

Association between polymorphisms in the *STAT5A* and *GH* genes and beef cattle productivity parameters

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ABSTRACT. The study analysed single nucleotide polymorphisms (SNP) of the growth hormone (*GH*) (*GH1*-exon 5, 2141C>G, *GH2*-exon 5, 2291A>C, *GH3*-intron 3, 1548C>T) and signal transducer and activator of transcription 5A (*STAT5A*) (exon 7, 6853C>T) in three beef cattle breeds (Angus, Charolais, and Limousin; n = 124), and their association with productivity traits. Significant associations were identified between the *GH1* (2141C>G) polymorphism and traits like daily weight gain and carcass weight, with the CC genotype showing the most favourable effect. The AA genotype of the *GH2* (2291A>C) polymorphism and the CC genotype of the *GH3* (1548C>T) polymorphism were the most common and were associated with higher weight gain and improved carcass characteristics. For the *STAT5* gene, the CC genotype also demonstrated a significant positive association with productivity traits. These findings highlight the potential utility of these polymorphisms as genetic markers for beef cattle selection. This study is the first to report on the frequency of these alleles and their associations with productivity traits in beef cattle raised in Lithuania.

Introduction

Livestock productivity traits, such as meat yield, growth rate, and milk production, are heritable and genetically determined (Ribeca et al., 2014; Akçay et al., 2020). Traditional improvement methods rely on quantitative genetics using phenotypic data to select animals with high genetic potential. This approach is most effective for highly heritable traits that can be measured before reproductive age. Genomic selection, on the other hand, employs genome-wide molecular markers to predict the breeding value of selection candidates (Pogorzelska-Przybyłek et al., 2018; Odzemir et al., 2018; Motmain et al., 2022; Kostusiak et al., 2023).

The growth hormone (*GH*) gene codes for a bone and muscle development regulator that plays a key role in postnatal somatic growth and skeletal maturation. The *GH* gene is located on the autosome 19th chromosome (BTA19) of *Bos taurus* (Kiyici et al., 2019). It is considered a candidate gene for predicting growth, milk, and meat quality traits in livestock (Omer et al., 2018; Akçay et al., 2020). The signal transducer and activator of transcription 5 (*STAT5A*) gene encodes a key mediator of GH signalling, activating target genes within the somatotrophic axis to promote cellular growth. The gene is located on the 19th chromosome (BTA19) of cattle (Kiyici et al., 2019). Given the dual role of *STAT5* in mediating prolactin and growth hormone

effects, its gene has been proposed a potential quantitative trait locus for characteristics such as meat yield and milk composition (Selvaggi et al., 2015; Kiyici et al., 2019). The analyzed polymorphisms offer valuable insights into marker-assisted selection (MAS) in beef cattle breeding. By identifying genotypes associated with improved performance traits, breeders can make more informed decisions to accelerate genetic improvement in their herds. To date, *GH* (2141C>G, 2291A>C, 1548C>T), and *STAT5A* (6853C>T) polymorphisms have not been studied in beef cattle raised in Lithuania, nor has their association with productivity traits. Considering this, the present study focused on three most popular high-value beef breeds in the country, i.e., Angus, Limousin, and Charolais, selected for their rapid growth, high carcass yield, and meat quality. The aim of the present study was to analyse the polymorphisms in the *GH* (2141C>G), (2291A>C), (1548C>T), and *STAT5A* (6853C>T) genes in Lithuanian beef cattle, and to determine their association with productivity traits.

Material and methods

The study was conducted following the methodology of the Law on the Welfare of Farm Animals of the Republic of Lithuania and complied with Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes. All procedures involving animals followed institutional guidelines for animal care and use, and were approved by the Institutional Local Ethics Committee (Approval No. 2024-BEC2-1055).

The study was conducted at “Šilutė Breeding” located in Armalėnai village, Šilutė district, southwestern Lithuania, between 2023 and 2024. “Šilutė Breeding” is the only licensed performance testing station in Lithuania that evaluates the offspring of purebred beef breed bulls based on growth performance and carcass traits. In Lithuania, a continuous evaluation of beef cattle sires is carried out using the fattening performance of their male offspring to determine the heritable productivity characteristics of each sire. During the study period, animals for performance testing were selected from farms located in various regions of Lithuania. The selection included male offspring (young bulls) of sires representing the most popular beef cattle breeds in the country, i.e., Charolais, Limousin, and Angus. A total of 10 sires were selected from each of the Charolais and Limousin breeds, and 11 sires from the

Angus breed. Four offspring were randomly selected per sire. Genetic analysis involved 124 animals (44 Angus, 40 Limousin, and 40 Charolais) using hair follicle samples collected from bulls maintained under uniform fattening conditions. The test bulls were not castrated. Purebred bulls were selected for control fattening at 210 days of age. Intermediate weighings were performed every three months. Animal age (in days), full fattening period duration (90–270 days), and daily weight gain (kg) were recorded at the Šilutė control station. Hair samples for genetic analysis were obtained from all animals, while slaughter data and carcass quality parameters (including chilled carcass weight in kg and muscle development scores according to the EUROP classification system) were provided by participating private slaughterhouses. All bulls included in the study were classified as category A, indicating animals up to 24 months of age. Prior to carcass chilling, warm carcass weights were recorded. These were subsequently adjusted by deducting 2% to calculate chilled carcass weight. The EUROP scoring system was used to assess both fat coverage and muscular conformation, which directly influence carcass valuation within the European beef market.

Molecular genetic analysis was conducted at the Dr. K. Janušauskas Laboratory of Animal Genetics of the Lithuanian University of Health Sciences. Bovine genomic DNA was extracted from hair follicles using the Chelex method (200 µl Chelex 100, 7.5 µl DTT, 10.7 µl proteinase K), followed by 10-minute inactivation step at 94 °C. DNA samples were stored at 4 °C (Miceikienė et al., 2002). SNP genotyping was conducted using the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) technique, with primer sequences and thermal cycling conditions provided in Table 1. A 404-bp *GH1* fragment was digested with *AhaI* restriction enzyme, *GH2* (404-bp) with *DdeI* (*HpyF3I*), and a *GH3* 329-bp fragment with *MspI* (*HpaII*). A 215-bp *STAT5A* PCR product was digested with *AvaI* (*Eco88I*) restriction nuclease. Digestions were carried out at 37 °C overnight (Miceikienė et al., 2002; Omer et al., 2018). RFLP products were separated by agarose gel electrophoresis using 3% gels for *STAT5A* analysis and 2% gels for *GH* gene fragments (Table 2).

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics, version 29.0.0.0 (241). Allele frequencies for *GH1*, *GH2*, *GH3*, and *STAT5A* polymorphisms were determined by direct allele counting.

Table 1. Primer sequences, amplified fragment sizes, and PCR conditions, for each SNP

Genes	SNP	Sequence (5'→3')	PCR profile			PCR product size, bp	References
<i>GH1</i>	Exon 5, 2141C>G	F: TAGGGGAGGGTGGAAAATGGA	94 °C	2 min		404	Silveira et al. (2008)
<i>GH2</i>	Exon 5, 2291A>C	R: GACACCTACTCAGACAATGCG	94 °C	30 s	40 cycles		
			59 °C	80 s			
			72 °C	90 s			
			72 °C	5 min			
<i>GH3</i>	Intron 3, 1548C>T	F: TAGGGGAGGGTGGAAAATGGA R: GACACCTACTCAGACAATGCG	95 °C	2 min		329	Sodhi et al. (2007)
			94 °C	30 s			
			60 °C	60 s			
			72 °C	60 s			
			72 °C	5 min			
<i>STAT5A</i>	Exon 7, 6853C>T	F: CTGCAGGGCTGTTCTGAGAG R: GGTACCAGGACTGTAGCACAT	95 °C	2 min	35 cycles	215	Cosier et al.(2012)
			94 °C	30 s			
			60 °C	60 s			
			72 °C	60 s			
			72 °C	10 min			

SNP – single-nucleotide polymorphism, PCR – polymerase chain reaction, *GH1* – growth hormone (exon 5, 2141C>G), *GH2* – growth hormone (exon 5, 2291A>C), *GH3* – growth hormone (intron 3, 1548C>T), *STAT5A* – signal transducer and activator of transcription 5 (exon 7, 6853C>T)

Table 2. Restriction endonucleases and RFLP fragment sizes for individual SNP genotypes

Genes	SNP	Restriction endonuclease	Genotype	Fragment size, bp
<i>GH1</i>	Exon 5, 2141C>G	<i>AluI</i>	CC	185, 131, 51, 37
			GG	236, 131, 37
			CG	236, 185, 131, 51, 37
<i>GH2</i>	Exon 5, 2291A>C	<i>DdeI</i> (<i>HpyF3I</i>)	AA	192, 157, 45, 9
			CC	349, 45, 9
			AC	349, 192, 157, 45, 9
<i>GH3</i>	Intron 3, 1548C>T	<i>MspI</i> (<i>HpaII</i>)	CC	224, 105
			TT	329
			CT	329, 224, 105
<i>STAT5A</i>	Exon 7, 6853C>T	<i>AvaI</i> (<i>Eco88I</i>)	CC	181, 34
			TT	215
			CT	215, 181, 34

SNP – single-nucleotide polymorphism, RFLP – restriction fragment length polymorphism *GH1* – growth hormone (exon 5, 2141C>G), *GH2* – growth hormone (exon 5, 2291A>C), *GH3* – growth hormone (intron 3, 1548C>T), *STAT5A* – signal transducer and activator of transcription 5 (exon 7, 6853C>T)

Data normality was verified using the Shapiro-Wilk test. Means and standard errors of productivity traits for each genotype were calculated. The effects of genotype and breed were evaluated using the general linear model, while pairwise comparisons were performed using the post hoc LSD test. Association between genotype and carcass characteristics (muscularity and adiposity) were evaluated using the χ^2 test. The z-test with Bonferroni correction was applied to compare proportions. Differences were considered statistically significant at $P < 0.05$.

Results

The genotypic results and fragment size patterns for the *GH1* (2141C>G), *GH2* (2291A>C), *GH3* (1548C>T), and *STAT5A* (6853C>T) variants

after restriction enzyme digestion are presented in Figure 1 and Table 2.

The C allele of *GH1* and A allele of *GH2* were more frequent than the G and C alleles, respectively, in all three breeds. The CC genotype of the *GH1* (*AluI*) polymorphic site was dominant in Charolais (90%), Limousin (75%), and Angus (52%) cattle. The heterozygous CG genotype was most frequent in Angus (41%), which also showed the highest G allele frequency (33%). The GG genotype (7%) was detected only in the Angus breed. For the *GH2* (*DdeI*) polymorphism, the AA genotype was most frequent in all breeds: Charolais (60%), Angus (59%), and Limousin (50%). The AC genotype was more common in Angus (41%) compared to Limousin (15%) and Charolais (30%). The CC genotype was most frequent in Limousin (35%) and

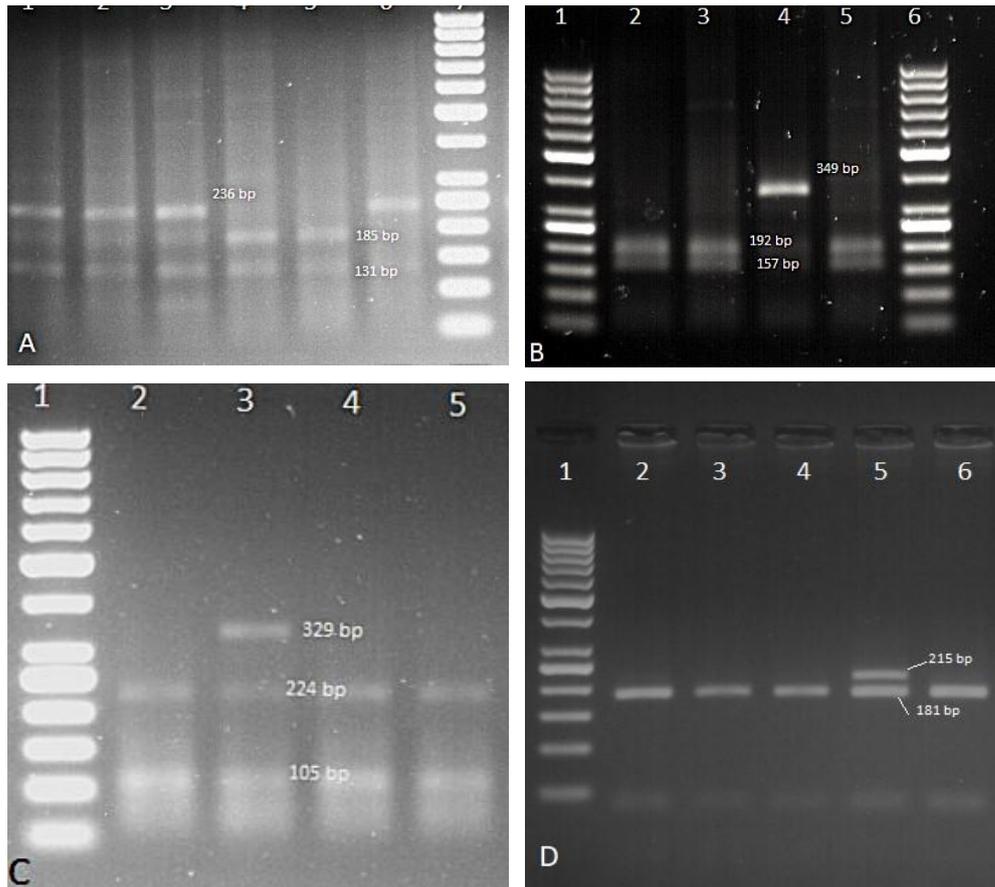


Figure 1. PCR-RFLP results for the *GH1* (2141C>G) (A), *GH2* (2291A>C) (B), *GH3* (1548C>T) (C), and *STAT5A* (6853C>T) (D) polymorphisms. *GH1* – growth hormone (exon 5, 2141C>G), *GH2* – growth hormone (exon 5, 2291A>C), *GH3* – growth hormone (intron 3, 1548C>T), *STAT5A* – signal transducer and activator of transcription 5A (exon 7, 6853C>T)

was not observed in Angus. Regarding the *GH3* (*MspI*) polymorphism, the CC genotype was the most common in all breeds, i.e., Charolais (95%), Angus (86%), and Limousin (75%). The CT genotype was most frequent in Limousin (20%) and least in Charolais (5%), while the TT genotype appeared only in Limousin (5%). The C allele frequency was similar across breeds: Charolais (97%), Angus (93%), and Limousin (85%). The frequency of the C allele of the *STAT5A* gene was high and relatively consistent in all studied bulls: Charolais (97%), Angus (93%), and Limousin (85%). The T allele was found at low frequencies in Charolais (5%) and Limousin (3%) breeds, but was more common in the Angus breed (17%). The *STAT5A* CC genotype was most frequent in all breeds: Limousin (95%), Charolais (90%), and Angus (66%). The TT genotype was not detected in any of the animals, while the CT genotype frequency was highest in Angus bulls (34%) compared to Charolais (10%) and Limousin (5%) bulls (Table 3).

Significant variation in average daily weight gain (kg) during the fattening period (90–270 days)

was observed between *GHI*, *GH2*, *GH3*, and *STAT5A* genotypes in Limousin bulls and the overall beef cattle population ($P < 0.05$). No such variation was detected in other breeds. In the Limousin breed, animals with the CC genotype of the *GHI* SNP showed a 25% higher average daily weight gain compared to CG genotype animals. In the general beef cattle population, the difference between these genotypes was 5% ($P < 0.05$). The *GH2* CC genotype was associated with the highest growth rates compared to AA genotypes in both Limousin and general populations. *GH3* genotype effects were breed-specific, with Limousin TT genotype bulls outperforming CC and CT genotypes by 24% and 21% respectively ($P < 0.05$). *STAT5A* CC genotype bulls showed significantly increased growth, with Limousin CC individuals gaining 73% more weight daily than CT counterparts, and a 13% advantage population-wide ($P < 0.05$). Interbreed comparisons revealed significant differences ($P < 0.05$) in daily weight gain (kg) between *GHI*, *GH2*, *GH3*, and *STAT5A* SNP genotypes. Charolais bulls consistently showed the highest average daily gain

Table 3. Genotypes and allele frequencies of *STAT5* and *GH* genes polymorphisms in beef cattle breeds (Angus, Charolais, and Limousin)

Breed		Angus n = 44		Charolais n = 40		Limousins n = 40	
Genes	Genotypes	Genotype frequency	Alleles frequency	Genotype frequency	Alleles frequency	Genotype frequency	Alleles frequency
<i>GH1</i> (Alul)	CC	0.523	C (0.670)	0.900	C (0.950)	0.750	C (0.875)
	CG	0.409	G (0.330)	0.100	G (0.050)	0.250	G (0.125)
	GG	0.068		0.000		0.000	
<i>GH2</i> (Ddel)	AA	0.591	A (0.795)	0.600	A (0.650)	0.500	A (0.575)
	AC	0.409	C (0.205)	0.300	C (0.350)	0.150	C (0.425)
<i>GH3</i> (MspI)	CC	0.000		0.100		0.350	
	CC	0.864	C (0.932)	0.950	C (0.975)	0.750	C (0.850)
	CT	0.136	T (0.068)	0.050	T (0.025)	0.200	T (0.150)
	TT	0.000		0.000		0.050	
<i>STAT5A</i> (Aval)	CC	0.659	C (0.830)	0.900	C (0.950)	0.950	C (0.975)
	CT	0.341	T (0.17)	0.100	T (0.050)	0.050	T (0.025)
	TT	0.000		0.000		0.000	

STAT5A – signal transducer and activator of transcription 5 (exon 7, 6853C>T), *GH* – growth hormone, *GH1* – growth hormone (exon 5, 2141C>G), *GH2* – growth hormone (exon 5, 2291A>C), *GH3* – growth hormone (intron 3, 1548C>T),

for all analysed genotypes. In contrast, Limousin bulls had the lowest daily weight gain (kg) for many of the gene variants compared to other breeds (Table 4).

We evaluated differences in chilled carcass weight (kg) of cattle between bull breeds and *GH* and *STAT5A* polymorphisms. Significant differences in chilled carcass weight were identified between *GH1* genotypes in all breeds and the general population ($P < 0.05$). The CC genotype was associated with higher chilled carcass weight compared to CG, with differences of 13% in Angus, 11% in Charolais, 15% in Limousin, and 29% in the

general population. For *GH2* and *GH3* genotypes, no meaningful variation was observed in Charolais and Limousin breeds; however, clear genotype-related differences were found in Angus and the general population, where AA (*GH2*) and CC (*GH3*) genotypes were associated with improved performance ($P < 0.05$). In contrast, bulls with the *GH3* TT genotype had lower chilled carcass weight compared to CC/CT in the general population ($P < 0.05$). For the *STAT5A* gene, significant genotype-related differences in carcass weight were found in Charolais and Limousin bulls. Bulls with the CC genotype showed higher values than those

Table 4. Differences in average daily gain between breeds and genotypes of the analysed genes during the fattening period (90–270 days), kg/day

Gene	Genotype	Overall	Angus	Charolais	Limousins
<i>GH1</i> (Alul)	CC	1.162 ± 0.031 ^a	1.200 ± 0.047 ^A	1.415 ± 0.021 ^B	1.165 ± 0.026 ^A
	CG	1.107 ± 0.034 ^b	1.118 ± 0.043 ^A	1.336 ± 0.021 ^B	0.934 ± 0.097 ^C
	GG	1.407 ± 0.02 ^{ab}	1.265 ± 0.109		
<i>GH2</i> (Ddel)	AA	1.203 ± 0.027 ^a	1.180 ± 0.040 ^A	1.381 ± 0.020 ^B	1.021 ± 0.046 ^C
	AC	1.229 ± 0.045 ^b	1.135 ± 0.048 ^A	1.536 ± 0.095 ^B	1.307 ± 0.072 ^C
	CC	1.269 ± 0.042 ^b		1.416 ± 0.037 ^A	1.144 ± 0.051 ^B
<i>GH3</i> (MspI)	CC	1.222 ± 0.022	1.173 ± 0.032 ^A	1.409 ± 0.021 ^B	1.046 ± 0.038 ^C
	CT	1.213 ± 0.050	1.090 ± 0.099	1.372 ± 0.002	1.266 ± 0.051 ^b
	TT	1.383 ± 0.050			1.383 ± 0.050 ^b
<i>STAT5A</i> (Aval)	CC	1.247 ± 0.021 ^a	1.184 ± 0.040 ^A	1.415 ± 0.021 ^B	1.135 ± 0.030 ^A
	CT	1.108 ± 0.054 ^b	1.119 ± 0.046 ^A	1.336 ± 0.021 ^B	0.657 ± 0.010 ^C

GH1 – growth hormone (exon 5, 2141C>G), *GH2* – growth hormone (exon 5, 2291A>C), *GH3* – growth hormone (intron 3, 1548C>T), *STAT5A* – signal transducer and activator of transcription 5A (exon 7, 6853C>T); ^{ab} – different lowercase letters in columns (for each gene separately) indicate statistically significant differences ($P < 0.05$); ^{ABC} – different uppercase letters in rows (between breeds) indicate statistically significant differences ($P < 0.05$); data are presented as mean values ± SEM

with the CT genotype, with differences of 11% in Charolais ($P < 0.01$), 6% in Limousin ($P < 0.001$), and 17% in the general population ($P < 0.01$). No significant differences were detected in Angus bulls. Overall, chilled carcass weight differences were associated with *GHI*, *GH2*, *GH3*, and *STAT5A* SNP genotypes ($P < 0.05$). Among the breeds studied, Charolais bulls consistently achieved the highest chilled carcass weight for all genotypes compared to Angus and Limousin bulls (Table 5).

GH3 genotype effects were significant in Angus, Charolais and the general population ($P < 0.05$), with CC genotypes consistently scored as U class carcasses, and CT/TT genotypes more frequently categorised as R class. The carcasses of bulls with the heterozygous *GH2* AC genotype were mainly classified in the lower R (good) conformation class, while *GH3* genotype analysis showed that CC homozygotes were associated with U (very good) class and CT/TT genotypes with R class

Table 5. Differences in average chilled carcass weight between breeds and genotypes of the analysed genes

Gene	Genotype	Chilled carcass weight, kg			
		Overall	Angus	Charolais	Limousins
<i>GHI</i> (<i>Alul</i>)	CC	425.1 ± 11.23 ^a	362.7 ± 9.66 ^{Aa}	535.8 ± 5.61 ^{Ba}	329.8 ± 5.58 ^{Ca}
	CG	329.0 ± 11.16 ^b	320.8 ± 7.69 ^{Ab}	483.4 ± 7.25 ^{Bb}	286.0 ± 14.92 ^{Cb}
	GG	320.7 ± 17.73 ^b	320.7 ± 17.73 ^{ab}		
<i>GH2</i> (<i>Ddel</i>)	AA	404.0 ± 11.87 ^a	352.5 ± 9.06 ^{Aa}	525.9 ± 6.51 ^B	324.6 ± 11.74 ^C
	AC	346.4 ± 16.00 ^b	316.8 ± 6.29 ^{Ab}	539.1 ± 24.86 ^B	306.5 ± 2.32 ^A
	CC	417.9 ± 22.92 ^a		537.0 ± 11.69 ^A	315.8 ± 6.32 ^B
<i>GH3</i> (<i>MspI</i>)	CC	404.8 ± 10.15 ^a	344.4 ± 6.84 ^{Aa}	532.4 ± 5.82 ^B	319.6 ± 8.16 ^C
	CT	333.2 ± 16.69 ^b	306.6 ± 6.85 ^{Ab}	496.0 ± 0.50 ^B	320.0 ± 7.32 ^A
	TT	302.9 ± 0.50 ^c			302.9 ± 0.50
<i>STAT5</i> (<i>Aval</i>)	CC	404.0 ± 10.29 ^a	344.1 ± 9.33 ^A	535.8 ± 5.61 ^{Ba}	324.7 ± 4.98 ^{Ca}
	CT	344.5 ± 17.34 ^b	325.9 ± 4.92 ^A	483.4 ± 7.25 ^{Bb}	306.3 ± 0.50 ^{Cb}

GHI – growth hormone (exon 5, 2141C>G), *GH2* – growth hormone (exon 5, 2291A>C), *GH3* – growth hormone (intron 3, 1548C>T), *STAT5A* – signal transducer and activator of transcription 5A (exon 7, 6853C>T); ^{ab} – different lowercase letters in columns (for each gene separately) indicate statistically significant differences ($P < 0.05$); ^{ABC} – different uppercase letters in rows (between breeds) indicate statistically significant differences ($P < 0.05$); data are presented as mean values ± SEM

The influence of the genotypes of all studied genes on carcass muscularity conformation and fat coverage classes in individual breeds varied according to the cattle carcass muscularity classification system (Figure 2). Most carcasses with the *GHI* CC genotype were assigned to the U (very good, EUROP system) carcass conformation class in Limousin, Charolais, and the general population, but not in Angus (Figure 2). In Charolais bulls, the *GHI* CC genotype was also associated with the highest muscularity class E (excellent) (Figure 2). In contrast, carcasses from bulls with the *GHI* CG genotype were more frequently ranked in the lower R (good) and even O (fair) classes (Figure 2), demonstrating significant differences between *GHI* genotypes ($P < 0.001$) in all breeds except Angus. For *GH2*, while AA and CC genotypes were predominantly associated with U (very good) class, the AC heterozygote showed reduced performance, particularly in Charolais and the general population, where it was associated with R class ($P < 0.05$ and $P < 0.01$, respectively).

in Angus, Charolais and the general population ($P < 0.05$). For *STAT5A*, the CC genotype bulls consistently produced U class carcasses, with significant CC-CT differences in Charolais and general population ($P < 0.001$), where CT genotype carcasses were typically graded as R or O (fair) conformation class. Most bull carcasses from all breeds were assigned to the 3rd (medium) fat coverage class, with genotypes showing limited influence on this trait. However, differences were observed for the Limousin breed. The *GH2* CC genotype was more frequently associated with the 3rd (medium) fat class compared to the AA and AC genotypes ($P < 0.01$). Bull carcasses with the *GH3* TT genotype were primarily ranked as the 2nd (low) fat class, while the CT genotype more frequently placed in the 3rd class ($P < 0.05$). *STAT5A* gene genotypes also showed a clear association with fat coverage in Limousin bulls. Most carcasses with the CT genotype were classified in the 2nd (slight) fat coverage class, whereas the CC genotype to the 3rd (medium) class ($P < 0.05$).

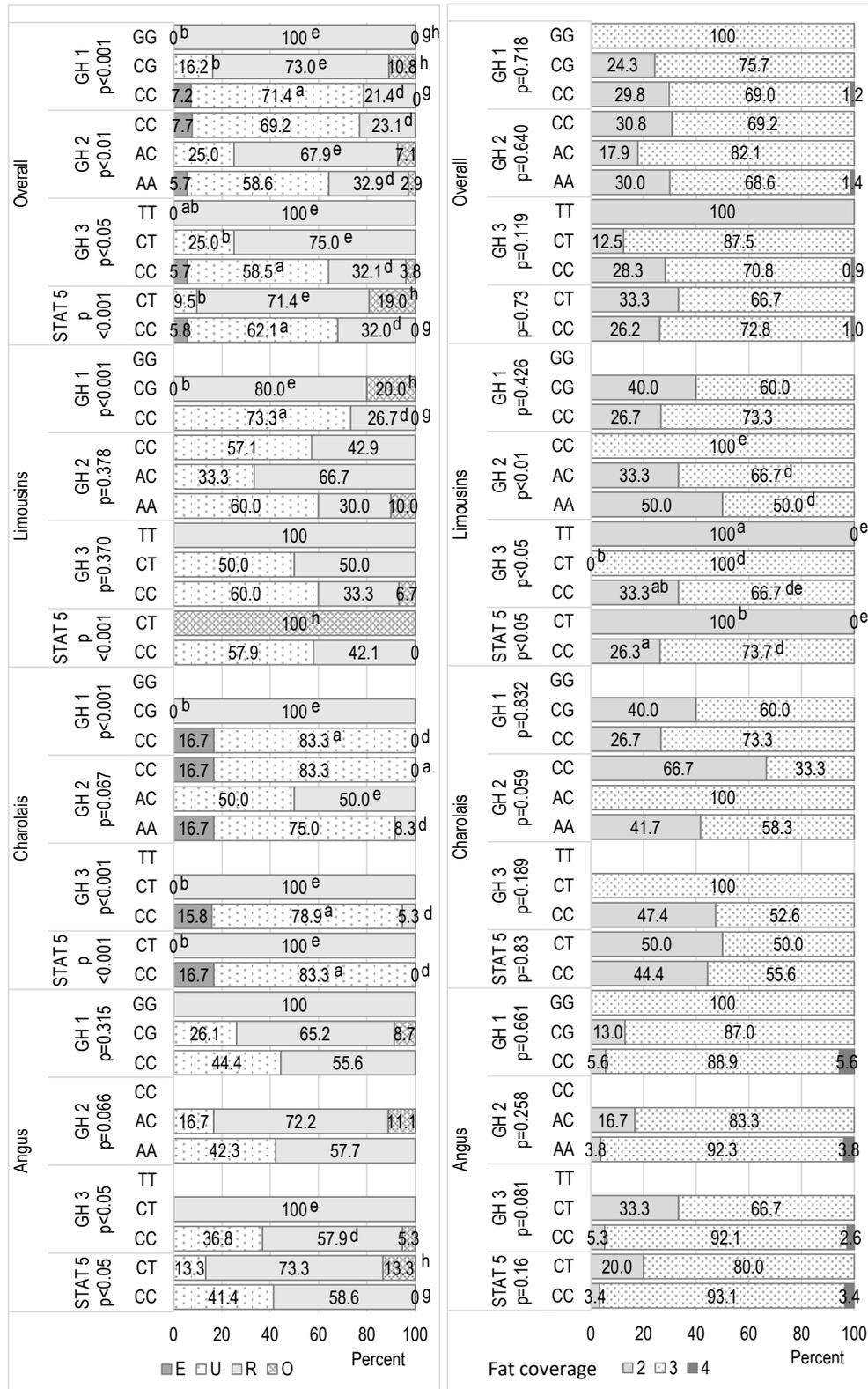


Figure 2. Influence of *GH1* (exon 5, 2141C>G), *GH2* (exon 5, 2291A>C), *GH3* (intron 3, 1548C>T), and *STAT5* (exon 7, 6853C>T) polymorphisms on carcass muscularity classes (EUROP, %), and fat coverage classes (%) in individual breeds

(A) *GH1* – lanes 1, 2, 3, and 6 – CG genotype; lanes 4 and 5 – CC genotype; lane 7 – 50 bp DNA ladder marker (Thermo Fisher Scientific Baltics, Vilnius, Lithuania); (B) *GH2* – lanes 2, 3, and 5 – AA genotype; lane 4 – AC genotype; lanes 1 and 6 – 50 bp DNA ladder marker; (C) *GH3* – lanes 2, 4, and 5 – CC genotype; lane 3 – CT genotype; lane 1 – 50 bp DNA ladder marker; (D) *STAT5A* – lanes 2, 3, 4, and 6 – CC genotype; lane 5 – CT genotype; lane 1 – 50 bp DNA ladder marker; frequencies marked with different letters indicate statistically significant differences in muscularity classes: ^{ab} – U class ($P < 0.05$), ^{de} – R class ($P < 0.05$), ^{gh} – O class ($P < 0.05$), for fat coverage classes: ^{ab} – class 2 ($P < 0.05$), ^{de} – class 3 ($P < 0.05$); *GH1* – growth hormone (exon 5, 2141C>G), *GH2* – growth hormone (exon 5, 2291A>C), *GH3* – growth hormone (intron 3, 1548C>T), *STAT5A* – signal transducer and activator of transcription 5A (exon 7, 6853C>T)

Discussion

This study investigated the association of *GHI*, *GH2*, *GH3*, and *STAT5A* polymorphisms on growth performance and carcass quality traits in beef cattle. Candidate gene studies are valuable tools in livestock breeding, as they support early selection and contribute to high economic returns. The *GHI* gene is a key candidate associated with economic traits such as growth, reproduction, and fattening performance (Bayraktar et al., 2022; Gerasimov et al., 2023.). In the present study, the *GHI* C allele and CC genotype were most frequent in all bull breeds studied: Angus (52%), Charolais (90%), and Limousin (75%). These findings are consistent with the results of Sedykh et al. (2020), who reported higher frequencies of the CC genotype (52.63%) compared to CG (35.96%) and GG (11.40%) in beef cattle. The C allele has been consistently associated with favourable traits, including higher birth weight (Lee et al., 2013), mature weight, and marbling (Fedota et al., 2016). In a subsequent study, Fedota et al. (2017) reported a clear association between *GHI* genotypes and live weight, with animals carrying the CC genotype consistently exhibiting higher body weight compared to heterozygous CG and homozygous GG animals. On average, CC individuals were 3–25 kg heavier than CG, and 10–25 kg heavier than GG cattle. Comparable results were obtained in the present study. The average daily weight gain of bulls with the *GHI* CC genotype was 25% higher than that of heterozygous CG genotype bulls in the Limousin breed and 5% higher in the general cattle population during the fattening period. In addition, the CC genotype was associated with higher chilled carcass weight (kg) and, according to the EUROP carcass classification system, was mostly assigned to the U (very good) class, with some carcasses even assigned to the E (excellent) class. In contrast, carcasses from animals with the CG genotype were more commonly classified in lower classes, with these differences between genotypes being statistically significant ($P < 0.05$). These findings align with results of Tatsuda et al. (2008), who reported that the animals with the *GH* CC genotype had significantly higher chilled carcass weight and meat content compared to those with the GG genotype—by 7.20% and 4.86% in Herefords, and 6.65% and 8.02% in Limousins, respectively. Although the findings of Gerasimov et al. (2023) differed from ours, as they showed that young animals with the GG genotype had significantly higher body weight at weaning and 1 year of age, as well as faster growth rate during the control rearing period. The present results regard-

ing the *GH2* polymorphism were similar to those obtained by other researchers. The *GH2* AA genotype predominated in our population (Angus 59%, Charolais 60%, Limousin 50%), which was consistent with Mullen et al. (2010), who reported genotype frequencies of 78% for AA, 20% for AC, and 2% for CC. Similarly, Lee et al. (2013) found that the most frequent *GH2* genotype (2291A>C) in the Hanwoo beef breed was homozygous AA (67%) followed by heterozygous AC (29%) and homozygous CC (4%). In the current study, significant differences in daily weight gain (kg) between the *GH2* AA, AC, and CC genotypes were observed only in Limousin bulls and the general beef cattle population. Significant differences in chilled carcass weight (kg) between the AA and AC genotypes of the *GH2* polymorphism were also found in Angus bulls and the general population. Bulls carrying the *GH2* AA genotype showed higher chilled carcass weight (kg) compared to bulls with other genotypes. Silveira et al. (2008) reported significant genotype-related differences only in weaning weight, with calves carrying the AA genotype weighing approximately 5 kg more than those with the AC genotype, and 4.5 kg more than those with the CC genotype. Our assessment using the EUROP carcass muscularity classification system showed that carcasses with the *GH2* AC genotype were more frequently classified in the lower R (good) muscularity conformation class. In contrast, most carcasses with the *GH2* AA and CC genotypes were assigned to the U (very good) class; however, the differences between genotypes were not statistically significant. Meanwhile, in Limousins, the CC genotype showed a significant association with 3 (medium) fat coverage compared to AA and AC genotypes ($P < 0.01$). These findings contrast with Lee et al. (2013), who observed no significant associations between the *GH2* 2291A>C polymorphism and carcass traits in Hanwoo cattle. Analysis of the *GH3* polymorphism revealed that the C allele and the CC genotype were the most frequent in all studied bull breeds. Meanwhile, the homozygous *GH3* TT genotype was found only in the Limousin breed (5%) and was absent in other breeds. Similar findings were reported by Putra et al. (2013), who observed lower frequencies of the T allele and TT genotype in Limousin and Simmental breeds (*Bos taurus*) compared to Indonesian Aceh cattle (*B. indicus*). The *GH3* 1548C>T SNP showed statistically significant differences between genotypes, observed exclusively in the Limousin breed. Bulls with the TT genotype had 24% higher daily weight gain (kg) compared to those with the CC genotype and 21% higher than bulls with the CT

genotype. This result aligns with findings by Arango et al. (2014), who recorded significant differences in growth traits associated with an intron 3 polymorphism in the *GH* gene, with the TT genotype animals weighing 73.6 kg and 68.7 kg more than those with the CC and CT genotypes, respectively. In contrast, Maylinda et al. (2011) observed that body weight was more strongly associated with the CC and CT genotypes than with the TT genotype. In our study, statistically significant differences were also found in chilled carcass weight (kg), with bulls carrying the *GH3* TT genotype both in the Angus breed and the general cattle population showing lower values. Additionally, carcasses of bulls with the TT genotype in the general population were predominantly assigned to the lower R (good) carcass conformation class.

The results of our study on the *STAT5A* SNP were consistent with those obtained by other researchers. Selvaggi et al. (2015) reported C and T allele frequencies of 83% and 17%, respectively. In our study, the C allele was predominant in all investigated bull breeds, with frequencies of 97% in Limousin, 95% in Charolais, and 83% in Angus. Regarding genotype distribution, the CC genotype was the most prevalent in our study: 95% in Limousin, 90% in Charolais, and 66% in Angus, while the TT genotype was not detected. Likewise, a study examining the *STAT5A*-*AvaI* polymorphism in cattle breeds of European origin (Red Angus, Charolais, Limousin, and Hereford) reported that the CC genotype was the most common and the TT genotype was not detected (Daldaban et al., 2020). The present results showed that bulls with the *STAT5A* CC genotype had significantly higher daily weight gain than those with the CT genotype, both within the Limousin breed and the general cattle population. Additionally, statistically significant differences in *STAT5A* genotypes between breeds were observed for chilled carcass weight. The advantage of the CC genotype over the CT genotype was evident in Charolais and Limousin bulls, as well as in the general cattle population. Similarly, Oprządek et al. (2005) showed that beef cattle with the CC variant of the *STAT5A* polymorphism outperformed CT animals in terms of weight gain, several carcass traits, and feed conversion, which was consistent with the aforementioned studies of Daldaban et al. (2020) and Selvaggi et al. (2015). Our study also demonstrated that, according to the EUROP carcass classification system, the CC genotype of the *STAT5A* polymorphism was significantly associated with higher carcass muscularity conformation classes as opposed to the CT genotype. Statistically significant differences between the CC and CT genotypes were observed

in Charolais cattle and the general population. Most bulls of all breeds with the CT genotype had carcasses classified in the lower R (good) and O (fair) conformation classes. Daldaban et al. (2020) also found that animals with the CC genotype presented better carcass traits compared to those with the CT genotype.

This study has confirmed that polymorphisms in the *GH*, and *STAT5A* genes are associated with important growth and carcass traits in beef cattle. The *GH1* CC and *GH2* AA genotypes were most frequent and associated with improved daily weight gain and chilled carcass weight. The *GH3* CC genotype was dominant across breeds and associated with better carcass conformation, while the rare TT genotype increased weight gain but reduced carcass weight. The *STAT5A* CC genotype showed the strongest overall association with increased weight gain, carcass weight, and muscularity, particularly in Limousin and Charolais bulls.

Conclusions

Charolais bulls raised in Lithuania demonstrated better growth and carcass performance among all analysed genotypes compared to Angus and Limousin breeds, which displayed greater genotype-dependent variation. These findings underscore the potential utility of *GH* and *STAT5A* gene polymorphisms as molecular markers in selective breeding programmes aimed at improving productivity and carcass quality in beef cattle. Future research should build upon these findings by increasing the sample size and incorporating additional beef cattle breeds to improve the reliability and broader applicability of the results.

Conflict of interest

The Authors declare that there is no conflict of interest.

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