Determination of *STAT5* and *GH* Genes Polymorphisms and Their Influence on Productivity Traits of Beef Cattle Reared in Lithuania

Nijolė Pečiulaitienė[1], Ramutė Mišeikienė[1], Kristina Morkūnienė[1], Renata Bižienė[1], Ugnė Meškauskaitė[1], Šarūnas Nenartavičius[2], Laimutis Kučinskas[1]

[1]Institute of Biology Systems and Genetic Research, Lithuanian University of Health Sciences, Lithuania

[2]Lithuanian control bulls feeding station, Lithuania

Keywords: *cattle, GH gene, STAT5 gene, PCR-RFLP.*

Abstract. *The aim of this study was to investigate the prevalence of polymorphisms of STAT5 and GH genes and to determine their influence on the productivity traits in beef cattle. A total of 95 animals were genotyped, belonging to the breeds Angus, Limousin, Galloway and Simmental. Polymorphisms of STAT5 and GH locus were identified using a PCR-RFLP method. The evaluation of the STAT5 gene polymorphism (7 exon, 6853C> T) demonstrated that C allele (frequency 0.959) and CC genotype (frequency 0.918) were the most common in beef bull populations reared in Lithuania. This polymorphism had a statistically significant effect on the live weight index of animals. The examination of the GH gene polymorphism (5 exon, 2141C> G) revealed that the G gene allele (frequency 0.612) and heterozygous CG genotype (frequency 0.424) were the most common. This polymorphism had a statistically significant effect on daily bull weight and live weight. Bulls of the homozygous CC genotype exhibited better economic characteristics. In conclusion, our results demonstrated the potential of polymorphisms of GH and STAT5 genes as candidates for the investigation of quantitative traits in cattle.*

Introduction

Carcass composition, meat quality growth and weight gain are multifactorial quantitative traits; they are influenced by both environment and genes. In particular, they are under the control of multiple genes (Keady et al., 2011; Ribeca et al., 2014; Selvaggi et al., 2015). Traditional trait improvement has centered on quantitative genetics, using statistical analysis of phenotypic data to determine animals with the highest genetic merit. This selection approach is most effectively implemented for highly heritable traits that are easily recorded before reproductive age. Genomic selection refers to the use of genome-wide genetic markers to predict the breeding value of selection candidates. This method relies on linkage disequilibrium between the markers and the polymorphisms that cause variation in important traits (Hayes et al., 2013; Odzemir et al., 2018). Molecular genetic markers in animal breeding programs could make selection precise and efficient.

Signal transducer and activator of transcription 5 (*STAT5*) is known as a main mediator of growth hormone (*GH*) action on target genes (Selvaggi et al., 2009; Dario et al., 2009). The *STAT5* transcription factors are members of the somatotropic axis. They initiate the growth process in the target cells, a process mediated by the pituitary growth hormone (Cosier et al., 2010). *STAT5* exists in two isoforms (*STAT5A* and *STAT5B*) that differ by a few amino acids in the carboxylic end of the protein molecule and are coded by two different genes (Kmiec et al., 2010; Cosier et al., 2012). These two forms of *STAT5* have been identified in sheep, mouse, human, rat and cattle cells. Owing to its mediator role in the effects of the prolactin and growth hormones, it is suggested that the *STAT5A* gene is a potential quantitative trait locus for the quantitative traits of livestock, such as meat yield and milk composition (Arslan et al., 2015).

Growth hormone (*GH*) gene acts and mediates the growth of bones and muscles. It is known that *GH* is the main regulator of postnatal somatic growth, stimulating anabolic processes and skeletal growth (Sodhi et al., 2007; Hadi et al., 2015; Omer et al., 2018). The *GH* gene is located on the 19th chromosome in the q26-qter band region. This gene is approximately 1.8 kb in size and contains 5 exons and 4 introns (Ozkan-Unal et al., 2015). The growth hormone (*GH*) gene is a candidate gene for predicting growth and meat quality traits in animal genetic improvement since it plays a fundamental role in growth regulation and development (Omer et al., 2018). The aim of this study was to investigate the prevalence of polymorphisms of *STAT5* and *GH* genes and to determine their influence on the productivity traits in beef cattle. The polymorphism of *STAT5* and *GH* genes in beef cattle raised in Lithuania has not been studied so far. The association of these genes with signs of cattle productivity has not been studied either.

Correspondence to Nijolė Pečiulaitienė, Institute of Biology Systems and Genetic Research, Lithuanian University of Health Sciences, Lithuania.
E-mail: nijole.peciulaitiene@lsmu.lt

Materials and Methods

Samples and DNA extraction

The study was carried out following the methodology of the Law on the Welfare of the Farm Animals of the Republic of Lithuania and complied with the Directive 2010/63/EU of the European Parliament and the Council on the Protection of Animals Used for Scientific Purposes.

Samples of cattle hair follicles were collected from 95 bulls consisting of Angus (41), Limousin (19), Galloway (19) and Simmental (16) cattle. The cattle were kept under the same rearing conditions at the bull fattening station. The data on daily weight gain records were obtained from Šilutė control bulls feeding station (Lithuania, Šilutė region, Armalėnai village). The hair samples and slaughter data were obtained from private slaughterhouses, where animals were slaughtered. Molecular genetic analysis was done at the Lithuanian University of Health Sciences, Dr. K. Janušauskas Laboratory of Animal Genetics. Bovine genomic DNA was extracted from hair follicles using Chelex DNA extraction method: 200 µL Chelex 100, 7.5 µL DTT (1M), and 10.7 µL Proteinase K (20 mg/mL). After extraction, the inactivation step was performed at 94°C, for 10 minutes. DNA samples were stored in the refrigerator at 4°C (Miceikienė et al., 2002).

Restriction fragment length polymorphism – polymerase chain reaction (PCR-RFLP)

The PCR was done in a reaction volume of 30 µL. The reaction consists of 2.5 µL of 10X Dream Taq Buffer, 1.5 µL each primer forward and reverse 1µM, 2.5 µL of dNTP Mix 0.2mM, 0.25 µL Dream Taq DNA Polymerase, 11.75 µL ddH2O and 10 µL genomic DNA. The reactions were done in a Thermal Cycler 2700. The primer sequences and thermal cycling programs for each SNP are represented in Table 1, respectively (Flisikowski et al., 2002; Silveira et al., 2008).

PCR product of *STAT5* gene was digested with *AvaI (Eco88I)* restriction nuclease and the *GH* gene amplified 404-bp-long DNA fragment was digested with *AluI* restriction endonuclease. Amplified DNA fragments were digested with restriction endonucleases at 37 °C for 1–16 h. The reaction volume was 20 µL consisting of 10 µL PCR product, 7.5 µL ddH2O, 2 µL 10X Buffer Tango and 0.5 µL restriction enzyme 10 U/µL. The PCR-RFLP product of each sample (8 µL) and GeneRuler 50 bp DNA Ladder (0.1 µg/µL, Thermo Fisher Scientific, Waltham, USA) were loaded in 3% (w/v, for *STAT5* gene SNP) and in 2% (w/v, for *GH* gene SNP`s) agarose gels in tris-acetate-EDTA (TAE) buffer (50X TAE Electrophoresis Buffer, staining using 10 mg/mL ethidium bromide). The electrophoresis was carried out for 60 min at 100 V. The electrophoresis gel was examined on an UV transilluminator MiniBIS Pro (Bio-Imaging Systems, Israel) and bands were visualized and photographed. Polymorphisms of *STAT5* and *GH* genes were identified based on the length of the band. The following DNA restriction fragments were obtained for locus *STAT5* (Exon 7, 6853C>T): 181 and 34 bp for the CC genotype; 215, 181, and 34 bp for the CT and 215 bp for the TT genotype. After restriction, fragments for locus GH (Exon 5, 2141C>G) were obtained: 185, 131,51 and 37 for the CC genotype; 236, 185, 131, 51 and 37 bp for the CG genotype; and 236, 131, and 37 for the GG genotype.

Statistical analysis

Statistical analysis was done using IBM SPSS Statistics software package and Microsoft Excel spreadsheets. The influence of genes on each indicator (bull weight and carcass weight) was calculated by using one-way ANOVA, and the influence of polymorphisms on economic traits was evaluated by calculating average means and standard errors of productivity traits. Differences between genotypes was evaluated by the *Fisher least significant difference* (LSD) test.

Table 1. Primer sequences and size of the amplified fragments and reaction conditions, PCR programs for each SNP (single nucleotide polymorphism)

Genes	SNP	Sequence	PCR profile			PCR product size	References
STAT5	Exon 7, 6853C>T	F: 5`– CTG CAG GGC TGT TCT GAG AG – 3` R: 5`– GGT ACC AGG ACT GTA GCA CAT – 3`	95 °C	2 min		215	Flisikowski et al., 2002
			94 °C	30 s	35 cycles		
			60 °C	60 s			
			72 °C	60 s			
			72 °C	10 min			
GH	Exon 5, 2141C>G	F: 5`– TAG GGG AGG GTG GAA AAT GGA– 3` R: 5`– GAC ACC TAC TCA GAC AAT GCG – 3`	94 °C	2 min		404	Silveira et al., 2008
			94 °C	30 s	40 cycles		
			59 °C	80 s			
			72 °C	90 s			
			72 °C	5 min			

Results

Polymorphism of STAT5 gene, exon 7, 6853C>T

Both C and T alleles of the *STAT5* gene were detected in the Lithuanian beef cattle population. Frequency of C allele was found to be the highest and that of T allele – the lowest. The C and T allele frequencies were 0.961 and 0.039, respectively. The *STAT5* gene CC genotype was the most common in the studied population (92%) followed by the CT genotype (8%) while the TT genotype was not found in the analyzed population (Table 2).

The *STAT5* gene had a statistically significant effect on the live weight index of the animals. Significant differences between the CC and CT genotypes were found for live body weight at slaughter (slaughter age of cattle from 12 to 24 months) ($P < 0.05$). The animals carrying the CT genotype were 86.5 kg (632.9 ± 23.01 kg vs 546.4 ± 10.69 kg) heavier than CC homozygotes, and the difference was significant. The mean value, standard errors and influence of *STAT5* gene polymorphism on five productivity traits are shown in Table 3.

Polymorphism of GH gene, exon 5, 2141C>G

The C allele of the *GH* gene was found most frequently compared with the G allele. The CG genotype of the *GH* gene was the most frequent in the studied population (41%) followed by the CC genotype (36%) while the GG genotype demonstrated the lowest frequency (23%) (Table 4).

Significant differences between genotypes were found for live body weight at slaughter, hot carcass weight, carcass weight ($P < 0.01$), weight gain and average daily gain ($P < 0.05$). The live weight at slaughter was the highest for the CC homozygotes which benefited by gained 16.7 kg more than CG heterozygotes and 95.5 kg more than GG homozygotes (CC: 577.4 ± 13.29 kg, CG: 560.7 ± 15.36 kg, GG: 481.9 ± 27.83 kg). Moreover, the CC genotype was associated with a higher hot carcass weight (+16.3 kg compared with the CG genotype and +68.6 kg compared with GG genotype), and carcass weight (+15.5 kg compared with CG genotype and +66.5 kg compared with GG genotype). Average daily gain also was higher in CC genotype compared with those of GG genotype. In general, the homozygous CC genotype appeared superior in all the traits measured. The mean value and standard errors for the five productivity traits are shown in Table 5.

Discussion

The polymorphism of *STAT5* gene was not reported previously for Lithuanian beef cattle and the frequencies of alleles obtained in this study were like those reported in other cattle breeds. Flisikowski and Zwierzchowski (2002) studied polymorphism in the bovine *STAT5* gene (6853C>T) and its association with meat production traits in beef cattle. The overall frequencies of alleles C and T were 0.82 and 0.18, respectively.Frequencies of C and T alleles obtained by Selvaggi (2009) were 0.83 and 0.17, respectively. In our study, frequencies of the *STAT5* gene alleles were quite similar (C – 0.961, T – 0.039). However, Selvaggi et al. (2015) found that the T allele was more common than the C allele in native Podolica

Table 2. Genotypes and allele frequencies of STAT5 gene polymorphism (6853C>T)

Breeds	N	nCC	nCT	Alleles frequency		Genotype frequency	
				C	T	CC	CT
Angus	41	35	6	0.927	0.073	0.854	0.146
Limousin	19	19	–	1	0	1	0
Galloway	19	19	–	1	0	1	0
Simmental	16	14	2	0.917	0.083	0.833	0.167
Total and average	95	87	8	0.961	0.039	0.921	0.078

Table 3. Effect of *STAT5* polymorphism (6853C>T) on productivity traits

Trait	Influence of polymorphism	Genotype means ± standard errors	
		CC	CT
Number of cattle		85	10
Weight gain (kg)	2.4%	103.8 ± 2.83	118.0 ± 11.71
Live weight (kg)	6.4%*	546.4 ± 10.69a	632.9 ± 23.01b
Hot carcass weight (kg)	3.9%	309.7 ± 7.35	355.8 ± 14.81
Carcass weight (kg)	3.8%	303.2 ± 7.19	347.4 ± 14.83
Average daily gain (kg)	4.5%	0.96 ± 0.02	1.10 ± 0.04

a, b* – values with different superscript letters show statistically significant differences ($P < 0.05$) between different genotypes in the trait

Table 4. Genotypes and allele frequencies of *GH* gene polymorphism (2141C>G)

Breeds	N	n_{CC}	n_{GG}	n_{CG}	Allele frequency		Genotype frequency		
					C	G	CC	GG	CG
Angus	41	17	3	21	0.671	0.329	0.415	0.073	0.512
Limousin	19	13	1	5	0.816	0.184	0.684	0.053	0.263
Galloway	19	3	9	7	0.342	0.658	0.158	0.474	0.368
Simmental	16	3	5	8	0.417	0.583	0.167	0.333	0.500
Total and average	95	36	18	41	0.562	0.438	0.356	0.233	0.411

Table 5. Effect of *GH* polymorphism (2141C>G) on productivity traits

Trait	Influence of polymorphism	Genotype means ± standard errors		
		CC	GG	CG
Number of cattle		36	18	41
Weight gain (kg)	7.6%*	95.2 ± 4.69a	70.0 ± 8.47b	86.1 ± 5.69
Live weight (kg)	13.0%**	577.4 ± 13.29a	481.9 ± 27.83b	560.7 ± 15.36c
Hot carcass weight (kg)	14.2%**	332.5 ± 9.27a	263.9 ± 17.95b	316.2 ± 10.31c
Carcass weight (kg)	14.0%**	325.1 ± 9.08a	258.6 ± 17.62b	309.6 ± 10.09c
Average daily gain (kg)	7.6%*	1.02 ± 0.03a	0.88 ± 0.05b	0.97 ± 0.03

a, b, c – values with different superscript letters show statistically significant differences ($P < 0.05$) between different genotypes in the trait.

cattle breed. The observed frequencies of C and T alleles were 0.344 and 0.656, respectively. Besides, it was found that the most frequent genotype in the Podolica breed was TT genotype (45.70%), followed by TC (39.79%) and CC (14.51%). Meanwhile, in our studies, the CC genotype was found to be the most common, and the TT genotype was not found at all. So, further studies of *STAT5* polymorphism are also needed in other cattle breeds to better clarify the role of this SNP prevalence and influence on production traits in cattle.

In our study, the influence of *STAT5* gene on the rate of live weight was found to be statistically significant. However, Flisikowski and Zwierzchowski (2002), by studying beef cattle breeds, found different associations of *STAT5* gene genotypes with productivity traits than in our study. Their study revealed a statistically significant association between *STAT5* gene polymorphism and beef production traits in cattle (Flisikowski and Zwierzchowski, 2002). They found that in the animals of the CC genotype the live body weight, weight gain and carcass weight were more favorable than in CT animals. Also, Oprzadek et al. (2005) found that the CC genotype was associated with a significantly faster growth rate from 8 to 15 months (1.04 kg daily vs 0.97 kg). However, the results of our research were opposite: we found that the CT heterozygotes were heavier than CC homozygotes. It is necessary to underline that the genotype frequencies observed in some breeds were obtained from a small sample of animals, so they cannot be considered representative for the beef breed. Only 10 animals tested had a CT genotype. Therefore, the present result can be interpreted only as an association between the marker and production trait at this time and in this population. To confirm these results, further investigations including bigger cattle populations of different beef breeds are necessary. Increasing the sample of animals could change the result; therefore, deeper investigation of this aspect may be an interesting perspective to remove all doubt.

Similar to our study according to the polymorphism of *GH* gene, Fedota et al. (2016) have also found the higher frequencies of genotype CG = 46.6% compared with CC = 8.6% and GG = 44.8% genotypes in Aberdeen-Angus cattle population. However, unlike in our study, they obtained a higher frequency of the G allele compared with the C allele. Meanwhile, the highest frequency of C allele was found in our study. Our results are consistent with those reported by Ruban et al. (2016) who studied the effects of polymorphism in GH gene (2141C>G) on growth traits in Angus cattle. They found that in the animals of the CC genotype the live weight at birth and live body weight (at slaughter) were more favorable than in CG and GG animals. Similar results were obtained in our study, CC genotype appeared superior in all traits measured. In fact, this observation is explained by the more intense secretion of the growth hormone in animals with CC genotype (Selvaggi et al., 2015). In general, due to the crucial role of GH in animal growth, the GH gene is thought to be a candidate marker for performance traits in livestock animals

such as cattle (Aytac et al., 2015). Fedota et al. (2017) have also found a statistically significant correlation between polymorphism and birth weight; cattle of the CC genotype weighed more than CG heterozygous and GG homozygous animals.

Conclusions

In conclusion, our results demonstrate the potential of polymorphisms of *GH* and *STAT5* genes as candidates for the investigation of quantitative traits in cattle. These SNP can be used as reliable genetic markers for productivity traits in cattle breeding. Therefore, we will extend this study by increasing the number of animals analyzed and including more beef cattle breeds.

Conflict of interest

There are no conflicts of interest involving the publication of this work, according to the authors.

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Received 20 May 2024
Accepted 10 September 2024