LITHUANIAN UNIVERSITY OF HEALTH SCIENCES
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CATTLE GROWTH HORMONE, LEPTIN AND MYOSTATIN GENE POLYMORPHISM AND INFLUENCE TO FARM TRAITS IN CATTLE BRED IN LITHUANIA

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GALVIŲ AUGIMO HORMONO, LEPTINO IR MIOSTATINO GENŲ ĮVAIROVĖ BEI ĮTAKA ŪKINOS SAVYBĖMS LIETUVOJE VEISIAMŲ GALVIŲ TARPE

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INTRODUCTION

It is known that the productivity of livestock and other farming characteristics, such as meat yield, growth rate, milk production, milk composition, and others 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 synthetic markers are used for cattle genotyping that recognize and determine such characteristics as meat yield, milk yield, but less is known of genes that are related to reproductive performance. 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 the meat and milk production. This is 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. 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 use them in the selection process as genetic markers.

Use of genetic markers for livestock breeding offers the opportunity to evaluate the animal from 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 in a young age to identify the genes controlling the selective and technological value of livestock, to assess 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.

Aim of the present study
To investigate polymorphism of growth hormone, leptin and myostatin genes in cattle grown in Lithuania and their influence to farm traits.

Tasks of the present study
1. Genotype polymorphisms in cattle GH (growth hormone), LEP (leptin) and MSTN (myostatin) genes.
2. Estimate allele and genotype frequencies in GH, LEP and MSTN locuses in cattle grown in Lithuania.
3. Investigate influence of one nucleotide polymorphisms (VNPs) in GH and LEP genes for cattle milk yield, milk composition and weight gain.

Novelty of the present study
In this study first time in Lithuanian cattle population (11 breeds and 3 crossbreeds) are presented SNPs polymorphisms in candidate genes GH, LEP, MSTN and their influence on farm traits.

Practical importance
The polymorphisms of the bovine growth hormone and leptin gene found and their influence on the characteristics of farming such as milk production, composition and fattening indicators enable them to be used as biomarkers in the selection process thus accelerating it through the molecular technologies, increasing the breeding value of livestock and improving their farming characteristics and quality of products.

MATERIAL AND METHODS

Selected animals
In different stages of this research in total were genotyped 359 animals belonging to Lithuanian Black and White (LBW), Old type 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), Sharolais (Sa), Angus (An) and crossbreds of Lithuanian Black and White with Belgian Blue, Sharolais and Limousins. 270 animals were genotyped for myostatin gene polymorphisms, 128 cows for GH and LEP gene polymorphisms investigating influence to milk traits, 163 bulls for GH and LEP gene polymorphisms investigating influence to beef traits, 33 bulls for GH gene polymorphisms to investigate influence to semen quality.

The data of productivity of animals were obtained from Record Processing Center SE “Kaimo verslo pletros ir informacijos centras”, daily gain
records from SE “Silutes veislininkyste”, semen quality records from SE “Panevezio veislininkyste”.

**Research methods**

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 (Miller et al., 1988). Hair root samples for DNA genotyping were extracted using method received from Van Haeringen laboratory (Holland) and deep-frozen sperm using standard phenol-chloroform purification method according Dr. J. Kantanen MTT (Finland).

The method of polymerase chain reaction (PCR) and restriction length polymorphism (RFLP) (Sakai et al., 1988) were used to genotype GH, LEP and MSTN polymorphisms. All PCR reactions were performed using Applied Biosystems 2700 Thermal Cycler. The PCR based detection of polymorphisms was carried out using primers’ sequencies, polymerase chain reaction conditions and restriction endonucleases according to growth hormone gene (Sadeghi et al., 2005), for leptin gene (Choudhary et al., 2005) and for myostatin gene (Smith et al., 2000; Stasio and Rolando, 2005), techniques and procedures used at the Janusauskas Laboratory of Animal Genetics, Lithuanian Veterinary Academy. Visualization of the different GH, LEP and MSTN genetic types was carried out by agarase gel electrophoresis after staining the gels with ethidium bromide, using Heliorab video documentation system.

**Statistical analysis**

Observed number of alleles, genotypes, observed and expected heterozigosity for each locus and each breed, 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, milk composition and weight daily gain in GH and LEP genes of Lithuanian cattle were analysed using single factorial and multifactorial analysis of variance (ANOVA) with R package (Gentlemen and Ihaka, 1997). Following General Linear Models were used for calculations of effects:

\[
\text{Milk yield, kg}_{ijklm} = \mu + \text{GH}_i + \text{LEP}_j + \text{breed}_k + \text{lactation}_l + \text{lactation length}_\text{reg} + e_{ijklm}
\]

\[
\text{Fat, kg}_{ijklm} = \mu + \text{GH}_i + \text{LEP}_j + \text{breed}_k + \text{lactation}_l + e_{ijklm}
\]

\[
\text{Protein, kg}_{ijklm} = \mu + \text{GH}_i + \text{LEP}_j + \text{breed}_k + \text{lactation}_l + e_{ijklm}
\]

\[
\text{Fat, %}_{ijklmn} = \mu + \text{GH}_i + \text{LEP}_j + \text{breed}_k + \text{lactation}_l + e_{ijklm}
\]

\[
\text{Protein, %}_{ijklmn} = \mu + \text{GH}_i + \text{LEP}_j + \text{breed}_k + \text{lactation}_l + e_{ijklm}
\]

\[
\text{Average milk yield per day, kg}_{ijklm} = \mu + \text{GH}_i + \text{LEP}_j + \text{breed}_k + \text{lactation}_l + e_{ijklm}
\]

\[
\text{Daily gain, g}_{ijklm} = \mu + \text{GH}_i + \text{LEP}_j + \text{breed}_k + \text{bull}_l + e_{ijklm}
\]

**RESULTS AND DISCUSSION**

In recent years, there is a significant increase in interest in the beef and dairy cattle breeding based on genetic technologies. Quantitative traits, such as animal growth rate, feed conversion efficiency, carcass and internal fat mass, or such as milk, fat and protein content and some technological properties of milk are controlled by multiple loci in the chromosomes. Genes candidates are selected on the basis of known relationships between physiological and biochemical processes and properties. Sequence alterations may be used as genetic markers (Beckmann and Soller, 1983).

**Distribution of alleles’ and genotypes’ frequencies in single nucleotide polymorphisms between growth hormone (GH) and leptin (LEP) genes**

The polymorphism of the growth hormone and leptin gene were studied in 10 dairy and beef cattle breeds. Frequencies of alleles and genotypes of the growth hormone GH and leptin LEP genes 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 the allele B because it is associated with improvement of milk processing characteristics (Strzalkowska et al., 2002; Lien et al., 1999; McLean et al., 1984) 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., 1991) and Jerry (Lucy et al., 1993) breeds, low frequency in Hereford breed (Vukasinovic et al., 1999). According to our results, two alleles A and B of the leptin gene were found. The frequency of allele A of leptin LEP gene in the investigated cattle ranged from (0.700) in the Danish Red breed to (0.875) in Simmental breed. Comparing our findings with literature data, similar results of studies were obtained. These results strongly suggest that selection forces could act against the LEP gene allele B or the selection environment was more favourable to allele A. Our research results show that cattle breeds bred in Lithuania usually have the same LEP alleles, as the European cattle breeds. No statistically significant differences were found between dairy and beef cattle breeds for the growth hormone and leptin genes’ allele frequencies (Table 1).

Table 1. Frequencies of growth hormone GH and leptin LEP gene alleles in 10 cattle breeds bred in Lithuania

<table>
<thead>
<tr>
<th>Breed / Veislė</th>
<th>Frequency of GH gene alleles / GH geno alelijai</th>
<th>Frequency of LEP gene alleles / LEP geno alelijai</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>DBW / DJ</td>
<td>0.692</td>
<td>0.308</td>
</tr>
<tr>
<td>DR / DZ</td>
<td>0.600</td>
<td>0.400</td>
</tr>
<tr>
<td>H</td>
<td>0.692</td>
<td>0.308</td>
</tr>
<tr>
<td>LBW-OT / SG-LJ</td>
<td>0.722</td>
<td>0.278</td>
</tr>
<tr>
<td>LBW / LJ</td>
<td>0.823</td>
<td>0.177</td>
</tr>
<tr>
<td>LR / LZ</td>
<td>0.739</td>
<td>0.261</td>
</tr>
<tr>
<td>Milk breeds / Pieninės veislės</td>
<td>0.751</td>
<td>0.249</td>
</tr>
<tr>
<td>HE</td>
<td>0.900</td>
<td>0.100</td>
</tr>
<tr>
<td>SI</td>
<td>0.563</td>
<td>0.438</td>
</tr>
<tr>
<td>SA</td>
<td>0.850</td>
<td>0.150</td>
</tr>
<tr>
<td>LI</td>
<td>0.639</td>
<td>0.361</td>
</tr>
<tr>
<td>Beef breeds / Mėsinės veislės</td>
<td>0.750</td>
<td>0.250</td>
</tr>
<tr>
<td>Tested group / Tirta grupė</td>
<td>0.751</td>
<td>0.249</td>
</tr>
</tbody>
</table>

Three different genotypes – AA, AB and BB – were found in both the growth hormone GH and leptin LEP genes loci. 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 Lithuanian genotype Black and White cattle breed, GH BB genotype was not found. The genotype AA of leptin gene ranged from (0.522) to (0.750) frequencies. Meanwhile, genotype LEP AB ranged from (0.222) in Limousin and in the old genotype Lithuanian Black and White cattle breeds to (0.450) in Charolais cattle breed. LEP BB genotype was not found in

Table 2. Frequencies of growth hormone GH and leptin LEP gene genotypes in 10 cattle breeds bred in Lithuania

<table>
<thead>
<tr>
<th>Breed / Veislė</th>
<th>GH genotypes / GH genotipai</th>
<th>LEP genotypes / LEP genotipai</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AB</td>
</tr>
<tr>
<td>DBW / DJ</td>
<td>0.487</td>
<td>0.410</td>
</tr>
<tr>
<td>DR / DZ</td>
<td>0.450</td>
<td>0.300</td>
</tr>
<tr>
<td>H</td>
<td>0.462</td>
<td>0.462</td>
</tr>
<tr>
<td>LBW-OT / SG-LJ</td>
<td>0.444</td>
<td>0.556</td>
</tr>
<tr>
<td>LBW / LJ</td>
<td>0.731</td>
<td>0.183</td>
</tr>
<tr>
<td>LR / LZ</td>
<td>0.565</td>
<td>0.348</td>
</tr>
<tr>
<td>Milk breeds / Pieninės veislės</td>
<td>0.604</td>
<td>0.294</td>
</tr>
<tr>
<td>HE</td>
<td>0.900</td>
<td>-</td>
</tr>
<tr>
<td>SI</td>
<td>0.500</td>
<td>0.125</td>
</tr>
<tr>
<td>SA</td>
<td>0.750</td>
<td>0.200</td>
</tr>
<tr>
<td>LI</td>
<td>0.583</td>
<td>0.111</td>
</tr>
<tr>
<td>Beef breeds / Mėsinės veislės</td>
<td>0.681</td>
<td>0.138</td>
</tr>
<tr>
<td>Tested group / Tirta grupė</td>
<td>0.629</td>
<td>0.244</td>
</tr>
</tbody>
</table>

Three different genotypes – AA, AB and BB – were found in both the growth hormone GH and leptin LEP genes loci. 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 Lithuanian genotype Black and White cattle breed, GH BB genotype was not found. The genotype AA of leptin gene ranged from (0.522) to (0.750) frequencies. Meanwhile, genotype LEP AB ranged from (0.222) in Limousin and in the old genotype Lithuanian Black and White cattle breeds to (0.450) in Charolais cattle breed. LEP BB genotype was not found in
Hereford, Simmental and Charolais breeds of cattle. According to researcher Bonvillani (2000) the LEP gene genotype BB was not detected in Argentinian Holstein cattle; Simmental and Hereford cattle breeds showed the highest frequency of genotype AA as well as in our study (Table 2).

All the tested breeds had lower observed heterozygosity than expected value with the exception of two breeds: old genotype Lithuanian Black and White cattle and Charolais, where the observed heterozygosity was higher than the expected, but the differences were not statistically significant. Statistically significant deviation from Hardy-Weinberg equilibrium's law and little observed heterozygosity was found in the Lithuanian Black and White and Limousine breeds. After grouping the stock into dairy and beef breeds, both groups showed a statistically significant deviation from Hardy-Weinberg equilibrium with the downward heterozygosity trend, with 7.23 (p<0.007) and 14.6 respectively (p<0.001) (Table 3).

Table 3. Mean expected and observed heterozygosity in 10 cattle breeds bred in Lithuania

<table>
<thead>
<tr>
<th>Breed / Veislė</th>
<th>Observed Heterozygosity / Faktinis heterozigotiskumas</th>
<th>Expected Heterozygosity / Teorinis heterozigotiskumas</th>
<th>$\chi^2$ – test (p – meaning) / (p – reikšmė)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBW / DJ</td>
<td>0.321</td>
<td>0.421</td>
<td>3.24 (0.072)</td>
</tr>
<tr>
<td>DR / DZ</td>
<td>0.300</td>
<td>0.450</td>
<td>2.04 (0.154)</td>
</tr>
<tr>
<td>H</td>
<td>0.346</td>
<td>0.368</td>
<td>0.31 (0.580)</td>
</tr>
<tr>
<td>LBW-OT / SG-LJ</td>
<td>0.389</td>
<td>0.373</td>
<td>0.93 (0.335)</td>
</tr>
<tr>
<td>LBW / LJ</td>
<td>0.204</td>
<td>0.270</td>
<td>3.98 (0.046)</td>
</tr>
<tr>
<td>LR / LZ</td>
<td>0.370</td>
<td>0.396</td>
<td>0.1 (0.756)</td>
</tr>
<tr>
<td>Milk breeds / Pieninės veislės</td>
<td>0.274</td>
<td>0.355</td>
<td>7.23 (0.007)</td>
</tr>
<tr>
<td>HE</td>
<td>0.150</td>
<td>0.218</td>
<td>1.88 (0.170)</td>
</tr>
<tr>
<td>SI</td>
<td>0.188</td>
<td>0.355</td>
<td>2.23 (0.136)</td>
</tr>
<tr>
<td>SA</td>
<td>0.325</td>
<td>0.302</td>
<td>1.65 (0.199)</td>
</tr>
<tr>
<td>LI</td>
<td>0.167</td>
<td>0.350</td>
<td>9.62 (0.002)</td>
</tr>
<tr>
<td>Beef breeds / Mėsinės veislės</td>
<td>0.234</td>
<td>0.332</td>
<td>14.6 (0.001)</td>
</tr>
<tr>
<td>Tested group / Tirta grupe</td>
<td>0.261</td>
<td>0.348</td>
<td>14.84 (0.0001)</td>
</tr>
</tbody>
</table>

The assessment of genetic diversity in the loci of growth hormone and leptin gene showed that taking the whole investigated group of animals, the observed heterozygosity was found to be less than expected, but the deviation from Hardy - Weinberg equilibrium's law was not statistically significant (p>0.05). Less genetic diversity of the growth hormone gene locus in both dairy cattle and beef group was found than theoretically expected, while in the leptin gene locus in beef group a statistically significantly higher heterozygosity was found than expected (Table 4).

Table 4. Mean expected and observed heterozygosity in growth hormone and leptin gene locus

<table>
<thead>
<tr>
<th>Breed / Veislė</th>
<th>Growth hormone gene / Augimo hormono genas</th>
<th>Leptin gene / Leptino genas</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBW / DJ</td>
<td>0.294</td>
<td>0.374</td>
</tr>
<tr>
<td>DR / DZ</td>
<td>0.138</td>
<td>0.375</td>
</tr>
<tr>
<td>Tested group / Tirta grupe</td>
<td>0.244</td>
<td>0.374</td>
</tr>
</tbody>
</table>

Association study between different SNPs in GH and LEP genes and milk productivity traits

In the investigated cow group, the mean amount of milk and milk component content obtained was 6193.5 ± 124.35 kg of milk over lactation, 4.46 ± 0.03% milk fat, 276.2 ± 7.4 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 and leptin 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 and leptin LEP gene, effects of breed, lactation and combined factors on milk yield and milk components. Breed and lactation significantly influenced all indicators of the milk yield and composition. Breed influenced 2.3% of the milk yield diversity, 6.9% fat percent and 6.1% percent protein diversity. Lactation affected 1.4% of milk yield diversity, 1.6 % fat kg and 2.5% protein kg diversity. The growth hormone, the gene significantly influenced 2.1% of the milk yield diversity, 1.9% milk fat percentage and 0.1% milk protein kg diversity. Leptin gene significantly influenced 1.7% of the milk yield diversity, 1.7% milk fat kg and 1.6% protein kg (Table 5).

Table 5. Influence of genetic factors to milk yield and composition

<table>
<thead>
<tr>
<th>Genetic factors / Genetiniai veiksnių šaltinis</th>
<th>Number of classes / Klasių sk.</th>
<th>Milk per lactation, kg / Pieno per laktaciją, kg</th>
<th>Fat, % / Riebalai, %</th>
<th>Fat, kg / Riebalai, kg</th>
<th>Protein, % / Baltymai, %</th>
<th>Protein, kg / Baltymai, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH gene / GH genas</td>
<td>3</td>
<td>2.1*</td>
<td>1.9*</td>
<td>0.1</td>
<td>0.9</td>
<td>0.1*</td>
</tr>
<tr>
<td>LEP gene / LEP genas</td>
<td>3</td>
<td>1.7***</td>
<td>0.2</td>
<td>1.7***</td>
<td>0.5*</td>
<td>1.6***</td>
</tr>
<tr>
<td>Lactation dur. / Laktacijos trukė</td>
<td>Cov</td>
<td>71.4***</td>
<td>–</td>
<td>71.7***</td>
<td>–</td>
<td>78.0***</td>
</tr>
<tr>
<td>Breed / Veisle</td>
<td>5</td>
<td>2.3***</td>
<td>6.9***</td>
<td>1.1**</td>
<td>6.1***</td>
<td>2.4***</td>
</tr>
<tr>
<td>Lactation / Laktacija</td>
<td>4</td>
<td>1.4***</td>
<td>0.2</td>
<td>1.6***</td>
<td>5.2***</td>
<td>2.5***</td>
</tr>
<tr>
<td>Breed x lact. / Veisles x laktacija</td>
<td>12</td>
<td>1.9**</td>
<td>5.2*</td>
<td>2.6***</td>
<td>3.8</td>
<td>1.2**</td>
</tr>
<tr>
<td>Breed x GH / Veisles x GH</td>
<td>12</td>
<td>0.5</td>
<td>4.5**</td>
<td>0.5</td>
<td>1.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Lact x GH / Lakt x GH</td>
<td>12</td>
<td>0.4</td>
<td>0.3</td>
<td>0.5</td>
<td>1.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Breed x Lep / Veisles x LEP</td>
<td>15</td>
<td>1.1*</td>
<td>3.1</td>
<td>0.7</td>
<td>3.1*</td>
<td>0.9**</td>
</tr>
<tr>
<td>Lact x Lep / Lakt x LEP</td>
<td>12</td>
<td>0.5</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

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

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

Fig. 1. Comparison of milk yield according GH genotypes

It was found that the cows of BB genotype of growth hormone gene yielded 823.9 kg (p <0.01) more milk over lactation than the cows with AA genotype and 759.5 kg (p<0.05) than the cows with AB genotype (Figure1), 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 (Figure 2) and their milk protein content was 0.07% less than for AB and 0.10% less than of AA genotype cows (Figure 3). Our data correlate with scientists 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 GH1 locus to dairy properties.
Fig. 2. Comparison of fat amount (%) in milk according GH genotypes
2 pav. GH genotipų palyginimas pagal pieno riebumą (%)

Fig. 3. Comparison of protein amount (%) in milk according GH genotypes
3 pav. GH genotipų palyginimas pagal pieno baltymingumą (%)

Fig. 4. Comparison of milk yield (in lactation) according LEP genotypes
4 pav. LEP genotipų palyginimas pagal pieno kiekį (per laktaciją)

Fig. 5. Comparison of fat amount (%) in milk according LEP genotypes
5 pav. LEP genotipų palyginimas pagal pieno riebumą (%)
It was found that the leptin gene, genotype AA cows yielded an average of 639.9 kg (p <0.001) less milk per lactation than cows with genotype AB and 670.6 kg (p<0.05) than cows with BB genotype (Figure 4), and they had 0.12% lower fat content than genotype AB cows and 0.17% lower than genotype BB cows (Figure 5). Nevertheless, the milk of genotype AA cows had higher protein content by 0.02% than that of AB genotype cows, but 0.13% less than of genotype BB cows (p<0.05) (Figure 6).

Association study between different SNPs in GH and LEP genes and fattening traits

In order to highlight the influence of the growth hormone and leptin gene and eliminate other factors contributing to one of the cattle fattening indicators – the weight gain, a multifactor analysis was performed. Linear mixed model was applied for the analysis of individual factors such as growth hormone GH and leptin LEP genes, effects of breed, sire, and combined factors on the weight gain of animals. The breed and the sire statistically significantly affected weight gain rates in all the fattening periods. Breed influenced by an average 16.4% and the sire – 17.6% weight gain characteristics. Both growth hormone and leptin genes statistically significantly influenced weight gain indicators only during the first half of fattening. Growth hormone gene influenced the 2.4% rate of weight gain per day, and leptin gene 4.2% (Table 6).
The influence of bovine GH gene polymorphism to meat properties was studied among Italian Podolian cattle. Animals with BB genotype showed a lower growth rate than individuals with two other genotypes (Dario et al., 2005). According to the research of Zwierzchowski et al. (2001), it was reported that the bull-calves with genotype BB had higher daily weight gain than bull-calves with other genotypes. Di Stasio et al. (2002) found no relationship between the GH polymorphism and meat production characteristics of Piedmont cattle. According to our data bull-calves with the growth hormone GH genotype AA had 68.9 kg greater weight gain than those with genotype AB and 44.7 kg greater weight gain than those with genotype BB (Figure 7).

A comparison of different indicators of the average daily weight gain in accordance with LEP leptin AA, AB and BB genotypes showed that bull-calves with leptin LEP gene AB had 32.8 kg greater weight gain than bull-calves with genotype AA and 6 kg greater weight gain than those with genotype BB (Figure 8).

Association study between different SNPs in GH gene and semen quality
To assess the GH gene influence on semen quality, database of the following phenotypic characteristics has been formed - semen volume, semen concentration, semen mobility. Bulls of the GH gene, genotype BB had better semen characteristics than the bulls with genotypes AA or AB, but statistically significant effect was found only for the semen mobility index. Bulls with GH genotype BB matured faster in comparison with AB and BB genotype bulls. BB genotype bulls had a better exterior score, which statistically significantly exceeded the exterior evaluation of the genotype AA bulls (Table 7). There is also scientific evidence that growth hormone may affect sexual development and testicular function (Lin T., 1996).
Table 7. Influence of growth hormone gene to semen quality
7 lentelė. Augimo hormono įtaka galvijų spermos kokybei

<table>
<thead>
<tr>
<th>Genotype / Genotipas</th>
<th>AA</th>
<th>AB</th>
<th>BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume, ml / Spermos tūris, ml</td>
<td>4.22±0.27</td>
<td>4.07±0.19</td>
<td>4.30±0.4</td>
</tr>
<tr>
<td>Semen concentration, m lbl/ml</td>
<td>0.89±0.05</td>
<td>0.98±0.06</td>
<td>0.98±0.05</td>
</tr>
<tr>
<td>Semen mobility, scores / Spermos judrumas</td>
<td>11.77±1.30</td>
<td>8.93±0.68</td>
<td>14.06±1.74</td>
</tr>
<tr>
<td>Weight at 3 month age, kg / Svoris 3 m.</td>
<td>117.94±3.39</td>
<td>114.89±3.56</td>
<td>116.5±7.68</td>
</tr>
<tr>
<td>Weight at 6 month age, kg / Svoris 6 m.</td>
<td>226.83±7.63</td>
<td>227.56±6.49</td>
<td>243.17±18.42</td>
</tr>
<tr>
<td>Weight at 9 month age, kg / Svoris 9 m.</td>
<td>424.33±10.43</td>
<td>429.56±6.23</td>
<td>445.17±17.89</td>
</tr>
<tr>
<td>Evaluation of exterior, scores / Eksterjero vertinimas</td>
<td>13.46±0.15</td>
<td>13.62±0.15</td>
<td>14.08±0.12</td>
</tr>
</tbody>
</table>

a,b – averages, (p≤0.05)

Distribution of alleles’ and genotypes’ frequencies in two cattle myostatin gene mutations – MSTN1 and MSTN2

The study of 270 different breeds of animals, belonging to Lithuanian Black and White (LBW), Lithuanian Red (LR), Hereford (He), Limousin (Li), Simmental (Si), Sharolais (Sa), Angus (An) and crossbreds of Lithuanian Black and White with Belgian Blue, Sharolais and Limousins took place in order to identify the bovine myostatin gene mutation MSTN1 (11 bp deletion, at 821 bp exon 3) and MSTN2 (single nucleotide change, G→A, in the 938th base pair in exon 3). The study in the investigated group of animals resulted in the identification of two MSTN1 alleles: L1 - wild-type, and MSTN1-a mutated allele and one MSTN2 allele – L2. The MSTN2 locus is monomorphous in the investigated group of cattle regardless the breed. Neither heterozygous animals, carriers of a mutated gene, nor homozygous animals having two copies of the modified gene were found.

The wild-type allele L1 of the myostatin gene was found with the frequency of 0.95, while the mutant allele MSTN1 – with the frequency of 0.05. A total of 89% of the animals tested had a homozygous wild L1/L1 genotype, 11% had a heterozygous L1/MSTN1 genotype and were the carriers of the double-muscle allele. Animals, having two mutated myostatin alleles with the genotype MSTN1/MSTN1 were not found in the test group. It was found that 100% of Charolais, Limousine, Simmental, Angus, Lithuanian Black and White, Lithuanian Red, Lithuanian Black and White crossbreeds with Charolais and Limousine cattle had a wild, i.e. unchanged myostatin gene genotype L1/L1, and the Lithuanian Black and White crossbreeds with the Belgian Blue all had one copy of the mutated myostatin gene and had the genotype L1/MSTN1. Since the myostatin gene mutation (11 bp deletion) was not found in Lithuanian Black and White cattle breed, the crossbreeds inherited this altered allele from the parental side, i.e. Belgian Blue sires. Examination of the phenotype showed that all animals had partly expressed double-muscled phenotype characteristics.

Table 8. Frequencies of myostatin MSTN1 gene alleles and genotypes in cattle bred in Lithuania
8 lentelė. Galvijų miostatino MSTN1 genotipų ir alelių dažniai tirtoje Lietuvoje veisiamų galvijų grupėje

<table>
<thead>
<tr>
<th>Alleles / Aleliai</th>
<th>Frequencies / Dažniai</th>
<th>Genotypes / Genotipai</th>
<th>Frequencies / Dažniai</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1 0.95</td>
<td>L1/L1 0.89</td>
<td>MSTN1 0.05</td>
<td>L1/ MSTN1 0.11</td>
</tr>
<tr>
<td>MSTN1/ MSTN1 0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Our results correlate with those of other researchers. Grobet with co-workers examined the prevalence of seven mutations of the myostatin gene in eleven breeds and found that different myostatin gene mutations are specific to specific breeds - the F 94 L mutation was specific to the Limousine breed, 414 C-T mutation was found in all breeds studied with low frequency, but was characteristic to Galloway and Salers breeds, the mutations of 7-ins 10 nt 419, the mutation Q 204 X, E 226 X and C 313 Y were only found in Limousine breed, but they did not determine the double-muscled phenotype except for F 94 L, the mutation 11 base pair deletion was found in Belgian Blues like in our study and in the South Devon cattle breeds (Grobet et al.,1998). Understanding of double muscling phenotype and creation of specific diagnostic PCR tests for miostatin gene polymorphism identification gives possibilities to farmers to perform genotypic selection according their needs.
CONCLUSIONS

1. In the investigated group of cattle, the growth hormone gene allele A was found with the frequency of 0.751, allele B with the frequency of 0.249. Allele A was found with the maximum frequency (0.900) in Hereford breed, and the allele B (0.438) in Simmental breed. Leptin gene allele A was found with the frequency of 0.799, and the allele B was found with the frequency of 0.201. Allele A was found with the maximum frequency (0.875) in Simmental breed, and B allele B (0.300) in the Danish Red breed. Growth hormone allele frequencies between dairy and beef cattle breeds did not differ. The frequency of leptin gene allele B in dairy cattle breeds group was higher than in the group of beef cattle breeds.

2. In the investigated group of cattle the growth hormone gene genotype AA was found in 62.9% of the cattle, heterozygous AB genotype in 24.4%, 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 genotype. Leptin gene genotype AA was found in 66.0% animals, heterozygous genotype AB in 27.8%, BB genotype in 6.2%. Genotype AA with the highest frequency was found in Simmental and Limousin cattle, genotype AB in the Lithuania's Red and genotype BB in Danish Red cows. Genotype BB was not found on all in Hereford, Simmental and Charolais breeds.

3. Examination of the 11 base pairs deletion mutation, MSTN1 myostatin gene’s wild L allele was found with the frequency of 0.89, whereas the mutant allele MSTN1 with 0.11. As many as 85% of the tested animals had a homozygous wild L/L genotype, 15% had a heterozygous L/MSTN1 genotype and were the carriers of a double-muscled allele. Animals, having two changed myostatin alleles with genotype MSTN1/MSTN1 were not found in the analysed group. It was found that 100% of the Lithuanian Black and White, Limousine, Simmental and Charolais cattle had a wild, i.e. unchanged myostatin gene genotype L/L, and the Lithuanian Black and White crosses with the Belgian Blue all had one copy of the mutated myostatin gene and had the genotype L/MSTN1.

4. After the examination of the single nucleotide mutation in myostatin gene MSTN2 all animals were found to be similar under the genotype L/L, with the wild-type myostatin gene allele. Mutated allele was not found in the investigated group of animals.

5. The investigation of 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 per day was determined. It affected around 2 percent of the diversity of these indicators. The growth hormone’s allele A increases milk fat percentage, and the allele B increases milk volume during lactation. Statistically significant effect of the leptin gene was found to the protein and fat content and milk yield during lactation. It influenced around 2.5% of the diversity of these indicators. Leptin gene’s allele B increases the amount of milk during lactation.

6. The investigation of genetic factors on the weight gain of animals in different fattening periods showed that the leptin gene statistically significantly influenced weight gain in the first two fattening quarters. Leptin gene affected 0.1 to 4.4% of the variety of these indicators. Leptin gene’s allele A increased weight gain of cattle. The cattle with growth hormone genotype AA had the greatest weight gain, but the differences were not statistically significant.

7. Cattle with growth hormone genotype BB had the best semen quality and exterior evaluation.

8. Cattle growth hormone GH, leptin LEP, myostatin MSTN1 genes were found polymorphic, and it allows them to be used as genetic biomarkers for selection. The myostatin gene MSTN2 was found monomorphic in the investigated group of animals.

PROPOSALS

The polymorphisms of the cattle growth hormone and leptin gene found and their influence on the characteristics of farming such as milk production, composition and fattening indicators enable them to be used as biomarkers in the selection process thus accelerating it through the molecular technologies, increasing the breeding value of livestock and improving their farming characteristics and quality of products.
LIST OF PUBLICATIONS ON DISSERTATION TOPIC

Scientific papers in journals listed by ISI:

Scientific papers in International reviewed journals:

Conference thesis:

REZIUMĖ

Darbo tikslas – iššvysti augimo hormono, leptino ir miostatino genų genetinių tipų paplitimą Lietuvoje veisiamų galvijų veislėse ir nustatyti jų įtaką ūkinėms savybėms.

Darbo uždaviniai:
1. Genotipuoti galvijų GH (augimo hormono), LEP (leptino) ir MSTN (miostatino) genų polimorfizmus.
3. Įvertinti genotipų vietų nukleotido polimorfizmų (VNP) GH ir LEP genuose įtaką pieno kiekiui, sudėčiai ir galvijų priesvoriui.
4. Įvertinti GH geno polimorfinių vietų įtaką galvijų spermos kokybei bei eksterjerui.

Darbo naujumai:
Pirmą kartą Lietuvoje auginamų galvijų populiacijoje (11 veislės ir 3 mišrūnų) buvo ištirti vieno nukleotido polimorfizmai GH, LEP ir MSTN genuose kandidatuose bei nustatyta jų įtaka ūkinėms savybėms.

Darbo praktinė reikšmė:
Rasti galvijų augimo hormono ir leptino genų polimorfizmai bei jų įtaka tokioms galvijų ūkinėms savybėms kaip pieno kiekiui, sudėčiai ir penųjios rodikliai sudaro pagrindą pagrindinės veislių selekcijos procese. Šie sienkūnai turėtų būti naudojami kaip viena iš veislių, kurios gali turėti didesnį galvijų veislės vertę.

TYRIMŲ REZULTATAI IR APTARIMAS


Tiek augimo hormono genų, tiek leptino LEP genų lokais buvo nustatyta té社会科学第四次元的研究，以及加速度、加減速度等係數的簡易法則。其中，速度的變化率方程為。以速度的基本概念為基礎，引入加減速度等係數的簡易法則，可以簡化計算過程，提高計算效率。
augimo hormono GH AA, AB ir BB. Augimo hormono GH geno AA genotipas mažiausiai dažnį (0,444) rastas senojo genotipo Lietuvos juodmargių galvijų veislių, didžiausiai (0,900) Herefordų galvijų veislių. Tuo tarpu AB genotipas įvairiai dažniau (0,111) Limuzinų galvijų veislių, didžiausiau (0,556) senojo genotipo Lietuvos juodmargių galvijų veislių. Herefordų galvijų veislių AB genotipas nebuvo nustatytas. Augimo hormono GH BB genotipas didžiausiai dažnai rastų (0,375) Simentalių veislių. Senojo genotipo Lietuvos juodmargių galvijų veislių BB genotipas nebuvo nustatytas. Leptino geno AA genotipas įvairiai nuo (0,522) iki (0,750) dažnio. Tuo tarpu AB genotipas įvairiai nuo (0,222) Limuzinų ir senojo genotipo Lietuvos juodmargių galvijų veislių (0,450) Šarolę galvijų veislių. Herefordų, Simentalių ir Šarolę galvijų veislių BB genotipas nebuvo nustatytas (2 lentelė). Visose tirtose veislių rastas mažesnis faktinis heterozigotiškumas nei teorinis išskyris dvi veisles: senojo genotipo Lietuvos juodmargių galvijų ir Šarolę, kur faktinis heterozigotiškumas buvo didesnis nei teorinis, tačiau skirtumai nebuvo statistiškai reikšmingi. Statistiškai reikšmingas nuokrypis nuo Hardžio–Weinbergo puosiuosvros dėsnio bei mažai išreikštas heterozigotiškumas nustatytas Lietuvos juodmargių ir Limuzinų veislių. Sugrupavus veisles į pieninių ir mėsinių, abiejose veislių grupėse nustatytas statistiškai reikšmingas nuokrypis nuo Hardžio–Weinbergo puosiuosvros dėsnio heterozigotiškumo mažėjimo kryptimi, atitinkamai 7,23 (P<0,007) ir 14,6 (P<0,001) (3 lentelė). Įvertinus genetinę įvairovę augimo hormono ir leptino geno lokuose nustatyta, kad imant visą tūrą rastų galvijų grupę, faktinis heterozigotiškumas rastas mažesnis nei teorinis, bet nuokrypis nuo Hardžio–Weinbergo puosiuosvros dėsnio nebuvo statistiškai reikšmingas (P>0,05). Augimo hormono geno lokuose tiek pieninių, tiek mėsinių rastą mažesnę genetinę įvairovę nei teoriskai planuota, tuo tarpu leptino geno lokuose mėsinių galvijų grupėje rastas statistiškai reikšmingai didesnis heterozigotiškumas nei prognozuotas (4 lentelė). Pieno kiekie ir pieno sudėtinų dalių vidurkiams paskaičiuoti panaudoti Lietuvoje auginamų pieninių veislų - Danijos juodmargių, Danijos žalųjų, Holšteinų, Lietuvos juodmargių, senojo genotipo Lietuvos juodmargių karvų 2008–2009 m. kontrolės produktųvono rodikliai, gauti iš „Zemės ūkio informacijos ir kaimo verslo centro“. Tirtos karvų grupėje vidutinis pieno kiekis ir pieno sudėtinų dalių kiekis gautas 6193,5±124,35 kg pieno per laktaciją, 4,46±0,03 proc. pieno riebalų, 276,2±5,74 kg pieno riebalų, 3,41±0,02 proc. pieno baltymų, 210,6±4,25 kg pieno baltymų, 27,8±0,31 kg vidutiniškai pieno per parą, 8021,6±164,04 kg bazinio pieno per laktaciją, 35,7±0,36 kg vidutiniškai bazinio pieno per parą.
gimo hormono genotipas atskiriems įtakos neturėjo (6 pav.). Falyginus skirtingų priesvorių rodiškių vidurkius pagal leptino LEP AA, AB ir BB genotipus nustatyta, kad leptino geno LEP AB genotipo buliukai turėjo 32,8 kg didesnį priesvorių nei AA ir 6 kg didesnį priesvorių nei BB genotipo buliukai (7 pav.).

Siekiant įvertinti GH geno įtaką spermos kojąbei buvo suformuota sekančių fenotipų požymių duomenų bazė: spermos tūris; spermos koncentracija; spermos judrumas. GH geno BB genotipo buliukai turėjo geresnį spermos rodiškius nei AA ar AB genotipų, tačiau statistiškai reikšminga įtaka nustatyta tik spermos judrumo rodiškiai (7 lentelė).

Leptino geno B alelis didina pieno kiekį per laktaciją.


7. Augimo hormono geno BB genotipo galvijai turėjo didžiausią spermos tūrį, koncentraciją, ju buvo geriausias spermos judrumas ir eksterjero vertinimas.


PASIŪLYMAI

Rasti galvijų augimo hormono ir leptino genų polimorfinzmai bei jų įtaka tokioms galvijų ukinėms savybėms kaip pieno kiekis, sudėtis bei penėjimais rodikliai sudaro priešingas ukinės savybės bei produkčios kokybę.