The use of single-nucleotide polymorphism in creating a cross-line of meat Simmentals

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Abstract. The article presents the materials of the study on bull cross of the Bredy meat type Simmentals, which include the genotyping of animals for the CAST and CAPN1 genes with the identification of frequencies of genotype and alleles, as well as testing the production of these animals expressed in the evaluation of aged within 8 days of meat for the indicators tenderness, juiciness, organoleptic assessment of taste. The results of the studies did not confirm, with certainty, the influence of the CAST gene polymorphism on the beef tenderness indicators, as well as no associations of this gene with the studied juiciness and meat taste indicators, while the carriers of the homozygous CC genotype CAPN1 exceeded other genotypes of the sample in tenderness, juiciness and taste of beef. And indicators of bull meat with a combination of TT* genes on CAST and CC* on CAPN1 had superiority over the average indicators of the total sample (P <0.001), as well as over the group of animals with the most preferred CC genotype for the CAPN1 gene by 1.22 points or 15.4% (P <0.01) by tenderness, by 1.44 points or 18.2% (P <0.05) by juiciness, by 1.56 points or 19.2% (P <0.001) by taste. Studies have confirmed the effect of the CAPN1 gene CC homozygotes polymorphic state itself, as well as the CAST and CAPN1 genes on beef tenderness in the part where the combination of TT genotypes in the CAST gene and CC in the CAPN1 gene gives a positive effect on beef tenderness, probably associated with activation the activity of μ-calpain in connection with the weakening of the effect of calpastatin as an inhibitor on μ-calpain, as a result of which the enzymatic effect on myofibrillary proteins acquires a different intensity, and possibly a different manifestation. What was the reason for the increase in sensory sensitivity to taste of prepared samples, tenderness and juiciness of meat.

1. Introduction

Russia is the first in the world in terms of territory, occupying 1/6 of the planet’s land area. Huge areas of pasture land is the potential for meat production. At the same time, own beef production is only 1.7-1.9 million tons, that is 2.5 times less than the required one [1]. At the same time, beef is traditionally preferable to other types of meat for the Russian inhabitant, for majority of the peoples of Russia. But the main advantage of cattle is that the production of high-quality red meat, especially in meat breeds, is carried out with minimal, in comparison with poultry and pig breeding, strategic costs important in
human nutrition, grain crops [2]. The breeding of meat breeds of animals in various natural and economic zones of the Russian Federation, their ability to process grain straw and other coarse feed, contribute to the ecological restoration of fallow lands, can be successfully used to create a production structure in remote agricultural areas, with the creation of jobs and social infrastructure, with the restoration of destroyed farms, villages and settlements, not to mention the creation of the export potential of beef in the country. This is possible only in the presence of breeds and types of beef cattle, with high potential indicators of productivity and quality of beef, adapted to certain climatic zones for which Russia is so rich.

The questions of creating new breeding achievements and improving existing ones on given qualitative and quantitative indicators of productivity are shifted in recent years to the use of innovative, fundamental areas of knowledge that can significantly improve the efficiency of breeding. These achievements include decoding the genome of biological objects [3,4], including farm animals and the associated search for the association of genes with the economically useful animal traits. The use of achievements in the field of molecular genetics in the breeding of beef cattle is gaining momentum in different countries of the world [5,6,7]. This is especially true of the quality of meat, including beef. For example, since 1996, when data on the effects of the expression products of the CAPN1 genes (μ-calpain - calcium-activated neutral protease) and CAST (calpastatin - μ-calpain inhibitor) on meat tenderness were presented for the first time [8].

2. Statement of the problem
In our research on meat simmentals, we have achieved some success in creating cross lines. The best results in absolute animal growth, carcass weight at the age of 15 months, the total amount of edible muscle mass obtained from the use on the Bredy meat type of the Pharaoh line of Canadian seed manufacturer Expert (Expert 598604) of the company "SEMEX ALLIANCE". The compatibility of this manufacturer with the uteri of the Pharaoh line allowed us to offer this cross for use in the production of industrial partners. At the same time, there was no data on the taste qualities of meat and the possibility of their improvement in the resulting cross. Taking into account the established associations of beef tenderness with the presence of gene polymorphism (OTL) - CAST and CAPN1 and taking into account the contradictory data of genotyping of different breeds [9-13], we attempted to study the meat quality of 15-month bulls of our crosses. At the same time, given that μ-calpine is related to the enzymatic degradation of the most important proteins that represent the myofibrillar muscle carcass, which affects the tenderness of the meat, it can be assumed that this enzymatic destruction of proteins can also affect such important indicators as the taste and juiciness of the aged meat, histological pattern of myofibrils [14].

3. Materials and methods
The complex of tasks including the study of meat productivity, interior features of bulls included the definition of meat quality, including juiciness, tenderness and taste of beef. The object of the study was bullhe - the cross obtained from the use of Canadian bull- producer Expert (Expert 598604) from the company "SEMEX ALLIANCE" on the uterus of the Bredy meat-type line of the Pharaoh, owned by LLC "Joint management Bredy". In carrying out research, efforts were made to minimize the suffering of animals and reduce the number of samples used, according to the instructions and recommendations of the Russian Regulations 1987 (Order No. 755 on 12.08.1977 the USSR Ministry of Health) and "The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, DC 1996)". The selected bulls (n-84) were fed on rations providing an average daily increase in live weight of 1000-1200 g to 15 months of age, consisting of hay of cereal and legumes - 20%, of grain-bean grain-senage - 45% and a mixture of concentrated feed - 35%. Before slaughter at the age of 15 months animals were bled, including for genetic studies. For this, blood samples were taken from each bull in vacuum tubes with EDTA anticoagulant. The blood was urgently transported to the genetic laboratory. The Test Center of the CKP BST RAS (accreditation certificate RA.RU.21PF59 from 10.10.2015; www.ckp-bst.rf; http://ckp-raf.ru/ckp/77384), where DNA was immediately isolated from blood using a set of
reagents "DNA-Extran 1" ("Syntol", Russia). For this, reagents from the set were added to the blood and the biological substrate was subjected to lysis. In the finished lysate, RNase A and the solution were added to precipitate the proteins, after which the mixture was centrifuged and the supernatant liquid layer was removed and placed in a test tube, isopropyl alcohol was added thereafter, and the DNA precipitated. In this state, the DNA could be frozen to 20 ºС and stored until genetic studies.

For genotyping a programmable amplifier ANK-32 ("Syntol", Russia) was used. For the implementation of the polymerase chain reaction (PCR) for replicating the genome region, the CAPN1316 gene component (GenBank accession No. AF248054) primers found in the open seal and synthesized in the company "Syntol" were used:

5’-AGCAGCCCACCATCAGAGAAA – 3’
5’-TCAGCTGGTTCGGCAGAT – 3’

Real-time PCR for the CAST gene at position 2857 C/T (GenBank accession No. AF159246) was performed using the following direct and reverse primers:

5’-ACATTCTCCCCACAGTGCC-3’
5’-GACAGAGTCTGGTTCGGCAGAT-3’

The conditions of the reaction according to the temperature and time of the initial denaturation, denaturation, annealing, synthesis and final synthesis, as well as the volume and composition of the used reaction mixture, were carried out according to the manufacturer's instructions.

Standard formulas were used to assess compliance with the Hardy-Weinberg equilibrium and to compare the frequencies of genotypes and alleles. The frequency of occurrence of genotypes was determined by the formula:

\[ P_i = \frac{n_i}{N} \]

where \( P_i \) is the frequency of occurrence of the \( i \)-th genotype; \( n_i \) is the number of animals characterized by the presence of the \( i \)-th genotype; \( N \) is the total number of animals in the sample.

The frequency of the alleles of the analyzed genes was calculated by the following formula:

\[ p_i = \frac{2n_{(homozygotes)} + n_{(heterozygotes)}}{2N} \]

where \( p_i \) is the frequency of occurrence of the \( i \)-th allele; \( n \) is the number of animals homo and heterozygotes; \( N \) is the total number of animals in the sample.

Slaughter of bulls was carried out at the age of 15 months in which a sample of the longest back muscle (1 kg) of *longissimus thoracis* (LT) was cut out from the right half of the carcass, after a 24-hour exposure, in the area of 9-11 ribs and was placed in a vacuum package in a refrigeration chamber for storage at temperature of 4 ºС. On the 8th day, samples were taken and a tenderness assessment was made (resistance to force cut by a Warner-Bratzler instrument + organoleptic evaluation of boiled and fried meat), succulence (p/h, using a LoT406-M6-DXK-S7/25 piercing electrode + moisture holding capacity, method of measuring the percentage loss of sample moisture after aging and after boiling + organoleptic evaluation of boiled and fried meat) and taste (tasting assessment of boiled and fried meat). All indicators were unified under the points on a 10-point system [15].

4. Research results

Genotyping for the CAPN1 gene (Table 1) showed a high degree of manifestation of the homozygous CC genotype. Among 84 heads of bulls in which biosubstrates were taken in the form of blood - 60 heads, which accounts for 71.4% of the total population, were carriers of the tenderness gene, 20 heads or 23.8% of bulls had a heterozygote for this gene and only 4 heads (4.8%) were homozygous for genotype GG. According to the frequency of occurrence of the alleles of this gene, the G allele was 0.153 and the C allele was 0.847. The high degree of presence of the allele C and in general of the CC genotype for the CAPN1 gene suggest, according to many authors, a high possibility of the degree of beef tenderness.
Table 1. The frequency of occurrence of the desired genotypes and allele frequencies of the CAPN1316 gene in the micro-population of animals of the received cross

| The frequency of occurrence of the genotype, % | GG  | GC  | CC* |
|----------------------------------------------|-----|-----|-----|
| n                                           | 84  | 20  | 60  |
| n                                           | 4   | 23.81 | 71.43 |
| n                                           | 4.76 |       |     |
| n                                           | 20  |       |     |
| n                                           | 60  |       |     |
| n                                           | 71.43 |      |     |
| G                                           | 0.167 |       |     |
| C                                           | 0.833 |      |     |

Genotyping for another gene - CAST, which is also (but not so clearly) associated with the tenderness of beef associated with the homozygous state of the T allele showed that the desired genotype of the CAST gene in the micro-population of the resulting cross, indicated by the TT symbol, appeared, unlike its predecessor, slightly (Table 2). Only 7.1% of their total number had this genotype, whereas individuals with a heterozygous component of 15.5% were twice as large. Most of the carriers were neutral homozygous CC associated with the expression of calpastatin, namely 65 heads of calves, which is 77.4% of the total sample.

Table 2. The frequency of occurrence of the desired genotypes and allele frequencies of the CAST2857 gene in the micro-population of animals of the received cross

| The frequency of occurrence of the genotype, % | CC  | CT  | TT* |
|-----------------------------------------------|-----|-----|-----|
| n                                           | 84  | 16  | 6   |
| n                                           | 65  | 15.48 | 7.14 |
| n                                           | 77.38 | 13  |     |
| n                                           | 15.48 | 6   |     |
| n                                           | 7.14 |     |     |
| C                                           | 0.851 |     |     |
| T                                           | 0.149 |     |     |

When studying such phenotypic characteristics of meat quality as pH and the moisture-holding capacity of meat, reliable results were not obtained with the results of genotyping (data not shown), and the results of statistical processing of material of genotypes with less than 3 animals in the sample are not presented, although absolute results are shown to indicate the trend. The reinforcement of the obtained results with measurement methods - sensory methods (organoleptic), is an integral part of modern methods for studying the taste qualities of meat.

Table 3 presents the results of the combined score of tenderness, juiciness and taste of beef, depending on the combination of genotypes for the genes CAST and CAPN1. It should be noted that in our studies, the desirable homozygous TT * genotype of the CAST gene did not receive reliable confirmation by association with the sign of tenderness of beef, and no associations with indicators such as juiciness and taste of aged 8 days of meat were found. At the same time, high rates were noted across the entire spectrum of meat's taste advantages, regardless of the combinations of genotypes for the CAST and CAPN1 genes in animals with the CC genotype for the CAPN1 gene. Their superiority over other genotypes amounted to tenderness - 1.5 points (22.5%), juiciness - 1.64 points (25.9%), tasting taste evaluation - 1.51 points (22.5%) (P < 0.01). All indicators of bull meat with a combination of TT * genes on CAST and CC * on CAPN1 had a superiority over the average indicators of the total sample on the 3rd confidence threshold (P <0.001), and also over the group with the most prevalent in the sample of animals (n = 54), having a combination of genotypes for CAST (CC) and CAPN1 (CC *) by 1.22 points or 15.4% (P <0.01) by tenderness, by 1.44 points or 18.2% (P <0.05) by juiciness, by 1.56 points or 19.2% (P <0.001) to taste.
Table 3. The degree of association of polymorphic states of the CAPN1 and CAST genes with a complex of indicators of tenderness, juiciness and taste of beef calves of the received cross (n = 84)

| Genotype by genes | The proportion of genotypes in the sample, % | Average score on meat quality indicators | Overall grade |
|-------------------|---------------------------------------------|-----------------------------------------|---------------|
| CAST | CAPN1 | tenderness | juiciness | taste |
| CC | GG | 2.4 | 6.50 | 6.00 | 6.50 | 19.00 |
| CC | GC | 10.7 | 6.11±0.31 | 5.78±0.22 | 6.00±0.24 | 17.89 |
| CC | CC* | 64.2 | 8.11±0.15 | 7.89±0.12 | 8.11±0.14 | 24.11 |
| CT | GC | 10.7 | 7.11±0.35 | 6.67±0.29 | 7.33±0.50 | 21.11 |
| CT | CC* | 3.6 | 8.00±0.58 | 8.00±0.58 | 8.67±0.33 | 24.67 |
| CT | GG | 1.2 | 7.00 | 5.00 | 6.00 | 18.00 |
| TT* | GG | 1.2 | 6.00 | 6.00 | 6.00 | 18.00 |
| TT* | CC* | 3.6 | 9.33±0.33 | 9.33±0.67 | 9.67±0.33 | 28.33 |
| TT* | GC | 2.4 | 7.50 | 7.00 | 7.00 | 21.50 |

Considering that the taste qualities of meat depend on a combination of individual qualities like tenderness, juiciness, taste and smell directly and their combinations, there is a high interdependence of these qualities. In our studies, the highest correlative dependence was established between the organoleptic taste rating and the total amount of points for taste qualities (r = 0.90), juiciness and total points (r = 0.86), tenderness and total points (r = 0.76), juiciness and taste (r = 0.78). A slightly smaller, but stable correlation (P <0.01) is established between tenderness and organoleptic taste evaluation (r = 0.48), tenderness and juiciness (r = 0.40).

5. Discussion of the results
In recent years, many works have appeared on confirming the association of polymorphic states of the CAST and CAPN1 gene with the tenderness of meat from different breeds of cattle in different zones of our planet. Despite the presence of unequivocal conclusions, most studies confirm the existence of such a connection [16,17]. Moreover, many works aimed at finding new associations of these genes with signs of animal productivity have appeared [18,19,20]. In this regard, the results obtained in our experiments are consistent with the data of many authors.

6. Conclusion
Our studies confirmed the effect of the polymorphic state of the homozygote CC gene CAPN1 itself and of the genes CAST and CAPN1 on tenderness of beef in that part when the combination of TT genotypes in the CAST gene and CC in the CAPN1 gene gives a positive effect on the tenderness of beef, probably associated with activation of μ-calpain in connection with the weakening of the effect of calpastatin as an inhibitor on μ-calpain, as a result of which the enzymatic effect on myofibrillary proteins acquires a different intensity, and possibly a different manifestation. In addition, the results of our experiments have shown that this may be the cause of a different sensory sensitivity in taste of prepared samples, tenderness and juiciness of meat.

Acknowledgments
The studies were carried out in accordance with the research plan for 2019–2020 of the Federal Research Center for Biological Systems and Agrotechnology’s of the Russian Academy of Sciences (# 0761-2019-0012).

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