFLP. Встановлено наступне розміщення генотипів: Leu/Leu 0.574, Leu/Val 0.408, Val/Val 0.018. У зв'язку з дуже низькою частотою генотипа Val/Val його було виключено з досліджень. Частота GH^L алелі склала 0,774. Вивчався взаємозв'язок між поліморфізмом Leu127Val і молочною продуктивністю корів за першу лактацію, і перших три лактації. В першу лактацію корови з генотипом Leu/Leu характеризувалися на 0,15 % вищим вмістом протеїну в молоці ($p \leq 0,05$), у порівнянні з коровами з генотипом Leu/Val. Генотип не вплинув на вихід молока, протеїну і жиру, так само як і на вміст жиру в молоці.

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ВЗАИМОСВЯЗЬ МЕЖДУ ПОЛИМОРФИЗМОМ ГЕНА ГОРМОНА РОСТА LEU127VAL И НАДОЕМ МОЛОКА ПОЛЬСКОГО КРАСНОГО КРУПНОГО РОГАТОГО СКОТА

Резюме

Идентификация Leu127Val (Alu II) полиморфизма в локусе гормона роста (GH) польской красной породы ВРХ была осуществлена, используя технику PCR-RFLP. Установлено следующее размещение генотипов: Leu/Leu 0.574, Leu/Val 0.408, Val/Val 0.018. В связи с очень низкой частотой генотипа Val/Val его было исключено из исследований. Частота GH^L алели составила 0,774. Изучалась взаимосвязь между полиморфизмом Leu127Val и молочной производительностью коров за первую лактацию, и первых три лактации. В первую лактацию коровы с генотипом Leu/Leu характеризовались на 0,15 % высшим содержанием протеина в молоке ($p > 0,05$), в сравнении с коровами с генотипом Leu/Val. Генотип не повлиял на выход молока, протеина и жира, так же как и на содержание жира в молоке.

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