The association of polymorphic variants of growth hormone gene with slaughter traits and carcass composition in crossbred Red Angus × Kalmyk bull-calves

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Abstract. The purpose of research was to study the association of polymorphic variants of growth hormone gene with development, slaughter traits and carcass composition in crossbred Red Angus × Kalmyk bull-calves. The experiment was carried out at LLC “Agrofirma Aduchi” in Republic of Kalmykia. The object of the study was crossbred Red Angus × Kalmyk (F2) bull-calves. The use of Red Angus sires with a high concentration of G allele in GH gene in the reproduction system of a herd made it possible to obtain second-generation crossbred progeny with a genotype distribution of 0.0625: 0.5: 0.4375, respectively, VV: LV: LL. The frequency of V allele reached 0.3125. The superiority of heterozygous individuals in live weight reached 18.0 kg by the age of 18 months (P>0.05). The maximum carcass weight was established in the group of heterozygous individuals, their superiority was 25.3 kg (P <0.01). The advantage in carcass yield of carriers of the minor allele V was 1.7% (P<0.05). The heterozygous animals were superior in flesh part weight in half-carcass by 11.1 kg (P <0.001). A less pronounced intergroup difference was found in bone weight, which reached 1.2 kg (P > 0.05). The monitoring the genetic structure of the herd taken into account the allelic distribution in growth hormone gene will provide the development of meat productivity potential.

1. Introduction
Improving the efficiency of beef production is an important task of agriculture. Modern achievements of molecular genetics have recently been involved for its successful solution. Thus, the use of DNA markers allows predicting quantitative and qualitative indicators of meat productivity at an early stage of ontogenesis. In particular, traits determined only after slaughter of animals (tenderness of meat, “marbling”, carcass weight, etc.) are predictable with sufficient accuracy based on the allelic profile of quantitative trait loci (QTL). This was a significant impetus to the cultivation of beef cattle herds, because for a long time the selective improvement of meat productivity indicators was limited by the difficulties in their lifetime assessment [1]. However, the genetic variability of productive traits is controlled by the action of many genes [2, 3]. In this regard, the reducing the list of key genes is advisable to search for reliable associations with economically useful traits for the applied introduction into the breeding program. Many biological processes in animals are ultimately related to the level of beef cattle productivity [4, 5]. Thus, genes involved in the molecular mechanisms of digestion, metabolism,
respiration and thermoregulation act as potential candidates for quantitative trait loci [6, 7]. Single nucleotide substitutions in such genes can be associated with variability in productive traits [8].

The use of growth hormone gene has found a wide application in MAS-selection as a marker of productive properties in cattle due to its early sequencing [9]. A lot of research have been conducted to study the associations of polymorphic states of this gene with growth rate, milk and meat productivity in livestock of various breeds in different countries, populations and herds [10]. There are at least 10 single nucleotide substitutions in different regions of bovine growth hormone gene [11]. The substitution of C-G bases in the 5th exon of the growth hormone gene is related with AluI polymorphism, as a result the amino acid Leucine is replaced with Valine (C2141G, rs 4192384).

The purpose of our research was to study the association of polymorphic variants of growth hormone gene with development, slaughter traits and carcass composition in crossbred bull-calves Red Angus × Kalmyk (F₂).

2. Materials and methods.

The research work was carried out in LLC “Agrofirma Aduchi” in Kalmykia Republic. The object of study were crossbred bull-calves Red Angus × Kalmyk (F₂). Rearing of experimental animals was carried out in a single herd under the same conditions, which were organized in accordance with the traditional technology adopted in beef cattle breeding.

Genotyping by the growth hormone (GH) gene was performed on the basis of DNA extracted from the whole blood using reagents "DIAtom™ DNA Prep" (IsoGene Lab, Moscow) in Laboratory of immunogenic and DNA-technologies of All-Russian Research Institute of sheep and goat breeding – branch of the North Caucasus federal agricultural Research Center (Stavropol). Blood samples were picked from jugular vein and collected into test tubes with 600 µl EDTA.

A “GenePak™ PCR Core” kit (IsoGene Lab, Moscow) were applied to conduct the polymerase chain reaction. PCR-RFLP was performed using a “Tertsik” programmable thermal cycler (DNA technology, Russia). The amplification of the sites was conducted using primer with following sequence: F: 5’-gct-gct-cct-gag-ggc-cct-tcg-3’; R: 5’-gcg-gcc-gca-ctt-cat-gac-cct-3’. The size of the amplified fragment was 223 p.n. PCR program: “hot start” - 5 min. at 95°C; 35 cycles: denaturation - 45 seconds at 94°C, annealing - 45 seconds at 65°C, synthesis - 45 seconds at 72°C; completion - 7 minutes at 72°C. The endonuclease AluI was used for restriction of the amplified regions of the genes. The splitting of the products was carried out at 37°C, the genotypes were identified by gel electrophoresis with visualization under UV light. Product identification for growth hormone gene: GH⁹⁹⁻ – 223 p.n.; GH¹¹⁻ – 223, 171, 52 p.n.; GH¹²⁻ – 171, 52 p.n.

Experimental groups were formed after genotyping in accordance with the allelic variant of the GH gene: I group – GH¹¹⁻ heterozygous bull-calves (n = 8 individuals), II group – homozygous animals GH¹¹⁻ gene (n = 7 individuals). Observations on the changes in live weight were carried out by monthly weighing in the morning before feeding. Growth traits included live weight at different ages: birth, 8, 12, 15 and 18 months. Average daily gain (g/d) was calculated for different age periods.

Experimental bull-calves (3 individuals from each group) were slaughtered at the age of 18 months. Carrcass traits included hot carcass weight, carcass dressing percentage, internal fat weight, slaughter weight and slaughter yield. Carrcasses were dressed and halved into half-carrcasses, chilled for 24 h at a temperature of around +2.4°C and weighted. Right half carrcasses were dissected into 5 anatomical parts: I – neck; II – humeroscapular; III – back rib cut; IV – lumbar cut ; V – hip cut. Net meat, total bones, cartilage and tendons were weighted during half-carrcasses dissection.

**Statistical analysis.** Data were processed with one-way analysis of variance with GLM procedure using Statistica 10.0 software (Statsoft Inc., USA). Least squares differences and probability values for differences were calculated using Tukey’s test.

3. Results

The use of Red Angus sires with a high concentration of the “desirable” allele for the growth hormone gene in the herd reproduction system made it possible to obtain crossbred progeny (F₂) with a genotypes distribution of 0.0625: 0.5: 0.4375, respectively for VV: LV: LL. The frequency of V allele reached 0.3125. Thus, half of the sons were carriers of the heterozygous genotype for the GH gene. There was
only one bull-carrier of the homozygous GHVV variant among the genotyped livestock (n = 16), therefore its productivity indicators were not taken into account when processing the results of the studies. Table 1 shows the live weight dynamics of bull-calves with genotypes LV (n = 8) and LL (n = 7) in growth hormone gene by age periods.

Table 1. Live weight in Red Angus × Kalmyk (F2) bull-calves depending on genotype of GH gene (M±SE), kg

| Age       | Genotype  | GHLV   | GHLL   |
|-----------|-----------|--------|--------|
| At birth  | 24.7±0.88 | 27.4±0.78* |
| 8 months  | 214.3±4.88 | 211.6±3.26 |
| 12 months | 323.9±7.56 | 314.6±7.54 |
| 15 months | 412.0±6.05 | 402.4±11.18 |
| 18 months | 476.1±6.60 | 458.1±13.67 |

Bull-calves with homozygous genotype LL surpassed their peers in live weight at birth by 2.7 kg (10.93%; P<0.05). However, heterozygous individuals showed the best weight growth at the next stages of ontogenesis. Thus, animals with the LV genotype had a minimum superiority by 2.7 kg (1.28%; P>0.05) at the age of weaning (8 months). Further, the advantage of heterozygous individuals increased and reached 18.0 kg (3.93%; P> 0.05) by 18 months of age.

The superiority of GHLV genotype carriers in average daily gain was established at all stages of weight growth control (Fig. 1). However, the intergroup differences were not statistically significant.

Figure 1. The variability of average daily gain in Red Angus × Kalmyk (F2) bull-calves depending on genotype of GH gene (M±SE), g

It should be noted that the highest superiority of heterozygous individuals in average daily gain was established in the final rearing period (15-18 months), amounting to 91.1 g (14.88%; P> 0.05). This indicates a genetically determined duration of intensive growth.
Carcasses of unequal mass were obtained at slaughter of bull-calves of studied genotypes (Table 2). The most massive carcasses were found in the group of heterozygous individuals, their superiority was 25.3 kg (10.63%; P <0.01). The maximum carcass yield was also recorded in carriers of the V allele, the advantage reached 1.7% (P <0.05).

Table 2. Slaughter traits in Red Angus × Kalmyk (F2) bull-calves depending on genotype of GH gene (M±SE)

| Trait                        | Genotype   |
|------------------------------|------------|
|                              | GH<sup>LV</sup> | GH<sup>LL</sup> |
| Hot carcass weight, kg       | 263.3±2.73** | 238.0±2.65       |
| Carcass dressing percentage, % | 56.2±0.26*  | 54.5±0.52        |
| Visceral fat weight, kg      | 12.8±0.33   | 15.4±0.38**      |
| Visceral fat yield, %        | 2.8±0.05    | 3.5±0.08**       |
| Slaughter weight, kg         | 276.1±3.06**| 253.4±3.01       |
| Slaughter yield, %           | 59.0±0.31   | 58.0±0.59        |

The best development of adipose tissue was found in the group of homozygous individuals. Thus, from GH<sup>LL</sup> genotype was obtained more visceral fat at the slaughter of animals by 2.6 kg (20.31%; P <0.01) compared with the analogues. The advantage (P <0.01) in the yield of visceral fat was also observed on the side of bull-calves, whose genotype lacked the desired allele.

Leadership in terms of slaughter weight was found for heterozygous genotype carriers, who were 22.8 kg ahead of their peers (9.00%; P <0.01). This had provided them with 1.0% superiority in slaughter yield (P>0.05). Thus, the presence of V allele in the locus of the growth hormone gene contributed to the production of more massive carcasses with minimal fat deposition from crossbred bull-calves.

Analysis of half-carcass weight showed a significant (P<0.01) superiority by 11.9 kg of individuals with a heterozygous variant of the genotype in the growth hormone gene (Table 3). The composition of the obtained half-carcasses had some features depending on the presence of the desired allele. Thus, the maximum flesh content was found in carcasses from GH<sup>LV</sup> genotype carriers, the superiority reached 1.3%. On the contrary, their peers of the homozygous state of the gene were characterized by the highest development of bone tissue, and the bone yield in chilled half-carcasses was 0.6% more than analogues.

Table 3. Morphological composition of carcasses in Red Angus × Kalmyk (F2) bull-calves depending on genotype of GH gene (M±SE)

| Trait                        | Genotype   |
|------------------------------|------------|
|                              | GH<sup>LV</sup> | GH<sup>LL</sup> |
| Half-carcass weight, kg      | 131.0±1.79** | 119.1±0.70       |
| Meat weight, kg              | 106.0±0.72*** | 94.9±0.45        |
| Meat yield, %                | 80.9±0.55   | 79.6±0.17        |
| Bone weight, kg              | 21.3±1.01   | 20.1±0.22        |
| Bone yield, %                | 16.3±0.55   | 16.9±0.19        |
| Cartilage and tendons weight, kg | 3.7±0.06   | 4.1±0.23         |
| Cartilage and tendons yield, % | 2.8±0.01   | 3.5±0.18**       |
| Meat yield per 1 kg of bones, kg | 4.99±0.20  | 4.71±0.06        |

The animals of heterozygous genotype had an advantage over their peers by the absolute weight of the main body tissues. Thus, the superiority in weight of flesh part was 11.1 kg (11.70%; P <0.001). A less pronounced intergroup difference was found in bone weight, which reached 1.2 kg (5.97%; P> 0.05) in favor of individuals carrying the desired allele. In addition, there were not noted significant differences in the number of cartilage and tendons - 0.4 kg (10.81%). The most optimal ratio of flesh part and bones was recorded during the boning of half-carcasses from bull-calves with GH<sup>LV</sup> genotype.
4. Conclusion
Crossbred progeny (F$_2$) were obtained as a result of using Red Angus sires in the reproduction system of the Kalmyk herd with the allelic frequency in growth hormone gene locus: V - 0.3125 and L - 0.6875. Allelic profile of the 5 exon of the gene at position 2141 p.n. had an impact on weight growth and meat productivity of bull-calves. Heterozygous individuals were superior in live weight by 18.0 kg (3.93%; P<0.05) at the age of 18 months compared with homozygous GH$^{LL}$ contemporaries. The presence of V allele in genotype of animals provided the most massive carcasses by 25.3 kg (10.63%; P<0.01). The superiority for meat weight in chilled carcass was 11.1 kg (11.70%; P<0.001). Thus, the monitoring the genetic structure of the herd taken into account the allelic distribution in growth hormone gene will provide the development of meat productivity potential.

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