Polymorphism of genes among heifers with different types of constitution

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Abstract. The article presents the results of the manifestation of the polymorphic state of single nucleotide (SNP) genes CAPN1 and TG5 among 15-16 month old heifers of the Bredy meat type simmentals, evenly distributed over the constitution of the body type. For the study, the real-time PCR method was used with the using of oligonucleotide primers (GenBank) on an Applied Biosistems 3130 automatic DNA sequencer using a standard protocol with forward and reverse primers. In animals, in the corresponding constitutional ranks, the growth rates and the state of meat forms at the age of 15 months were studied in a comparative aspect. Significant differences were revealed in the assessment of meat forms of leptosomal and eirosomal animals in favor of more significant points in eirosomal animals. For the dense type, this superiority was 4.4 points (P <0.01), and for the loose type, respectively, 5.2 points (P <0.01). The results of genotyping revealed some interesting facts. If the even distribution of genotypes according to constitutional types was determined by the CAPN1 gene, then the TG5 gene was characterized by their significant displacement. Thus, the homozygous desired TT genotype of this gene, determined in 7.5% of the animals in the sample, was found only in heifers with a loose constitution type, and 2/3 of them were found in the group of eirosome animals. Consequently, for eirosome animals with a loose type of constitution, predominant saturation of DNA with the T allele of the TG5 gene is characteristic, which may be a sign of a possible manifestation of beef marbling associated with this gene.

1. Introduction
One of the topical issues for further breeding, a relatively young Bredy meat type, is the task of consolidating the characteristics of productivity inherent in this type, at the same time, assuming its further improvement [1, 2]. In this context, it is important to give the animals of the new type, created from the breed of the combined direction of productivity, more expressed meat forms. Evaluation of the exterior and constitution of animals allows for successful selection in this direction. In addition, it is known that the productive indicators of animals, the quality indicators of production depend on the exterior of animals, the type of constitution. The creation of several lines, standard in terms of body type and productivity, will allow successful breeding for the implementation of a new, modern breeding achievement. Taking into account the development, in recent decades, of genetic technologies, the establishment of the association of some genes (SNP markers) with the productive and qualitative indicators of animals, it became possible to influence selection not through selection, but directly replicating animals with the corresponding state of the polymorphic gene [3-7].
The aim of the study is to determine the dependence of the eirosomality and leptosomality of the body type of heifers on the distribution of allelic forms of some marker genes and to compare animals of different constitutional types in terms of live weight under conditions of moderate feeding.

2. Material and research methods

Heifers aged 15-16 months from the composition of animals of the Bredy meat type belonging to LLC “State farm Bredy” were considered as the object of the study. The instructions "Russian Regulations, 1987 (Order No, 755 on 12/08/1997 the USSR Ministry of Health) and” The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, DC 1996)" were fundamental documents which were guided by the manipulation of animals when performing our research. All experiments with animals were carried out in accordance with the principles expressed in the Declaration of Helsinki (https://www.wma.net/policy/current-policies/). And efforts were made to minimize animal suffering and to optimize the number of samples used.

The experiment involved 40 randomly selected 8-month-old heifers of the Bredy meat type from OOO “State farm Bredy”, which, based on eye assessment, measurements of animals and the use of the method of model deviations [8-10] with the calculation of the individual meat index, were distributed in equal proportions, according to the type of body, into: leptosomal, mesosomal and eirosomal. The animals that were kept according to the technology of beef cattle breeding were put to the assessment of their own productivity. All heifers were bled separately into APEXLAB vacuum tubes with anticoagulant (EDTA) for laboratory genetic analysis. Genomic DNA was isolated from the blood using DNA-Extran reagents from Syntol (RF). To identify animal genotypes by the planned SNP markers: CAPN1, TG5, the real-time PCR method was applied with using of oligonucleotide primers (GenBank), for each primer separately in the corresponding sequences. CAPN1 is associated with the tenderness of beef, and TG 5, which is responsible for lipid metabolism, is often mentioned in studies related to beef marbling [11].

| DNA marker | Primer sequence |
|------------|----------------|
| CAPN1      | F: 5′-AGCAGCCCACCATCAGAGAAA – 3′ | R: 5′- TCAGCTGGTTCGGCAGAT – 3′ |
| TG5        | F: 5′-GTGAAAATCTTTGTGGAGGCTGTA-3′ | R: 5′-GGGGATGACTACGAGTGATGACTG-3′ |

The platform scales VPS were used to weigh the animals. VPS scales meet the requirements of GOST OML R76-1-2011, intended for weighing cattle. For measuring heifers, standard measuring devices were used: a Lidtin stick, Wilkins’ compasses and a measuring tape. All laboratory studies were carried out at the testing center of the Federal Research Center for Biological Systems and Agricultural Technologies of the Russian Academy of Sciences (accreditation certificate RA.RU21PF59 dated 02.12.15; www.tskp-bst.ru; http://ckp-rf.ru/ckp / 77384) with the using of standard techniques. Sequencing was performed on an Applied Biosistems 3130 automated DNA sequencer with the using of a standard forward and reverse primer protocol. Fragments embedded in agarose gel were excised and purified with the Wizard PCR Preps DNA PurificationSystem (Promega) gel isolation kit according to the manufacturer's instructions. The frequencies of genotypes and alleles in the studied population were calculated by standard formulas based on the numerical expressions of the Hardy-Weinberg law.

In this publication, for processing experimental data we used a standard comparative statistical analysis by the method of variation statistics with the Microsoft Office software package, with the Excel program (Microsoft, USA), with data processing in the Statistica 10.0 program (StatSoftInc. , USA). Statistical comparison of the results was carried out with the parametric method of Student's test. The parameter P ≤ 0.05 was taken as the limit of significance.
3. Research results
The constitution of an animal, being an integral feature and determining the direction of productivity and resistance to environmental conditions, is largely due to heredity (table 2). As a result of the research we noted that large, massive animals, as well as small ones, were characteristic of all groups. At the same time, heifers with a leptosomal constitutional type had the highest measurements: height at the sacrum, at the withers, oblique body length. On the contrary, eirosomes were distinguished by higher latitudinal measurements, chest girth behind the shoulder blades, and better indicators of the half-girth of the rear. Some calculated body indices had regular differences in magnitude in leptosomal and eirosomal animals; in mesosomal animals these values were intermediate values. Thus, for leptosomal animals, more significant indices of long-leggedness, elongation, and bone structure were characteristic, and for eirosomal animals - thoracic, hind-size, broad-bodied.

| Indicator                     | Constitutional body type | leptosomal (I gr.) | mesosomal (II gr.) | eirosomal (III gr.) |
|-------------------------------|--------------------------|--------------------|--------------------|---------------------|
|                               |                          | dense              | loose              | dense               | loose              |
| Live weight                   |                          |                    |                    |                     |
| at 8 months                   | 235.3±0.88               | 237.8±1.55        | 234.7±1.12        | 237.4±0.94         | 234.1±1.78        | 238.1±1.34        |
| at 15 months                  | 392.2±1.81               | 395.9±2.19        | 394.4±2.34        | 395.2±1.89         | 396.0±2.56        | 397.5±2.00        |
| Average daily gain in live weight over a period of 8 - 15 months | 736.6±12.18             | 742.2±8.99        | 742.2±11.43       | 742.2±12.56        | 760.1±10.67       | 748.4±17.32       |
| Evaluation of meat forms, point | 53.2±0.95                | 52.9±1.23         | 55.1±0.88         | 55.3±0.67          | 57.6±0.79aa       | 58.1±1.04aa       |

aa - (P <0.01) - the level of confidence in comparison with the dense type of group I; ** - (P <0.01) - confidence level in comparison with loose type I group.

Taking into account these tendencies, it should be noted that these differences are weakly reliable; this is also indicated by the comparison of animals in live weight and growth rate, in which no significant differences were found. As can be seen from the table, the difference was not significant, although heifers of the loose type slightly prevailed in terms of growth rates over their analogues of the dense type.

At the same time, the judgment about meat forms, independently carried out within the framework of evaluating animals by their own productivity, showed clear differences in heifers assigned to the leptosomal constitutional type from animals assigned to the eirosomal type. The general assessment of meat forms according to a 60-point system revealed the superiority of eirosome animals over leptosome ones. Thus, the superiority of dense type eirosome heifers over dense type leptosome heifers for this indicator was 4.4 points (P <0.01), and loose type eirosomal heifers over leptosomal analogs 5.2 points (P <0.01) or 9.8%.

The distribution of polymorphic genotypes of marker genes CAPN1 and TG5 among heifers of various conformational-constitutional types had their own patterns (table 3). Thus, the CAPN1 gene was characterized by a uniform distribution of its polymorphic variant GG over all identified ranks of constitution types (from 12.5 to 17.5%). This indicator for the GC genotype varied from 0 to 2.5%.
Table 3. Distribution of genotypes of marker genes CAPN1 and TG5 among heifers of various exterior-constitutional types.

| Gene | Constitutional body type | leptosomal (I gr.) | mesosomal (II gr.) | eirosomal (III gr.) |
|------|--------------------------|-------------------|-------------------|-------------------|
|      |                          | dense | loose | dense | loose | dense | dense |
| CAPN1| GG                       | 7     | 17.5  | 6     | 15.0  | 5     | 12.5  |
|      | GC                       | 0     | 0     | 1     | 2.5   | 1     | 2.5   |
|      | CC                       | 0     | 0     | 0     | 0     | 0     | 0     |
|      | G                        | 0.175 | 0.1625| 0.1375| 0.1625| 0.1625| 0.1625|
|      | C                        | 0     | 0.0125| 0.0125| 0.0125| 0.0125| 0.0125|
| TG5  | CC                       | 5     | 12.5  | 7     | 17.5  | 6     | 15.0  |
|      | CT                       | 0     | 0     | 0     | 0     | 0     | 0     |
|      | TT                       | 0     | 0     | 0     | 0     | 1     | 2.5   |
|      | C                        | 0     | 0     | 0     | 0.150 | 0     | 0.125 |
|      | T                        | 0     | 0     | 0     | 0.025 | 0     | 0.05  |

The TG5 gene in this sample is characterized by a significant shift in the distribution of genotypes from the expected, expressed by the complete absence of heterozygotes. At the same time, a uniform distribution among heifers with different conformational-constitutional types was characteristic of the homozygous CC genotype, the presence of which was most revealed in 13 eirosome heifers (32.5%). At the same time, the desired homozygous TT genotype was revealed only in the mesosomal and eirosome groups in animals with a loose constitutional type. Moreover, this genotype was detected in two heifers with a loose constitution type from the eirosome group, which is 66.7% of animals with the TT genotype and 5% of the entire sample.

4. Discussion of the results
The learning of the types of constitution is based on the philosophical principles of the unity of the whole and the part, internal and external, the interdependence of form and function. In zootechnics, the applied aspect of this dependence is important. Even in the works of the outstanding Russian scientists I.P. Pavlov, I.M. Sechenov, P.N. Kuleshov and others, the connection between the constitution and exterior of animals with the internal manifestations of metabolism, in particular, productivity and product quality, was revealed. P.N. Kuleshov singled out contrasting: dense and loose types of constitution, animals of which, to some extent, differed in the level of productivity, to a greater extent - in the morphological composition of carcasses, localization of the main tissues, the composition of edible pulp [12]. With the decoding of the genome of cattle, it became possible not only to state the observed facts, but also to discover cause-and-effect relationships. Studies have shown that the presence of the homozygous TT genotype of the thyroglobulin gene (TG 5), which is responsible for the metabolism in the area of triglycerides and fats, is associated with the severity of the loose constitution type in the animal. Thus, out of 7.5% of the animals in the study sample with the desired polymorphic state of this gene - the TT genotype, all without exception were attributed to the loose type of constitution, while 2/3 of these individuals were found in the group of eirosomal animals.

5. Conclusions
Numerous modern scientific studies indicating the association of one of the polymorphic states of the TG5 gene associated with lipid metabolism on the formation of beef marbling, on the one hand, and established on the basis of observations and economic experiments many years ago - the fact of the
tendency of animals of a loose constitution type to active fat deposition, on the other hand, can have one base. The results of our experiments show that animals with a homozygous TT genotype of the TG 5 gene are directly related to the loose type of constitution in the eirosome group. Consequently, our assumption that among the eirosomal individuals with a loose type of constitution there are more animals with the probability of the formation of marbled beef is correct. To unambiguously confirm this conclusion, a larger-scale genotyping is required, an analysis of the qualitative characteristics of meat products, and histological studies, which forces us to use the obtained groundwork in in-depth studies.

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