Cattle growth hormone and leptin genes influence on fattening traits

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This article presents data about growth hormone and leptin gene polymorphism and its influence on cattle fattening traits. A total of 163 animals were genotyped, belonging to the breeds Lithuanian Black and White (LBW), Lithuanian Red (LR), Hereford (HE), Limousin (LI), Simmental (SI), and Charolais (CH). The records of daily weight gain were obtained from the feeding control station of Šilutė bulls feeding. The research method of polymerase chain reaction (PCR) and restriction length polymorphism (RFLP) were used to GH and LEP genotype polymorphisms. In the control group, the growth hormone gene allele A was found, with maximum frequency (0.875) in Simmental breed, and allele B (0.283) in the Lithuanian Red breed. Similarly, Leptin gene allele A was found with maximum frequency (0.875) in Simmental breed, and allele B (0.283) in the Lithuanian Red breed. The AA genotype growth hormone, with the highest frequency of 90%, was found in Hereford cattle, AB genotype in 34.8 % of Lithuanian Red and BB genotype in 37.5 % of the Simmental genotype. While, the leptin gene AA genotype with the highest frequency, was found in Simmental and Limousin cattle, AB genotype in the Charolais and BB genotype in Lithuania Red cows. The research on genetic effects on the weight gain of animals in different fattening periods showed that the leptin gene had a significant influence on the weight gain during the first two fattening quarters. Leptin gene varied from 0.1 to 4.4 %. Leptin gene A allele increased cattle weight gain. Cattle with AA genotype growth hormone had the greatest weight gain, but the differences were not statistically significant.

Key words: *cattle; growth hormone, leptin gene.*

Currently, with the help of new molecular methods, genetic evaluation of cattle is carried out directly through the DNA analysis, which contains all the genetic information of animal's inherited characteristics. These methods allow detailed research of bovine genome and determination of genes, how they operate and influence on the quantity and quality of meat or milk, what genetic factors influence on cattle health, genetic diversity and also, genetic relationships among breeds (Miceikienė *et al*., 2006). The genetic markers, properly used in animal genotyping, increase significantly the selection efficiency as they help to carry it out by one or more features, due to the presence of one or more bonded genes (Jakari *et al*., 2009). There are several genes affecting meat production, which are growth hormone (GH), growth hormone receptor (GHR), myostatin (MSTN), leptin (LEP). Various researches show 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 (Buchanan *et al*. 2002, Stasio Di *et al*. 2002, Choudhary *et al*. 2005, Curi *et al*. 2005, Silveira *et al*. 2008, Jakari *et al*. 2009 and Żukiewicz *et al*. 2012). Genetic markers can be used both for the identification of a single gene and a gene group with traits of economic interest. Another advantage of the genetic markers used in breeding is the reliance of livestock evaluation. It also allows to identify the genes by controlling the selective and technological value of livestock in a young age, to assess the genetic variability and genetic defects of farm animals, and avoids the appearance of unwanted traits on time. The 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 with foreign markets (Miceikienė *et al*. 2006).

The aim of the present study was to analyze growth hormone and leptin gene polymorphism and their influence on cattle fattening traits.

Materials and Methods

Selected animals. A total of 163 animals were genotyped, belonging to the Lithuanian Black and White (LBW), Lithuanian Red (LR), Hereford (HE), Limousin (LI), Simmental (SI) and Charolais (CH) breeds. Offspring of 18 bulls were genotyped. The data on daily weight gain records were obtained from Šilutė control bulls feeding station.

Research methods. Blood samples were extracted from the jugular vein to a vacuum test-tube (Venoject, Belgium) with EDTA (K3). The DNA was extracted using the standard phenol-chloroform purification method (Miller *et al*, 1988). Hair root samples for genotyping DNA were extracted using the van Haeringen laboratory (Holland) method 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) were used

to GH and LEP genotype polymorphisms. All PCR reactions were performed using Applied Biosystems 2700 Thermal Cycler. The PCR based on the detection of polymorphisms was carried out using primary sequencies, polymerase chain reaction conditions and endonucleases restrictions according to the growth hormone gene (Sadeghi *et al*., 2005), for leptin gene (Choudhary *et al*., 2005) techniques and procedures used at the Janusauskas Laboratory of Animal Genetics, Lithuanian Veterinary Academy. Different GH and LEP genetic types were visualized using agarose gel electroforesis after dying the gels with ethidium bromide, according to the documentation system of Heliorab video.

Statistical analysis. The frequency of alleles and genotypes were used to assess the genetic variability of the studied populations for GH and Leptin genes. Associations between different polymorphic sites and daily weight gain in GH and LEP genes of Lithuanian cattle were analysed using ANOVA model of mixed effects with R package (Gentlemen and Ihaka, 1997). The general linear models were used for calculations:

Daily gain, $g_{ijklm} = m + GH_i + LEP_j + breed_k + bull_l + e_{ijklm}$

Daily gain, $g_{ijklm} = m + GH_i + LEP_j + breed_k + progenitor_l + e_{ijklm}$

Fixed effects: GH - growth hormone gene (3 classes); LEP – leptin gene (3 classes); breed – breed of animal (6 classes). Random effect: progenitor – progenitor of animal (18 classes).

Results and Discussion

In recent years, there is a significant increase of interest in beef cattle breeding based on genetic technologies. Quantitative traits, such as animal growth rate, feed conversion efficiency, carcass and internal fat mass are controlled by multiple loci in the chromosomes. Genes candidates are selected on the basis of known relationships among physiological and biochemical processes and its properties, where sequence alterations may be used as genetic markers (Asalkar *et al*. 2005 and Passos *et al*. 2007).

Frequencies of alleles and genotypes of the growth hormone GH and leptin LEP genes were calculated for each tested breed. According to this study and other scientists, the growth hormone GH gene showed two alleles, A and B, with a predominance of A allele in all breeds (Silveira and Oliveira 2008, Mullen *et al*. 2010, Bujko *et al*. 2011, Żukiewicz *et al*. 2012 and Mullen *et al*. 2013). In this study, the frequency of A allele of the growth hormone GH gene varied from 0.563 in Simmental breed to 0.900 in Hereford breed. In Europe, selection is carried out regarding B allele and specific herds of cattle are formed. Great attention is paid to the B allele because it is associated with improvement of meat properties (Dario *et al*. 2004). The highest frequency of GH B allele was found in Simmental cattle and the lowest in Hereford. The data offered in this study agree with other scientists testing results. The highest frequency of GH B allele was identified in Slovak Simmental (Carnicela *et al*. 2003) and Jersey (Moravčíková *et al*. 2012) breeds and the lowest frequency in Hereford breed (Silveira and Oliveira 2008). According to the results, two alleles, A and B, of the leptin gene were found. The frequency of A allele of LEP gene in the studied cattle ranged from 0.717 in the Lithuanian Red breed to 0.875 in Simmental breed. The results of this study were similar to those found in the literature. These results suggest that selection forces may act against the LEP gene B allele or the selection environment was more favourable to A allele. These results show that cattle breeds bred in Lithuania usually have the same LEP alleles, as the European cattle breeds (table 1).

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 (0.500) was found in Simental cattle and the highest (0.900) in Hereford cattle breed. Meanwhile, the GH AB genotype with lowest frequency (0.111) was found in Limousin cattle breed, the highest (0.348) in the Lithuanian Red cattle breed. GH AB genotype was not identified, in the Hereford breed. The BB genotype of the growth hormone had the highest frequency (0.375) in Simmental breed. The

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

Breed	Frequency of GH gene alelles		Frequency of LEP gene alelles	
	A	B	A	B
LBW	0.823	0.177	0.855	0.145
LR	0.739	0.261	0.717	0.283
HE	0.900	0.100	0.850	0.150
SI	0.563	0.438	0.875	0.125
CH	0.850	0.150	0.775	0.225
LI	0.639	0.361	0.861	0.139
Tested group	0.752	0.247	0.822	0.177

AA genotype of leptin gene ranged from 0.522 to 0.750 frequencies. Meanwhile, LEP AB genotype ranged from 0.222 in the Limousin breed to 0.450 in the Charolais. LEP BB genotype was not found in Hereford, Simmental and Charolais cattle breeds. According to Bonvillani *et al*. (2000), the LEP gene BB genotype was not detected in Simmental and Hereford breeds, which showed the highest frequency of genotype AA as well as in this study (table 2).

In order to highlight the influence of the growth hormone and leptin genes and eliminate other factors contributing to one of the cattle fattening indicators – the weight gain, a multifactor analysis was performed. The linear mixed model was applied for the analysis of individual factors such as GH growth hormone and LEP leptin genes, effects of breed, progenitor, and factors combined with the liveweight gain of animals. The breed and progenitors affected significantly the liveweight gain rates in all the fattening periods. Breed influence was of at about 16.4 % and the progenitor influence was of 17.6 %, regarding the characteristics of the weight gain. Both, growth hormone and leptin genes, had a great influence on liveweight gain indicators only during the first half of fattening period. Growth hormone gene influenced on 2.4 % rate of liveweight gain per day, and leptin gene 4.2 % (table 3).

Differences among breeds and progenitors were statistically significant. Therefore, differences among the genotypes are presented in the charts after the evaluation of progenitors and breed influences.

The influence of bovine GH gene polymorphism on meat properties was studied among Italian Podolian cattle. Animals with BB genotype showed a lower growth rate than individuals with the two other genotypes (Dario *et al*., 2005). According to Zwierzchowski *et al*. (2001), calves with BB genotype had higher daily liveweight gain than calves with other genotypes. Stasio Di *et al*. (2002) found no relationship between the GH polymorphism and meat production characteristics of Piedmont cattle. According to this study, calves with the GH growth hormone AA genotype had 68.9 g, greater liveweight gain than those with AB genotype and 44.7 g greater weight gain than those with BB. No significant differences were found among growth hormone genotypes (figure 1).

A comparison of different indicators of the average daily liveweight gain in correspondence with LEP leptin AA, AB and BB genotypes showed that calves with leptin LEP AB gene were 32.8 g higher than those with AA genotype and 6 g higher than those with

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

Breed	GH genotypes			LEP genotypes		
	AA	AB	BB	AA	AB	BB
LBW	0.731	0.183	0.086	0.742	0.226	0.032
LR	0.565	0.348	0.087	0.522	0.391	0.087
HE	0.900	-	0.100	0.700	0.300	-
SI	0.500	0.125	0.375	0.750	0.250	-
CH	0.750	0.200	0.050	0.550	0.450	-
LI	0.583	0.111	0.306	0.750	0.222	0.028
Tested group	0.671	0.193	0.167	0.669	0.306	0.049

Table 3. Influence of genetic factors to cattle daily weight gain

Genetic factors	Number of classes	Daily gain 1 20-215 days, g	Daily gain 215-310 days, g	Daily gain 310- 405 days, g	Daily gain 405-500 days, g	Average daily gain 120 -500 days, g	Weight on 500 day., kg
GH	3	2.4*	1.2	2.3	1.2	0.9	0.2
Lep	3	4.2*	4.4*	0.2	1.9	0.2	0.004
Breed	6	18.2***	15.9***	17.3***	14.2***	10.8***	18.6***
Sire	18	26.8***	25.2***	5.4	13.2*	16.5**	12.8***
Breed x GH	16	2.1	3.8	7.8	3.3	4.5	20.4***
Sire x GH	40	13.4***	6.5	5.3	2.8	9.6	14.1***
Breed x Lep	15	1.8	1.8	2.1	3.5	2.7	7.9*
Sire x Lep	35	1.6	2.2	2.4	2.4	1.0	3.0

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

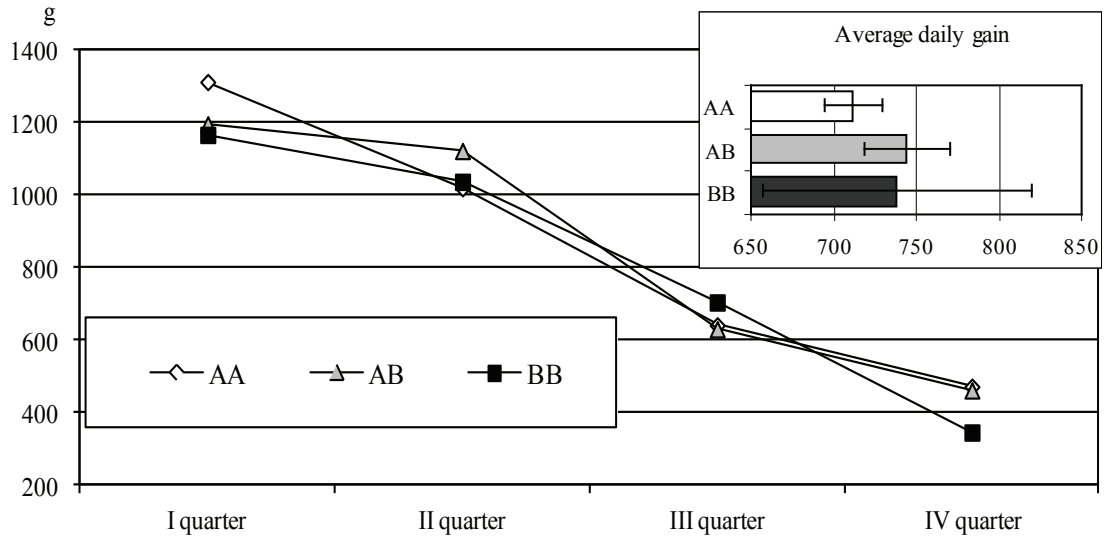

Figure 1. Daily weight gain in different fattening periods (quarters) according GH genotypes.

BB. However, the differences were not statistically significant (figure 2).

According to the analysis, it is possible to conclude that, in the studied cattle group, the growth hormone gene A allele was found with a frequency of 0.752 and B allele with 0.247. The A allele was found with the maximum frequency (0.900) in Hereford breed, and the allele B (0.438) in Simmental breed. Leptin 34.8% of Lithuanian Red and BB genotype in 37.5 % of the Simmental genotype. Leptin AA genotype was found in 66.9% animals, heterozygous genotype AB in 30.6%, BB genotype in 4.9%. AA genotype with the highest frequency was found in Simmental and Limousin cattle, AB genotype in the Charolais and BB genotype in Lithuanian Red cows. BB genotype was not found on all in Hereford, Simmental and Charolais breeds.

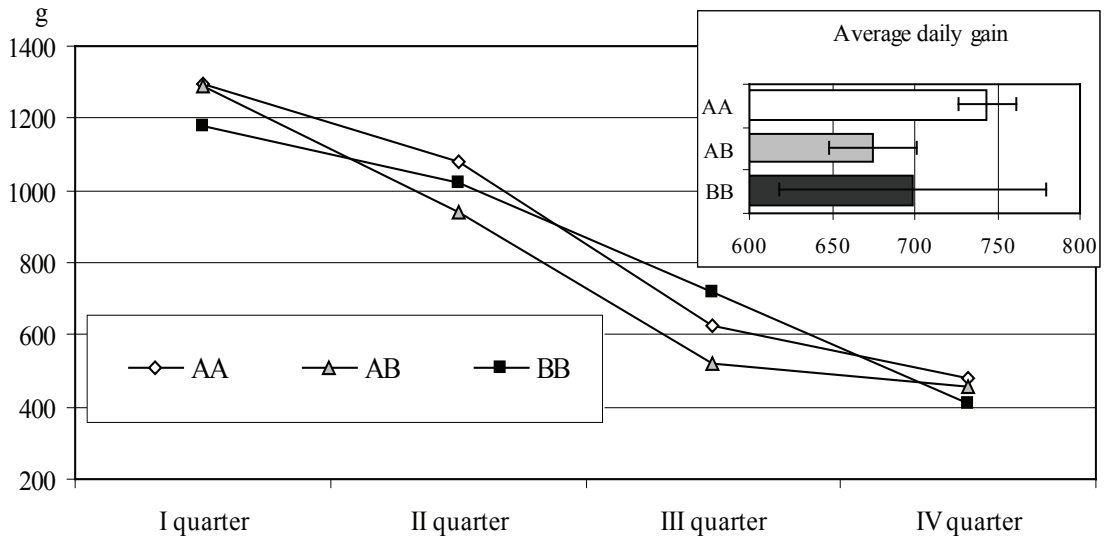

Figure 2. Daily weight gain in different fattening periods (quarters) according LEP genotypes

A allele was found with a frequency of 0.822, and the allele B with 0.177. The allele A was found with the maximum frequency (0.875) in Simmental breed, and B allele (0,283) in the Lithuanian Red breed. Growth hormone allele frequencies between dairy and beef cattle breeds were not different.The frequency of leptin B allele in dairy cattle breeds group was higher than in the group of beef cattle breeds. The growth hormone gene AA genotype was found in 67.1% of the cattle, heterozygous AB genotype in 19.3%, BB genotype in 16.7%. AA genotype, with the highest frequency of 90%, was found in Hereford cattle, AB genotype in

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

References

Asalkar C.S., Sawane M.P., Pawar V. D., Doiphode A. Y. &

Chilgunde S. 2005. PCR-Rflp of growth hormone gene in Deoni cattle. 13:1

Bonvillani A. G., Di Renzo M. A. & Tiranti I. N. 2000. Genetic polymorphism of milk protein loci in Angertinian Holstein cattle. Genet. Mol. Biol. Sao Paulo. 4:23

Buchanan F. C.,Fitzsimmons C. J., Van A. G., Thue T. D., Windkelman-Sim D. C. & Schmutz S. M. 2002. Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. Genetics Selection Evolution. 34:105

Bujko, J., Candrák, J., Strapák, P., Žitný, J. & Hrnčár, C. 2011. Factors effecting of milk productions in select herds of Slovak spotted breed. Scientifical Paper. Faculty of Animal Sciences and Biotechnologies. 44:176

Carnicela, D., Dario, C. & Bufano, G. 2003. Polimorphism del gene GH performances productive. Large Animals Review. 3: 3.

Choudhary, V., Kumar, P., Bhattacharya, T.K., Bushan, B. & Sharma, A. 2005. DNA polymorphism of leptin gene in Bos indicus and *Bos taurus* cattle. J. Genet. Mol. Biol. 28:740

Curi, R.A., Oliveira, H.N., Silveira, A.C. & Lopes, C.R. 2005. Effects of polymorphic microsatellites in the regulatory region of IGF1 and GHR on growth and carcass traits in beef cattle. Anim Genet. 36:58

Dario, C., Carnicela, D., Caroli, A. & Bufano, G. 2004. Analysis of growth hormone GH1-AluI polymorphism in Podolica cattle breed. XXIX International Symposium "Meat Science and Research" Roma. Italy. 303:308

Gentleman, R. & Ihaka, R. 1997. The R language. Fairfax Station. VA. USA.

Jakari, R.R., Noor, H.M. & Duryadi, B.D.T. 2009. Identification of growth hormone (Gh) gene Mspi and Alui Loci polymorphism in beef cattles. The 1st International Seminar on Animal Industry Bogor. 211:217

Miceikienė, I., Pečiulaitienė, N., Baltrėnaitė, L., Skinkytė, R. & Indriulytė, R. 2006. Association of cattle genetic markers with performance traits. J. Biol. 1:24

Miller, S.A., Dykes, D. & Polecky, H.E. 1988. A sample salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16:3.

Moravčíková, N., Trakovická, A. & Hazuchová, E. 2012. The association of bovine growth hormone gene polymorphism with milk performance traits in Slovak spotted cows. Anim. Sci. Biotechnol. 45:1

Mullen, M., Berry, D.P., Howard, D.J., Diskin, M.G., Lynch, C.O., Berkowicz, E.W., Magee, D.A., MacHugh, D.E. & Waters, S.M. 2010. Associations between novel single nucleotide polymorphisms in the *Bos taurus* growth hormone gene and performance traits in Holstein-Friesian dairy cattle. 93:5959

Mullen, M.P., Donagh, P., Howard, D.J., Diskin, M.G., Lynch, C. O.,1 Giblin, L.,3 Kenny, D.A., Magee, D.A, Meade, K.G. & Waters, S.M. 2013. Single nucleotide polymorphisms in the insulin-like growth factor 1 (IGF-1) gene are associated with performance in Holstein-Friesian dairy cattle. Acta Veterinaria Scandinavica. 55:2

Passos, D.T., Hepp, D., Moraes, J.C.F. & Weimer, T.A. 2007. Effect of polymorphisms linked to LEP gene on its expression on adipose tissue in beef cattle. In J. Anim. Breed. Genet. 124:157

Silveira, L.G.G., Furlan, L.R., Curi, R.A., Ferraz, L.J., Alencar, M.M., Regitano, L.C.A., Martins, C.L., Arrigoni, M.B., Suguisawa, L., Silveira, A.C. & Oliveira, H.N. 2008. Growth hormone 1 gene (GH1) polymorphisms as possible markers of the production of beef cattle using the Brazilian Canchim breed as a model. J. Genet. Mol. Biol. 31:874

Stasio, L.Di., Brugiapaglia, A., Destefanis, G., Albera, A. & Sartore, S. 2002. GH1 as candidate gene for variability of meat production traits. J. Anim. Breed. Genet. 120:358

Żukiewicz, A., Grzesiak, W., Szatkowska, I., Błaszczyk, P. & Dybus, A. 2012. Genetic factors of milk yield in dairy cattle – advances in the quest for universal markers. Israel J. Vet. Med. 67:2

Zwierzchowski, L., Oprzadek, J. Dymnicki, E. & Dzierzbicki, P. 2001. An association of growth hormone, K-casein, p-lactoglobulin, leptin and Pit-1 loci polymorphism with growth rate and carcass traits in beef cattle. Anim. Sci. 19:65

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