Polymorphism of the CAPN1 and GH genes and its relationship with the productivity of cattle of the Kazakh white-headed breed

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Abstract. The improvement of beef cattle is associated with the use of molecular genetic testing for genes associated with signs of meat productivity. The article presents the results of studies of gene polymorphism of calpain (CAPN1) and growth hormone (GH) in different sex and age groups of the Kazakh white-headed breed and its effect on the dynamics of live weight of young animals. It was found that polymorphism of the CAPN1 and GH genes is represented by three genotypes of CC, CG, GG and VV, LV, LL, respectively. The most common in the CAPN1 gene are the G allele and the GG genotype, the frequency of which ranged between 0.83-0.89 and 0.72-0.81, in the GH gene, the L allele and the LL genotype, the occurrence of which, respectively, was within 0.60-0.81 and 0.51-0.66. The carriers of the desired alleles, both in the homozygous and heterozygous state, were insignificant: in the CAPN1 gene, in the range of 0.03-0.22, in the GH gene, 0.03-0.31. In the CAPN1 gene, a lack of heterozygotes was observed in all groups, while in repair young animals it was higher than in animals of the parent herd. In the GH gene, a slight excess of heterozygotes was found in manufacturing bulls and repair bulls, while among cows and repair heifers they were deficient. Significant superiority in live weight at 8 and 12 months of age of gobies and heifers carriers of genotypes CAPN1CC and GHVV over peers CAPN1GG and GHLL was established by an average of 13.7 (P <0.05) and 6.8 %%, 12.1 (P <0.05) and 6.7 %.

1. Introduction

Specialized beef cattle breeding in Russia in recent years is characterized by dynamic development. In the period from 2008 to 2018, the volume of meat cattle breeding increased by 3.5 times and amounted to 15% of the total beef production. Further increase in the production of high-quality, including "marble", beef from meat breeds of livestock, involves the use of modern genetic technologies. In many countries of the world, animal genotyping and the use of DNA markers has become an integral part of the breeding process, since it allows the genotype to be evaluated and its prospects for further breeding to be used at a very young age [1, 2, 3].

One of the promising genetic markers of meat characteristics of cattle are the genes of calpain (calpain, CAPN1) and growth hormone (GH).

The calpain system controls the function of weakening the bonds between bundles of muscle fibers, due to the decomposition of the Z-disks of skeletal muscle of calcium-dependent cysteine protease, and creates conditions for a uniform distribution of intramuscular fat between the fibers, which ensures the “marbling” of the meat, its tenderness and juiciness [4] (Koohmaraie M., 1992). The CAPN1 gene is...
localized on chromosome 7 and consists of 22 exons and has a size of about 30 thousand pairs of nucleotides. In the coding part, two non-synonymous substitutions were found (C to G and A to G), which lead to changes in the amino acid sequence at positions 316 (glycine to alanine) and 530 (valine to isoleucine). It was found that in animals homozygous for these alleles (C316 and G530) meat has increased tenderness, and therefore they are of the greatest interest, both for study and for preferred use in practical selection [5, 6].

Growth hormone gene or somatotropin (gene hormone, GH) is an endogenous factor with lactogenic, insulin-like, fat-mobilizing and neurotropic effects. The gene is mapped on chromosome 19 and is a member of the multigenic family, which also includes prolactin, and placental lactogens [7]. Its length is approximately 2000 base pairs and includes five exons (I-V) and four introns [8]. Several polymorphic variants of the GH gene have been described, most of which are located in untranslated introns, several in exons, and only one of them is in the fifth exon, at position 2141 and is a C (Leu) → G (Val) transversion. It was she who attracted the most attention in studies related to the search for associations with signs of productivity in cattle [9, 10, 11].

Kazakh white-headed breed is one of the most common Russian breeds of beef cattle and is recognized as the most promising for breeding in the arid steppe regions of the country. In this connection, the goal of this study is to study the polymorphism of the CAPN1 and GH genes and to identify genotypes associated with the productivity signs of the Kazakh white-headed breed.

2. Material and methods

The object of the research was bulls-producers (n = 35), cows aged 3.5 years and older (n = 160), repair bulls (n = 93) and heifers (n = 64) of the Kazakh white-headed breed of the SPK collective farm “Gigant” of Blagodarnensky district of the Stavropol region.

The studies were carried out in the laboratory of immunogenetics and DNA technology, the laboratory of veterinary medicine and the laboratory of morphology and product quality VNIIOK - a branch of the Federal State Budget Scientific Institution “North Caucasian FNATS”. DNA was isolated from blood samples using the “DIAtom-nDNAPrep” kit (“Isogen”, Moscow) according to the protocol. Genotyping of the CAPN1 gene was performed on a nucleic acid analyzer (ANK-32) by PCR-RV using the CAPN1 reagent kit (“Synthol”, Russia), and by the GH gene, PCR-RFLP using primers (GH-F: 5' - gct-gct-cct-gag-cct-tcg -3' and GH-R: 5'- gcg-gcg-gca-ctt-cat-gac-cct-3') and Alu I restriction enzymes (NPO “SibEnzim”).

The frequency of alleles, genotypes, the number of effective alleles, the observed (Ho) and expected (He) heterozygosity were calculated using the GenAlEx 6.5 software [12].

The dynamics of the live weight of gobies and heifers of different genotypes was determined by weighing at birth, at 240 and 365 days. The average daily increase was determined by the difference in values and the accounting period.

The resulting material was processed biometrically using statistical methods, the program Microsoft Excel. The significance of differences in the compared indicators in groups was evaluated by Student's t criterion with the following significance level: * – P<0.05.

3. Results and discussion

An analysis of the obtained data allowed us to establish that the polymorphism of the CAPN1 and GH genes in cattle of the Kazakh white-headed breed, in both one and the second genes, is represented by two alleles - C, G and V, L and, accordingly, three genotypes - CC, CG, GG and VV, LV, LL. The most widespread in the CAPN1 gene in all age and gender groups was the G allele and GG genotype, whose frequencies ranged between 0.83-0.89 and 0.72-0.81, in the GH gene, the L allele and LL genotype, the occurrence of which, respectively was in the range of 0.60-0.81 and 0.51-0.66. The carriers of the desired alleles in the studied genes, both in the homozygous and heterozygous state, were insignificant: in the CAPN1 gene within 0.03-0.22, in the GH gene - 0.03-0.31. Moreover, a high frequency of occurrence of alleles and genotypes preferred for selection in the CAPN1 gene was noted in the group of repair heifers, while in the GH gene in the group of repair bulls (table 1).
In the GH gene, a greater number of effective alleles were observed in all age and gender groups than in the CAPN1 gene; the average herd difference between the genes was 16.0 abs. percent (table 1).

Table 1. Polymorphism of the CAPN1 and GH genes in cattle of the Kazakh white-headed breed of different sex and age groups.

| Index                        | CAPN1     | GH       |
|------------------------------|-----------|----------|
|                              | genotype  | genotype |
|                              | allele    | allele   |
| Bulls-producers              | CC/C      | CG       |
| Allele frequency             | 0.11      | 0.89     |
| Genotype frequency           | 0.03      | 0.17     |
| The number of effective alleles, % | 16.6    | 28.0     |
| Cows                         | GG/G      | VV/V     |
| Allele frequency             | 0.14      | 0.86     |
| Genotype frequency           | 0.04      | 0.19     |
| The number of effective alleles, % | 23.5    | 41.4     |
| Repair bulls                 | LV        | LL/L     |
| Allele frequency             | 0.13      | 0.87     |
| Genotype frequency           | 0.06      | 0.13     |
| The number of effective alleles, % | 21.5    | 46.9     |
| Repair heifers               | GG/G      | VV/V     |
| Allele frequency             | 0.17      | 0.83     |
| Genotype frequency           | 0.06      | 0.22     |
| The number of effective alleles, % | 26.6    | 35.9     |

The analysis of the data of the heterozygosity test, calculated by comparing the actually observed heterozygosity to the theoretically observed, according to the Hardy-Weinberg law, revealed that a lack of heterozygotes was observed in the CAPN1 gene in all groups. At the same time, it was higher in replacement young animals (-0.128) than the animals of the parent herd (-0.061) had. In the GH gene, among the groups of sire bulls and replacement bulls, albeit a very insignificant, but excess of heterozygotes (+0.008) was revealed, while among cows and replacement heifers, their deficiency (0.209) was found (table 2).

Table 2. Observed and theoretically expected heterozygosity for the CAPN1 and GH genes in cattle of the Kazakh white-headed breed in different age and gender groups.

| Index                              | Bulls-producers | Cows | Repair bulls | Repair heifers |
|------------------------------------|-----------------|------|--------------|----------------|
|                                    | CAPN1           | GH   | CAPN1        | GH             |
| The observed / theoretically       | 6.00/           | 11.00/| 30.00/       | 45.00/         |
| expected number of heterozygotes,  | 6.85            | 10.77/| 38.53/       | 67.20/         |
| heads                              |                 |      |              |                |
| Heterozygosity observed (Ho)       | 0.207/          | 0.458/| 0.231/       | 0.391/         |
|                                   | 0.243           | 0.444/| 0.317        | 0.724          |

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significant differences were established between animals of the GG genotype and their counterparts. However, meat obtained from such animals differed by a 14–20% greater severity of marbling in comparison with animals of the GG genotype [13, 14, 15].

The revealed polymorphism made it possible to separate the gobies and repair heifers in accordance with the established genotypes and study the dynamics of their live weight in different age periods of ontogenesis.

It was found that at 8 and 12 months of age, the advantage in live weight was with carriers of the C allele of the CAPN1 gene compared to genotypes in which such alleles were not detected (GHVV - GHLL) at 8 and 12 months in the group of calves was 14.6 (P <0.05) and 6.9 %, in the group of heifers - 9.6 (P <0.05) and 6.6 %, respectively (table 3).

Table 3. Dynamics of live weight of gobies and heifers of different genotypes by genes CAPN1 and GH.

| Index | CAPN1 | GH |
|-------|-------|----|
|       | CC    | GG | VV | LV | LL |
| Bulls |
| Live weight, kg at birth | 28.1±0.21 | 27.9±0.18 | 27.6±0.16 | 28.3±0.11 | 27.9±0.18 | 27.6±0.12 |
| in 8 months | 245.5±4.6 | 240.6±6.5 | 215.8±5.2 | 257.9±7.5 | 248.5±4.5 | 225.1±6.1 |
| in 12 months | 374.2±5.1 | 368.9±3.6 | 349.9±4.1 | 382.7±4.7 | 373.8±4.2 | 357.9±5.2 |
| Daily average gain, g | ±92.4 | ±72.6 | ±85.4 | ±65.4 | ±92.7 | ±71.3 |
| Repair heifers |
| Live weight, kg at birth | 27.9±0.57 | 26.1±0.37 | 25.6±0.65 | 26.9±0.68 | 27.1±0.43 | 27.6±0.34 |
| in 8 months | 196.9±2.6 | 179.8±1.7 | 173.4±1.4 | 192.1±2.7 | 181.9±1.9 | 175.2±1.8 |
| in 12 months | 357.5±4.8 | 339.9±4.2 | 335.7±3.8 | 356.7±6.4 | 341.8±3.4 | 334.9±3.2 |
| Daily average gain, g | 903.3 | 859.7 | 849.6 | 903.6 | 862.2 | 841.9 |

Thus, the conducted studies have established the polymorphism of CAPN1 genes and GH in different sex and age groups of the Kazakh white-headed breed and to identify its relationship with the dynamics of live weight of young animals. The carriers of the C and V alleles of the calpain and growth hormone genes are desirable for selection and further use in breeding improvement of the herd.

The data obtained in our own studies are in many respects consistent with the results of studies of other foreign and domestic scientists. So, in the breeds of beef cattle of Aberdeen-Angus and Hereford, the number of carriers of the desired genotype CC CAPN1 was less than the neutral GG genotype. However, meat obtained from such animals differed by a 14–20% greater severity of marbling in comparison with animals of the GG genotype [13, 14, 15].
Corva et al. (2007) found that meat from purebred and crossbred gobies of Limousine breed, Aberdeen-Angus and Hereford breeds with the SS genotype in the CAPN1 gene had less resistance to cutting, i.e. was more tender than meat from carriers of the GG genotype. The frequency of occurrence of the desired allele in all breed groups was 0.390 [16]. The Kalmyk breed of beef cattle also excelled in live weight of animals with the CC genotype in the CAPN1 gene. In addition, it was found that the meat of these animals had a larger average diameter of muscle fibers compared with meat obtained from gobies of the GG and GC genotypes, respectively, by 6.41 and 5.61 microns, or 14.11 and 12.13% [17].

In the work of Gorlov I.F. et al. (2015), the frequency of alleles and genotypes by GH was determined in animals of the Kazakh white-headed breed of Russian and Kazakhstan breeding. It was found that the frequency of the desired allele that controls meat productivity in animals of Russian selection was lower than in Kazakhstan [18]. In the works of Smith S.B. (2016) it is noted that in meat of animals in which the desired alleles in the GH gene were present, beneficial omega-3 and omega-6 fatty acids were found in greater quantities [19].

The foregoing allows us to conclude that genotyping of animals of the Kazakh white-headed breed by calpain and growth hormone genes, selection and targeted selection of carriers of the desired genotypes will increase the live weight and meat productivity of the herd as a whole.

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