GROWTH HORMONE GENE POLYMORPHISM AND ITS INFLUENCE ON MILK TRAITS IN CATTLE BRED IN LITHUANIA

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Abstract. The aim of the present study is to investigate the polymorphism of growth hormone gene in cattle grown in Lithuania and its influence on farming characteristics. In the investigated group of 291 cattle belonging to 10 breeds the growth hormone gene allele A was found with the frequency of 0.751 and allele B with the frequency of 0.249. Allele A was found with the maximum frequency (0.900) in Hereford breed and allele B (0.438) in Simmental breed. The growth hormone allele frequencies between dairy and beef cattle breeds did not differ. Growth hormone gene genotype AA was found in 62.9% of the cattle, heterozygous AB genotype in 24.4% and BB genotype in 12.7%. Genotype AA, with the highest frequency of 90% was found in Hereford cattle, genotype AB in 55.6% of Lithuanian Black and White and genotype BB in 37.5% of the old Lithuanian Black and White breed. Through investigation of the effects of genetic factors on the indicators of bovine milk yield and composition, the largest statistically significant impact of the growth hormone gene to the average percentage of fat content and milk yield was determined. It affected around 2% of the diversity of these indicators. Allele A of the growth hormone increases milk fat percentage, and allele B increases milk volume during lactation.

Keywords: growth hormone (GH), restriction fragments length polymorphism (RFLP), cattle.

AUGIMO HORMONO GENO POLIMORFIZMAS IR JO ĮTAKA LIETUVOJE VEISIAMŲ GALVIJŲ PIENINGUMO POŽYMIAMS

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Santrauka. Tyrimo tikslas – ištirti augimo hormono geno genetinių tipų paplitimą tarp Lietuvoje veisiamų galvijų veislių ir nustatyti jo įtaką ūkinėms savybėms. Tirtoje 291 galvijo, priklausančio 10 veislių, grupėje augimo hormono geno A alelis rastas 0,751 dažniu, B alelis – 0,249 dažniu. A alelis didžiausiu dažniu (0,900) rastas Herefordų veislėje, o B alelis (0,438) – Simentalio veislėje. Augimo hormono geno alelių dažnis tarp pieninių ir mėsinių galvijų veislių nesiskyrė. Augimo hormono geno AA genotipą turėjo 62,9 proc. galvijų, heterozigotinį AB genotipą – 24,4 proc., BB genotipą – 12,7 proc. Daugiausia AA genotipo (90 proc.) turėjo Herefordo veislės galvijai, AB genotipo – Lietuvos juodmargiai (55,6 proc.) ir BB – seno genotipo Lietuvos juodmargiai (37,5 proc.). Ištyrus genetinių veiksnių įtaką galvijų pieno kiekio ir sudėties rodikliams, nustatyta didžiausia statistiškai reikšminga augimo hormono GH geno įtaka riebalų procentui ir vidutiniam pieno kiekiui. Jis darė įtaką maždaug 2 proc. šių rodiklių įvairovės. Augimo hormono GH geno A alelis didina riebalų procentą piene, o B alelis – pieno kiekį per laktaciją.

Raktažodžiai: augimo hormonas (GH), restrikcinių fragmentų ilgio polimorfizmas (RFIP), galvijai.

Introduction. It is known that the productivity of livestock and other farming characteristics, such as meat yield, growth rate, milk production, milk composition, and other are inherited from generation to generation, and their formation and functional characteristics are determined by genes. Currently, with the help of new molecular methods, genetic evaluation of cattle is carried out directly analysing the DNA, which contains all the genetic information of animal's inherited characteristics. These methods allow for detailed investigation of bovine genome, and determination as which genes and how operate and influence meat and milk quantity and quality, what genetic factors influence cattle health, genetic diversity and genetic relationships among breeds (Blott et al., 1998). Specific genetic markers, if used in a targeted way in animal genotyping, significantly increase the efficiency of selection because they can help to carry out the selection by one or more features, which are manifested due to the occurrence of one or more bonded genes. A method for the determination of such locus of quantitative traits is called Marker Assisted Selection (MAS) (Lien et al., 1993). There is a number of genes discovered that affect milk production. They are: GH (growth hormone), GHR (growth hormone receptor), STAT5 (signal vector and transcription activator), MSTN (myostatin), LEP (leptin), IGF1 (insulin growth factor), PIT1 (pituitrin transcription factor), PREF1 (preadipocyte factor), casein and lactoglobuline genes (Lien et al., 1999; Minoshima et al., 2001; Zwierzchowski et al., 2002; Flisikowski et al., 2003; Peciulaitiene et al., 2007; Krasnopiorova et al., 2010).

Growth hormone (GH) and prolactin (PRL) are polypeptide hormones, which have evolved from a common ancestral gene. Although PRL and GH are produced by cells of the anterior pituitary that have a common stem cell, there are clear and distinct functions

of these two hormones. Both hormones have been shown to be important for control of mammary growth, lactogenesis and lactation (Bole-Feusot et al., 1998).

Bovine growth hormone (GH) plays a very important role in many physiological actions (Kratochvilová, 2000). It is made of 190 or 191 amino acids and alanine or phelynanine at the N-terminus (Wood et al., 1989). The GH gene consists of 5 exons and 4 introns, is mapped on chromosome 19 in cattle (Hediger et al., 1990). Growth hormone has wide physiological activities, which include the regulation of growth, lactation and mammary gland development, gluconeogenesis, the activation of lipolysis, and the enhancement of amino acid incorporation into muscle protein (Burton et al., 1994).

The **aim of the present study** is to investigate polymorphism of growth hormone gene and its influence on farming characteristics in cattle grown in Lithuania.

Material and methods. In total 291 cows and bulls were genotyped belonging to Lithuanian Black and White (LBW), Old Genotype Lithuanian Black and White (O-LBW), Lithuanian Red (LR), Danish Black and White (DBW), Danish Red (DR), Holstein (H), Hereford (He), Limousin (Li), Simmental (Si), and Charolais (Ca). The influence of GH gene on milk yield was investigated in a group of 128 cows. The data of productivity of animals were obtained from the Record Processing Centre SE "Kaimo verslo pletros ir informacijos centras".

Blood samples to vacuum test-tubes were collected from jugular vein (Venoject, Belgium) with EDTA (K3). DNA was extracted using standard phenol-chloroform purification method. All PCR reactions were performed using Applied Biosystems 2700 Thermal Cycler. GH gene was analysed by PCR-RFLP method, using GH forward (5´–GTGGGCTTGGGGAGACAGAT–3´) (Inter-lux) and GH reverse (5´–GTCCTCACTGCGCATG TTTG–3´) (Interlux) oligonucleotide primers (10pmol), 0.2 mM dNTP, 50 mM MgCl$_2$, 10x Taq Buffer (NH$_4$)SO$_4$, 1 U Taq DNA polymerase, BSA (20 mg/ml) (Fermentas). Polymerase chain reaction (PCR) was done with the following conditions: 95 $^{\circ}$C for 2 min, followed by 40 cycles of 94°C for 45 s, 60 $^{\circ}$C for 1 min. and 72 $^{\circ}$C for 1 min, with a final extension at 72 $^{\circ}$C for 5 min. After amplification, 10 μL of the PCR amplification product was digested with *AluI* (Grida) restriction enzyme. After PCR, the 282bp fragment was obtained. After digestion with restriction enzyme, fragments for A allele 150bp, 82bp, 50bp and for B allele –150 bp and 132 bp were obtained. Visualization of the different GH genetic types was carried out by 4 % agarose gel electrophoresis after staining the gels with ethidium bromide, using Heliorab video documentation system.

Statistical analysis. The observed number of alleles and genotypes, the observed and expected heterozigosity for each locus and each breed and the average heterozigosity over all loci were used to assess the genetic variability of studied populations. The Hardy Weinberg equilibrium was performed to evaluate the population differentiation. Associations between different polymorphic sites and milk yield and composition in GH gene were analysed using single factorial and multifactioriall analysis of variance (ANOVA) with R package (http://cran.r-project.org/bin/windows/base/). Following General Linear Models were used for calculations of effects:

Milk yield, kg $_{ijklm}$ = μ + GH$_i$ + breed$_k$ + lactation$_l$ + lactationlengh$_{reg}$ + e$_{ijklm}$

Fat, kg $_{ijklm}$ = μ + GH$_i$ + breed$_k$ + lactation$_l$ + lactationlengh$_{reg}$ + e$_{ijklm}$

Protein, kg $_{ijklm}$ = μ + GH$_i$ + breed$_k$ + lactation$_l$ + lactationlengh$_{reg}$ + e$_{ijklm}$

Fat, % $_{ijklmn}$ = μ = + GH$_i$ + breed$_k$ + lactation$_l$ + e$_{ijklm}$

Protein, %$_{ijklmn}$ = μ = + GH$_i$ + breed$_k$ + lactation$_l$ + e$_{ijklm}$

Results and discussion. The polymorphism of the growth hormone gene was studied in 10 dairy and beef cattle breeds. Frequencies of alleles and genotypes of the growth hormone GH gene for each tested breed were calculated. According to our findings and other scientists, the growth hormone GH gene showed two alleles A and B, with A allele predominant in all breeds (Sabour et al., 1997; Lucy et al., 1993 Kemenes et al., 1999; Vukasinovic et al., 1999). In our findings, the frequency of allele A of the growth hormone GH gene varied from (0.563) in Simmental breed to (0.900) in Hereford breed. Selection is carried out in Europe in respect of the allele B and specific herds of cattle are formed. Much attention is paid to allele B because it is associated with improvement of milk processing characteristics (Lien et al., 1999; Strzalkowska et al., 2002) and meat properties (Dario et al., 2004). The highest frequency of GH B allele we found in Danish Red and Simmental cattle, the lowest in Hereford. Our data correlate with other scientists, testing results. High frequency of GH B allele was identified in Slovak Simmental (Chrenek et al., 1998) and Jerzy (Lucy et al., 1993) breeds, low frequency in Hereford breed (Vukasinovic et al., 1999). No statistically significant differences were found between dairy and beef cattle breeds for the growth hormone gene' allele frequencies (Table 1).

Three different genotypes – AA, AB and BB – were found in growth. The AA genotype of the growth hormone GH gene in the lowest frequency (0.444) was found in the Old Genotype Lithuanian Black and White cattle breed, the highest (0.900) in Hereford breed of cattle. Meanwhile, the GH genotype AB with lowest frequency of (0.111) was found in Limousin cattle breed, the highest (0.556) in the Old Genotype Lithuanian Black and White cattle breed. In Hereford cattle breed, GH AB genotype was not identified. The genotype BB of the growth hormone GH was found the highest frequency (0.375) in Simmental breed. In the Old Genotype Lithuanian Black and White cattle breed, GH BB genotype was not found (Table 1).

The observed heterozygosity was found to be less than expected in growth hormone gene locus in the whole investigated group of animals as well as in dairy cattle and beef cattle groups. The deviation from Hardy-Weinberg equilibrium law was statistically significant (p 0.05).

Table 1. **Frequency of growth hormone GH gene alleles and genotypes in 10 cattle breeds**

Breed	Frequency of GH gene alleles		Frequency of GH gene genotypes		
	A	B	AA	AB	BB
DBW	0.692	0.308	0.487	0.410	0.103
DR	0.600	0.400	0.450	0.300	0.250
H	0.692	0.308	0.462	0.462	0.077
O - LBW	0.722	0.278	0.444	0.556	-
LBW	0.823	0.177	0.731	0.183	0.086
LR	0.739	0.261	0.565	0.348	0.087
Dairy breeds	0.751	0.249	0.604	0.294	0.102
HE	0.900	0.100	0.900	-	0.100
SI	0.563	0.438	0.500	0.125	0.375
CA	0.850	0.150	0.750	0.200	0.050
LI	0.639	0.361	0.583	0.111	0.306
Beef breeds	0.750	0.250	0.681	0.138	0.181
Tested group	0.751	0.249	0.629	0.244	0.127

Table 2. **Mean expected and observed heterozygosity in growth hormone gene locus**

Breed	Growth hormone gene		
	H Observed	H Expected	χ^2 – test (p - meaning)
Dairy breeds	0.294	0.374	8.87 (0.012)
Beef breeds	0.138	0.375	37.5 (0.000)
Tested group	0.244	0.374	35.2 (0.000)

Table 3. **Influence of genetic factors on milk yield and composition**

Genetic factors	Number of classes	Milk per lactation, kg	Fat, %	Fat, kg	Protein, %	Protein, kg
GH gene	3	2.1*	1.9*	0.1	0.9	0.1*
Breed	5	2.3***	6.9***	1.1**	6.1***	2.4***
Lactation	4	1.4***	0.2	1.6***	5.2***	2.5***
Breed x lactation	12	1.9**	5.2*	2.6***	3.8	1.2**
Breed x GH	12	0.5	4.5**	0.5	1.1	0.5
Lactation x GH	12	0.4	0.3	0.5	1.2	0.2

*P<0.05; **P<0.01; ***P<0.001

Table 4. **Influence of GH gene on milk yield and composition**

GH gene genotype	Milk per lactation, kg	Fat, %	Fat, kg	Protein, %	Protein, kg
AA	6085,0±114,44 [a]	4,56±0,05 [a]	275,9±5,25	3,46±0,03	209,5±3,24 [a]
AB	6011,5±124,67 [a]	4,45±0,06 [a]	269,6±5,71	3,43±0,03	204,2±3,52 [a]
BB	6844,5±278,53 [b]	4,08±0,13 [b]	280,2±12,77	3,36±0,06	230,9±7,87 [b]

a,b – the averages marked in the column of the table with different letters differ statistically significantly (P<0.05)

In the investigated cow group, the mean amount of milk and the content of milk components obtained was 6193.5 ± 124.35 kg of milk over lactation, 4.46 ± 0.03% milk fat, 276.2 ± 5.74 kg of milk fat, 3.41 ± 0.02% milk protein, 210.6 ± 4.25 kg of milk protein, 27.8 ± 0.31 kg of milk on average over day, 8021.6 ± 164.04 kg of base milk over lactation, an average of 35.7 ± 0.36 kg of base milk over day.

In order to highlight the effect of the growth hormone gene on milk yield and milk components and to eliminate other factors, a multifactor analysis was performed. Linear mixed model was applied for the analysis of individual factors such as growth hormone GH gene, effects of breed, lactation and combined factors on milk yield and milk components. Breed and lactation significantly influenced all indicators of milk yield and composition. Breed influenced 2.3% of milk yield diversity, 6.9% fat percentage and 6.1% protein percent

diversity. Lactation affected 1.4% of milk yield diversity, 1.6% fat kg and 2.5% protein kg diversity. The growth hormone gene significantly influenced 2.1% of the milk yield diversity, 1.9% milk fat percentage and 0.1% milk protein kg diversity (Table 3).

The differences between breeds and in the breeds between lactations were statistically significant, therefore the differences between the genotypes are presented in Table 4 after the evaluation of lactation and breed influence.

It was found that the cows of BB genotype of growth hormone gene yielded 759.5 kg (p <0.01) more milk over lactation than the cows with AA genotype and 833 kg (p<0.05) than the cows with AB genotype; they had lower milk fat content 0.37% (p<0.05) compared to AB and 0.48% compared to AA (p<0.001) genotype cows and their milk protein content was 0.07% less than for AB and 0.10% less than of AA genotype cows (Table 4). Our data correlate with the data obtained by Khatami (2005) and Zhou (2005) who found a link between the GH allele B with dairy properties. Similar trends for milk yield were found in the research of Lucy et al. (1993) and Lee et al. (1996). On the other hand, scientists Grochowska and Zwierzchowski (2000) found a greater allele A role in milk production in Holstein cattle. Meanwhile, researchers Van der Welf et al. (1996) in their study could not find a link in the GH locus to dairy properties.

Research shows that a new gene identification technology allows investigating the genotype of the animal and determining the genes encoding the productivity and quality traits and useing them in the selection process as genetic markers. The use of genetic markers for livestock breeding offers the opportunity to evaluate the animal from the inside and make full use of the inherent farming characteristics. Genetic markers can be used both for the identification of a single gene and a gene group of a decisive trait or traits group. Another advantage for the use of genetic markers in breeding is that this method of livestock valuation is reliable, allows identifying the genes controlling the selective and technological value of livestock in young age and assessing the genetic variability and genetic defects of farm animals and enables to avoid the manifestation of unwanted traits on time. Use of genetic markers in selection can greatly accelerate the breeding process, improve the quality of agricultural production, reduce its production costs and make production more competitive in foreign markets.

Conclusions. By investigation of the effects of genetic factors on the indicators of bovine milk yield and composition, the largest statistically significant impact of the growth hormone gene to the average percentage of fat content and milk yield were determined. It affected around 2 percent of the diversity of these indicators. Allele A of the growth hormone increases milk fat percentage, and allele B increases milk yield during lactation.

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