# The effect of GHR gene polymorphism on growth and carcass quality of heifers

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**Abstract**

This research was done to determinate the allele variants of the GHR gene in the heifer population of Simmental and Simmental x Holstein crosses. Furthermore, next objective was to determinate effect of GHR genotypes on growth characteristics, carcasses and meat quality. In analysed population frequencies of GHR genotypes were: AA (0.468), GA (0.426), and GG (0.106). Based on the determined genotypes, the allelic frequencies were as follows: A (0.681) and G (0.319). Statistically significant positive effect of GHRGG genotype was observed for higher net daily gain and muscle tissue percentage, and lower fat tissue percentage. Results indicate the potential usage of GHR gene variants in cattle selection associated to carcass and meat quality.

**Key words**: GHR gene, polymorphism, growth, carcass quality

**Introduction**

In the last decade selection in cattle breeding relay on genomics and direct selection to determine variants of polymorphic genes. Beef production is generated on genetic basis and non-genetic factors that affects body conformation, growth dynamics and meat quality which in economically developed countries turns the consumers attention. Growth hormone receptor (GHR) is considered as a strong and functional candidate gene for which is assumed to have a significant role in cattle metabolism, have affects in metabolic processes and growth of the organism. The bovine GHR gene has been mapped to BTA 20 and several polymorphism have been identified (Lucy et al., 1998; Hale et al., 2000). Ge et al. (2000) reported single nucleotide polymorphism (SNP) at position 257 (A/G) in exon 10 inducing an amino acid substitution of serine/glycine at protein position 555 (S555G). Exon 10 of the GHR gene is responsable for coding the cytoplasmic domain of the GHR. Polymorphisms in GHR have been found to be associated with drip loss (Di Stasio et al., 2005), marbling (Han et al., 2009) and some tehnological and sensory meat traits (Reardon et al., 2010). The aim of this study was to determine the effect of the GHR gene on growth performance, carcass and meat quality of heifers.

**Material and methods**

The research included samples of forty seven Simmental and crosses of Simmental x Holstein heifers. Animlas were kept in separate fattening facilities. During the fattening period they were fed with a total mixed ratio (TMR; corn silage, high moisture corn, concentrate and straw in weight ratio 45 : 40 : 10 : 5). Transport of animals to slaughterhouse, slaughter, and carcasses processing was carried out according to standard procedure. EUROP classification of conformation (E, U, R, O, P) and estimation of carcass coverage with fat tissue (score from 1 to 5) was done on the warm carcasses. Cold carcasses weight were mesured after cooling (24 h / 4 oC). The sample of MLD was cut off at the height of the 8th rib for chemical analysis by NIT spectrophotometry (Near Infrared Transmittance Spectroscopy) of Foodscan (Foss Electric A/S, Hillerød, Denmark). On the rib area (between the 10th and 12th rib), dissection of muscle and fatty tissue was perfomed to estimate their share in carcass.

The isolation of DNA was made from each individual tissue sample according to manufacturer’s protocol of Sigma-Aldrich, USA. The DNA strand length of 342 base pairs was multiplied using the oligonucleotide primers 5’-GCTAACTTCATCGTGGACAAC-3’ and 5’-CTATGGCATGATTTTGTTCAG-3’. Polymerase Chain Reaction (PCR) was performed according to the manufacturer’s protocol (Takara Bio Inc., Otsu, Shiga, Japan) in a total volume of 15 μL including 1.2 μL of genomic DNA, 7.5 μL EmeraldAmp® MAX HS PCR Master Mix, 0.45 μL of each oligonucleotide primers and 5.4 μL of water. Multiplication of the sequence involved activation of the Taq polymerase (98 oC/3 min), 35 cycles for multiplying DNA sequence (98 oC/ 10 s, 53 oC/ 30 s, 72 oC/ 50 s) and it’s final extension (72 oC/5 min). GHR allele variants were determinated by restriction with *AluI* enzyme (Promega Corporation, USA) and the visualization was performed on a stained ethidium bromide 3% agarose gel with a standard ladder of 50 base pair and visualized under UV light. The genotype and allele frequencies for polymorphism were calculated. The effect of genotype on growth characteristics, carcasses and meat quality was determined using the linear models (GLM procedure, SAS STAT, V8, 1999) with fixed effects of GHR genotype, breeds and slaughter age (months): Yijk=*μ* + Gi + Bj + Ak + eijkh (Yijkh is a phenotypic observation; *μ* is the overall mean; Gi is the fixed effect of GHR genotype; Bj is effect of breed; Ak is effect of slaughter age; eijkh is the random residual effect). The least square means were estimated for genotype groups (*differences were tested by Scheffe`s test*). The substitution effects of favourable alleles were calculated using linear regression instead of genotype effect in linaer model from above: Yijk=*μ* + βxi + Bj + Ak + eijkh, where Yijkh is a phenotypic observation; *μ* is the overall mean; β is the linear regression coefficient (allele substitution effect); xi is the number of desired alleles (0, 1, 2); Bj is effect of breed; Ak is effect of slaughter age; eijkh is the random residual effect).

**Results and discussion**

Descriptive statistics of slaughter age, weight of live animals, carcass weight, EUROP classification, ratio of different tissue types in rib clip area, and the basic chemical composition of meat from hifers (Table 1). The highest coefficients of variability are observed for the characteristics associated with the amount of fatty tissue in carcasses (carcass fatness score, fat tissue in carcass, content of fat in MLD).

Table 1 The mean, standard deviation, minimum, maximum and coefficient of variation of the studied traits (n=47)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Trait | Mean | S.D. | Min. | Max. | CV |
| Slaughter age (month’s) | 15.21 | 0.67 | 12.0 | 16.5 | 4.39 |
| Slaughter weight (kg) | 472.8 | 38.45 | 406.0 | 565.0 | 8.13 |
| Cold carcass weight (kg) | 267.7 | 21.03 | 222.6 | 320.0 | 7.85 |
| Net daily gain (g) | 578.8 | 59.79 | 500.2 | 874.3 | 10.33 |
| Carcass EUROP score (1-5) | 3.27 | 0.45 | 3.00 | 4.00 | 13.66 |
| Carcass fatness score (1-5) | 2.98 | 0.52 | 2.00 | 4.00 | 17.45 |
| Muscle tissue in carcass (%) | 62.93 | 3.83 | 52.84 | 70.55 | 6.08 |
| Fat tissue in carcass (%) | 20.31 | 3.67 | 13.09 | 31.35 | 18.06 |
| Bone tisue in carcass (%) | 16.75 | 1.89 | 11.86 | 20.85 | 11.27 |
| Content of protein (%) | 21.94 | 1.38 | 17.82 | 25.10 | 6.30 |
| Content of fat (%) | 3.23 | 1.58 | 0.87 | 9.50 | 48.84 |
| Content of collagen (%) | 1.41 | 0.14 | 1.21 | 1.87 | 10.26 |
| Content of ash (%) | 0.58 | 0.21 | 0.03 | 1.02 | 36.39 |

Using the *AluI* restriction enzyme GHR gene polymorphism was determined on 342 bp fragment codogene sequence. The homozygous GHRAA genotype was identified by three fragments (191 bp, 101 bp, 50 bp), GHRGG genotype by two fragments (191 bp, 151 bp), and heterozygous GHRGA genotype by four fragments (191 bp, 151 bp, 101 bp, 50 bp). Results of genotipisation of the GHR gene are presented in Table 2.

Table 2 Frequency of genotypes and allele variants of the GHR gene, observed and expected heterozygosity in the studied population of heifers

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Genotype | No. observed genotype | Genotype frequency | Ho  He | *χ2* | Allele | Frequency of allele | *S.D.* |
| GHRAA  GHRGA GHRGG | 22  20  5 | 0.468  0.426  0.106 | 0.426  0.434 | 0.0204ns | GHRA GHRG | 0.681  0.319 | ± 0.0481 |

Ho/He – observed/excepted heterozygosity; χ2 – Chi square; ns – non significant; *S.D.* – standard deviation

Allele variant GHRA in relation to GHRG allele dominates in the studied cattle populations (0.68 : 0.32). Ardicli et al. (2017) observed in Holstein population observed dominance of GHRA alelle variant (A vs. G; 0.76 : 0.24). Reardon et al. (2010) in population of Irish cross bred cattle observed the higher frequency of GHRA allele variants of the GHR gene (A vs. G; 0.58 : 0.42). Hadi et al. (2015) also noticed dominance of GHRA alelle variant (A vs. G; 0.64 : 0.36). Contrary, Di Stasio et al. (2005) observed equal distribution of both alelle variants (A vs. G; 0.49 : 0.51) in population of Piemontese bulls. The results of χ*2*‑test show that the observed genotypes of GHR gene in the examined population are not significantly different from those predicted for a population in Hardy-Weinberg equilibrium (Table 2).

Table 3 Effect of GHR genotype on carcass and meat quality indicators of heifers

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Indicator | Genotype | | | Sub. eff. of G allele |
| AA | GA | GG |
| Carcass | Weight (kg) | 270.5 ± 20.4 | 262.8 ± 19.3 | 274.1 ± 30.5 | -4.38 |
| Net daily gain (g) | 578.7 ± 46.8A | 567.8 ± 37.9a | 623.2 ± 142.8Bb | -8.09 |
| EUROP score (1-5) | 3.20 ± 0.41 | 3.30 ± 0.47 | 3.40 ± 0.55 | 0.07 |
| Fatness score (1-5) | 3.12 ± 0.45 | 2.85 ± 0.59 | 2.80 ± 0.45 | -0.28 |
| Muscle tissue (%) | 61.42 ± 4.00A | 63.89 ± 3.18 | 66.35 ± 1.73B | 2.85\*\* |
| Fat tissue (%) | 21.95 ± 3.50aA | 19.35 ± 3.23B | 16.33 ± 0.80b | -2.85\*\* |
| Bone tisue (%) | 16.63 ± 2.18 | 16.76 ± 1.56 | 17.32 ± 1.79 | 0.003 |
| In MLD | Protein (%) | 21.95 ± 1.00 | 22.11 ± 1.35 | 21.24 ± 2.76 | 0.10 |
| Fat (%) | 3.36 ± 1.22 | 3.44 ± 1.99 | 1.82 ± 0.68 | -0.41 |
| Collagen (%) | 1.36 ± 0.10 | 1.45 ± 0.17 | 1.51 ± 0.15 | 0.09\* |
| Ash (%) | 0.62 ± 0.21 | 0.60 ± 0.18 | 0.35 ± 0.22 | -0.07 |

Different large A-B letters in order signify p<0.05; different small a-b letters in order signify p<0.01

Although, heterozygous individuals have had slightly lower cold half carcasses weight the observed differences were not ststistically significant. However, higher net daily gain was determined in GHRGG genotype compared to homozygote GHRAA and heterozygote GHRGA genotype(p<0.01). Similar results to present study were obtain in Angus cattle by Ge et al. (2003), who failed to reveal significant effect of GHR gene on growth traits. Stasio et al. (2005) were generally observed unfavourable effect of the allele GHRA for all growth traits, but none of the values were significant. Share of muscle tissue in beef carcasses were significantly higher in animals of GHRGG genotypes compared to the GHRAA genotypes (66.35 vs. 61.42; p<0.05). Contrary, individuals with GHRAA genotype have significantly higher fat tissue percentage compared to GHRGA (p<0.05) and GHRGG (p<0.01). Reardon et al. (2010) also observed significant GHR gene polymorphisms association with the share of intramuscular fat (p<0.001). The effect of GHR allele substitution is present in Table 3. Changing one copy of the GHRA allele by the GHRG allele leads to significant (p<0.01) increase share of muscle tissue and decrease share of fat tissue by 2.85 %. In addition, the GHRG allele leads to significant (p<0.05) increase content of collagen by 0.09 %. The substitution effect of GHRG allele were non significant for EUROP carcass classification, fatness score, share of bone tisue, and protein, fat and ash content in MLD. However, due to low number of GHRGG genotypes, estimation of substitution effect of G allele for carcass weight and net daily gain was not reliable.

**Conclusion**

The dominance of A allele (0.681) of the GHR gene was determined in the researched sample of heifer. The observed genotypes of GHR gene statistically do not deviate from Hardy-Weinberg equilibrium. GHRGG genotype was associated with higher proportion of muscle tissue and colagen while to GHRAA genotype had higher proportion of fatty tissue. Substitution effect of G allele showes that one copy of this allele variant increases percenatge of muscle tissue (2.85%) and colagen (0.09%) and decreases fatt tissue percentage (2.85%). Results indicate the potential usage of GHR gene variants in cattle selection associated to carcass and meat quality.

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**Učinak polimorfizma GHR gena na rast i kvalitetu trupova junica**

**Sažetak**

Istraživanje je provedeno radi utvrđivanja udjela alelnih varijanti GHR gena u populaciji simentalskih junica i križanaca simentalskih × Holstein junica, te povezanosti alelnih varijanti GHR gena i genotipova s odlikama rasta, trupa i kakvoće mesa. U istraživanoj populaciji tvrđene su slijedeće frekvencije genotipova GHR gena: AA (0,468), GA (0,426) i GG (0,106), odnosno frekvencije alela kako slijedi A (0.681) i G (0.319). Utvrđen je pozitivan utjecaj GHRGG genotipa na veće dnevne priraste i veći udio mišićnog tkiva, te niži udio masnog tkiva u trupovima junadi koji su bili statistički značajni. Rezultati ukazuju na iskoristivost determinacije GHR genskih varijanti i njihove uporabe u selekciji mesnih pamina goveda.

**Ključne riječi**: GHR gen, polimorfizam, rast, kvaliteta trupa