The state of polymorphism of genes affecting the meat quality in micropopulations of meat simmentals

S D Tyulebaev, M D Kadysheva, V I Kosilov and V M Gabidulin

Federal Scientific Center of Biological Systems and Agrotechnologies of the Russian Academy of Sciences, Orenburg, Russia

E-mail: s-tyulebaev@mail.ru

Abstract. A comparative study of the biological characteristics of the heifers Bredy meat type of two populations located in Russia and Kazakhstan was carried out to determine the state of genes associated with the meat quality in the genotype CAPN1, GDF5, TG5, BGL. In genotyping, we used the real-time PCR method using oligonucleotide primers (Gen Bank), for each primer individually in the corresponding sequences. Studies for improve breeding by innovative methods revealed the frequencies of genotypes and alleles for marker genes CAPN1, TG5, BGL and GDF5, which are related to meat quality indicators, a certain displacement in the genotypes of the compared populations of the Bredy meat type of Kazakhstan and Russia according to the CAPN1 gene (χ² = 8.52) and the TG5 gene (χ² = 4.73).

The genotyping result of animals from LLC "Breeding plant Bredy" were analyzed from the point of view of attributing carriers of different genotypes to the offspring of bulls-producers Fakir, Kust and Chizhik, in order to determine the producer giving offspring with predictably high nutritional and biotechnological properties of beef. A comparison of the carriers of the identified genotypes of the studied genes by the characteristics used in the evaluation of young animals by their own productivity did not give significant results, however, showed trends by which scientific research can be directed. An assessment of the bulls-producers of the successors of the different line of meat simmentals on the quality of heifers – daughters showed that the largest comprehensive index was set for the bull-producer Bush 39046 – 102.7, which was recognized as an improver. The assignment of all, without exception, heifers of different groups, evaluated bulls-producers, to the highest class elite-record, indicates the value of all the studied genotypes.

1. Introduction

The greatest discoveries of recent decades in the field of molecular genetics, including decoding of the human genome and other living organisms, have opened up unprecedented opportunities in different fields of human activity: healthcare, ecology, agriculture, food industry, etc. [1]. Different forms and directions of genetic monitoring could provide control of both the expansion and narrowing of the biological diversity of living, including different types of farm animals.

For example, the introduction of new innovative methods in cattle breeding, in particular, DNA technology, makes it possible to identify genes - markers of all kinds of economically significant traits of animals, such as the level of milk and meat productivity, quality indicators of milk and meat, the growth rate of young animals and many other traits, the use of which in production could qualitatively change the traditional technologies used in breeding and significantly intensify the process of creating new breeding achievements with given characteristics of productivity and product quality [2, 3].
2. Statement of the problem
The Bredy meat type of Simmental cattle, created in 2006 in Russia, belongs to the meat breeds of the intensive type and has some differences from the meat breeds that are bred in the Russian Federation. They are characterized by high average daily gains in live weight in the suction period and intense growth in later periods of growth. These qualities contributed to the emergence of a tendency to spread a new type not only in the Russian Federation, but also abroad. A population of this type of simmental is also present in the Republic of Kazakhstan nowadays [4, 5]. At the same time, the improvement of breeds continues, new opportunities appear, for example, for the first time, it became possible to influence selection not only through selection, but also pointwise using genetic markers, which significantly reduces the time of breeding and improvement of breeds and types of livestock, and allows you to strictly control the genetic process of creation preset parameters of productivity, product quality and other economically and culturally significant traits [6–11]. The continuity and modernization of the breeding process for improving the breed involves testing bulls, including the new Bredy meat type Simmental, by the quality of the offspring, and their sons by their own productivity. At the same time, the possibility of using SNP markers, which open up new innovative biotechnological solutions in breeding methods, is an important stage in modern breeding work.

3. Materials and methods
The object of the study was heifers of the Bredy meat type of different populations. The experiments were carried out in agricultural enterprises with a livestock of pedigree meat simmental: LLC “Breeding plant Bredy” (Russia), farm “Reymkul” (Republic of Kazakhstan). Animal services and experimental studies were performed in accordance with the Russian Regulations, 1987 (Order No, 755 of 12/08/1997 the USSR Ministry of Health) and “The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, DC 1996)”. In carrying out the research, efforts were made to minimize animal suffering and reduce the number of used samples.

The studies were carried out using 40 heifers at LLC “Breeding plant Bredy” and 37 at the farm “Reymkul”. In each of the farms the animals were kept in accordance with the accepted diets. The heifers from LLC “Breeding plant Bredy” were the descendants of three manufacturing bulls and were conditionally divided into 3 groups: group I – the descendants of the bull Fakir 35024, group II – the descendants of the bull Bush 32036, group III – the descendants of the bull Chizhik 39046. Specifics of keeping animals on the farm is connected with the technology of beef cattle breeding and contains all its elements, including keeping animals in the autumn and winter periods in rooms equipped with walking yards. The cows were kept together with calves up to 8 months of age, after which weaning was carried out. Removable heifers of all groups were kept on a single diet with a calculated productivity potential of 800 g of average daily gain recommended for assessing heifers by their own productivity. The data obtained during the evaluation served as material for evaluating their fathers by the quality of the offspring. In this sense, the evaluation system for bulls adopted by the Ministry of Agriculture of the Russian Federation on the quality of offspring was used in the experiment [12].

As a biosubstrate in animals, blood was taken for analysis. For molecular genetic studies, blood was collected separately in APEXLAB vacuum tubes with anticoagulant (EDTA). Total DNA was isolated using the DNA Extran genomic DNA kit (Synthol, Russia). To identify animal genotypes by the planned SNP markers: CAPN1, GDF5, TG5, BGL, the real-time PCR method was used using oligonucleotide primers (Gen Bank), for each primer individually in the corresponding sequences (table 1).

The studies were carried out according to standardized methods in the testing center of the Federal State Budgetary Scientific Institution “Federal Scientific Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences” (accreditation certificate RA.RU21PF59 of 02/02/15; www.tskp-bst.rf; http://ckp-rf.ru/ ckp / 77384) using standard techniques. Sequencing was performed on an Applied Biosistems 3130 automated DNA sequencer using a standard protocol with forward and reverse primers. Fragments placed on an agarose gel were excised from the agarose gel and cleaned using the Wizard PCR Preps DNA Purification System (Promega) gel isolation kit.
according to the manufacturer's instructions. According to Hardy-Weinberg law, the expected genotype frequencies in the study population were calculated.

### Table 1. The sequence of oligonucleotide primers for genes

| DNA marker | Primer sequence |
|------------|-----------------|
| CAPN1      | F: 5' - AGCAGCCCACCATCAGAGAAA – 3’  |
|            | R: 5' - TCAGCTGGTTCGCCGAT – 3’      |
| TG5        | F: 5' - GTGAAAATCTTTGTGGAGGCTGTA-3’ |
|            | R: 5' - GGGGATGACTACGATGTGACTG-3’  |
| GDF 5      | F: 5' - TGTCCCGATGCTGACAAGG-3’      |
|            | R: 5' - GAGTGAGGTTAATCCAGATACCA-3’ |
| BGL        | F: 5' - CTCTGACCTGTCTTGTC-3’        |
|            | R: 5' - CAATAGGAGGCTTTCTTCCA-3’    |

In the processing of experimental data, methods of variation statistics were used with the office software package “Microsoft Office”, the Excel program (Microsoft, USA), and processing data in the Statistica 10.0 program (StatSoftInc., USA). Statistical comparison of the results was carried out using the parametric criterion method. The parameter \( P \leq 0.05 \) was taken as the limit of significance.

### 4. Results and Discussion

Our genetic monitoring of micropopulation in terms of the CAPN1 gene polymorphism showed that animals Farm “Reimkul” are superior in heterogeneity to analogues from LLC “Breeding plant Bredy” (table 2). In particular, the heifers from the I population did not have a single desired genotype, while in the II population, 5.4 \% of the animals possessed this genotype. The share of heterozygous genotypes for this gene in the II micro-population was 22.6 \% higher than that in the alternative group. The young bulls of LLC “Breeding plant Bredy”, in terms of the proportion of the GG homozygous genotype of the CAPN1 gene, had an advantage over animals of the II population by 28.0 \%. The corresponding alignment is observed in the frequencies of alleles in the population, where the desired C allele was more manifested in the micropopulation of animals belonging to the Farm “Reymkul”.

Analysis of the polymorphism of the TG 5 gene related to lipid metabolism in animals also indicates some differences in the manifestation of its state, depending on the ecological and genetic affiliation of the studied individuals. Despite the close frequency of alleles, genotypes have clear signs of differences. Thus, in the inert SS genotype, animals of LLC “Breeding plant Bredy” exceeded their analogues by 8.7 \%, and in the desired TT genotype by 2.1 \%. At the same time, not a single head of the heterozygous ST genotype was found in the I micropopulation, when as in II micropopulation their share was 10.8 \%.

The GDF5 gene polymorphism index (differentiating growth factor) associated with the main metabolic processes during the formation of the skeleton and muscle tissue did not appear in the sample of animals from Kazakhstan, while heifers from LLC “Breeding plant Bredy” showed minimal and, on the contrary, insignificant manifestation heterozygous CA genotype in the micropopulation Farm “Reymkul”, revealed by PCR to determine the polymorphism of the \( \beta - \) lactoglobulin gene – BGL, responsible for protein milk, fat milk and physiological processes physiology of feeding. Whereas, polymorphism was not detected in a sample of young animals from LLC “Breeding plant Bredy”.

Preservation of the Bredy meat type gene pool inherent for LLC “Experimental” and its autonomous breeding under the conditions of the farm “Raimkul” on the one hand, and selection pressure associated with combining Bredy and Bagan meat types and using Canadian meat simulations when creating a new breed in LLC “Breeding plant Bredy”, on the other hand, led to a certain shift in the displacement of the studied genes in the studied populations. Thus, the general criterion for Pearson's agreement on the CAPN1 gene was 8.52, which corresponds to a significant difference in the empirical series of the two micropopulations according to the first threshold \( P < 0.05 \), and on the
TG5 gene, the index $\chi^2$ was 4.73 and did not confirm the hypothesis about the micropopulation difference according to the state of polymorphism this gene.

Table 2. Indicators of CAPN1, TG5, GDF5, and BGL gene polymorphism in micropopulations of meat Simmental

| Gene name | Micropopulation Location | LLC “Breeding plant Bredy” | The Farm “Reymkul” |
|-----------|--------------------------|--------------------------|-------------------|
|           | n | % | n | % |
| CAPN1     | GG | 35 | 87.5 | 22 | 59.5 |
| Genotype frequency | CC | 0 | 0 | 2 | 5.4 |
| Allele frequency | G | 0.9375 | 0.7705 |
|               | C | 0.0625 | 0.2295 |
| TG5        | CC | 37 | 92.5 | 31 | 83.8 |
| Genotype frequency | CT | 0 | 0 | 4 | 10.8 |
| Allele frequency | C | 0.925 | 0.892 |
|               | T | 0.075 | 0.108 |
| GDF5       | TT | 38 | 95 | 37 | 100 |
| Genotype frequency | TC | 1 | 2.5 | 0 | 0 |
| Allele frequency | T | 0.9625 | 1.0 |
|               | C | 0.0375 | 0.0 |
| BGL        | CC | 40 | 100 | 35 | 94.6 |
| Genotype frequency | CA | 0 | 0 | 2 | 5.4 |
|               | AA | 0 | 0 | 0 | 0 |
| Allele frequency | C | 1.0 | 0.973 |
|               | A | 0.0 | 0.027 |

For LLC “The breeding plant Bredy” as the leading breeding reproducer of the Bredy meat type of Simmental, it is important to further improve the breed. One of the ways to improve livestock was the selection, when they left the best on the tribe, while paying attention to the bulls well transmitting their qualities to offspring. Especially intensively, this method began to be used in the late XIX and early XX centuries. Emerging and subsequently modernized methods for assessing bulls by the quality of offspring have become the basis of the methods that are currently undergoing a qualitative reassessment. In this regard, our analysis of genotyping of the livestock of heifers of LLC “The breeding plant Bredy” in the context of the assessment of bulls by producers on the quality of offspring is interesting (table 3).

Table 3. Test results of heifers by their own productivity when evaluating their fathers by the quality of offspring

| Live weight at the age of 8 months., kg | Live weight at the age of 15 months, kg | The average daily gain in age from 8 to 15 months, kg | Lifetime assessment of meat qualities, point index | Body type severity, point index | Total points | Class index | Complex index |
|----------------------------------------|----------------------------------------|-----------------------------------------------------|-----------------------------------------------|-------------------------------|----------------|-------------|---------------|
| 236.5± 0.45                            | 396.7± 2.75                            | 752.1± 13.24                                        | 55.2                                         | 99.8                          | 17.7          | 97.1        | 38.9          |
| I group                                |                                        | 101.2                                               |                                               |                               | 99.7           |             |               |
| 238.9± 1.52                            | 401.9± 1.83                            | 765.1± 12.76                                        | 55.7                                         | 101.3                         | 18.5          | 103.3       | 39.2          |
| II group                               |                                        | 103.8                                               |                                               |                               | 102.7          |             |               |
| 233.2± 2.05                            | 386.9± 3.48                            | 721.6± 10.76                                        | 54.9                                         | 99.1                          | 18.0          | 99.6        | 38.7          |
| III group                              |                                        | 95.1                                                |                                               |                               | 97.7           |             |               |
| 236.2                                 | 395.2                                 | 746.2                                              | 100                                          | 100                           | 100           | 100         |               |
| On average for all heifers             |                                       |                                                     |                                               |                               |               |             |               |

* (P<0.05) – confidence level in comparison with group III
As you can see, the live weight of heifers during setting was high 233.2–238.9 kg, which we associate with a good grass stand in the pasture and inherent simmental milk of mothers. All animals corresponded to the elite and elite-record eligibility classes; however, we did not pursue the purpose of obtaining maximum potential of live weight level, since the aim was the harmonious development of heifers as the future brood potential of the herd. Therefore, on average for all groups the average daily gain in live weight during the assessment period was quite moderate 746.2 kg, the largest intensity of growth in 8–15 months, possessed daughters-descendants of the bull-producer Bush 32036 (Kip-it-Klein 660K genealogical line). In group I, the live weight of 15 months averaged was 396.7 kg, which is higher than the elite-record by 31.7 kg (8.7 %). In group III at 15 months the studied indicator was 386.9 kg, which is higher than the elite-record by 21.9 kg (6.0 %). At the same time, heifers of group II in terms of live weight of 15 months exceeded peers of group III by 15.0 kg (P <0.05). And heifers of group I in live weight at 15 months occupied an intermediate position and, in turn, exceeded the analogues of group III by 9.8 kg (P > 0.05). During the test period of the tested bulls, the growth rate of all experimental heifers ranged from 615.0 to 873.2 g. Of all the bulls that are assessed for quality of the offspring, the best indicators were characteristic of the descendants of the bull Bush, which turned out to be an improver with a complex index of 102, 7. In their midst 15 heifers with a complex index above 100 (100.6-107) were identified.

The distribution of the results of genotyping by the offspring of bulls is shown in table 4. As can be seen, no large differences in the frequency of genotypes were revealed. According to the CAPN1 gene, the frequency of the GG genotype in the offspring of producer bulls ranged from 84.6 % in Chizhik to 92.3 % in the Bush. There were no differences in genotypes and TG5 gene. According to the GDF5 gene: one genotype homozygous CC and heterozygous TS were detected offspring of Fakir.

| Gene name | Genotype | Fakir n | %  | Bush n | %  | Chizhik n | %  |
|-----------|----------|-------|---|-------|---|-----------|---|
| CAPN1     | GG       | 12    | 85.7    | 12 | 92.3 | 11 | 84.6    |
|           | GC       | 2     | 14.3   | 1  | 7.7  | 2  | 15.4    |
|           | CC       | 0     | 0      | 0  | 0    | 0  | 0       |
| TG5       | CC       | 13    | 92.85  | 12 | 92.3 | 12 | 92.3    |
|           | CT       | 0     | 0      | 0  | 0    | 0  | 0       |
|           | TT       | 1     | 7.15   | 1  | 7.7  | 1  | 7.7     |
| GDF5      | TT       | 12    | 85.7   | 13 | 100  | 13 | 100     |
|           | TC       | 1     | 7.15   | 0  | 0    | 0  | 0       |
|           | CC       | 1     | 7.15   | 0  | 0    | 0  | 0       |

In our experiment, animals with different states of polymorphic genes did not differ in terms of live weight at the age of 8 and 15 months, the level of average daily gain in live weight. However, one noteworthy fact should be noted that the only heifer of the two genotyped micropopulations (Russian and Kazakhstan) that had the GDF5 SS gene genotype, when evaluated according to its own productivity, received a 60-point mark for evaluating meat forms, which indicates the advisability of continuing experiments with large-scale livestock, until statistically verified results are obtained.

5. Conclusion
The autonomous breeding of livestock purchased in the Russian Federation on the territory of Kazakhstan under the conditions of the farm “Reymkul” and the selection pressure associated with combining the Bredy and Bagan meat types using Canadian meat simulations when creating a new breed at LLC “Breeding plant Bredy” did not affect the appearance of significant differences in micropopulations of the studied genes. However, there was some displacement for the desired genes in the studied populations. Thus, the general criterion for Pearson's agreement on the CAPN1 gene was
8.52, which corresponds to a significant difference in the empirical series of two micropopulations at the first threshold $P < 0.05$, and for the TG5 gene, the index $\chi^2$ was 4.73 and did not confirm the hypothesis about the difference in micropopulation by state polymorphism of this gene. The data obtained are consistent with the opinion of other authors on the effect of the selection process on the genotype [13, 14]. The comparison of the carriers of the identified genotypes of the studied genes in the environment of the offspring of sire bulls, according to the traits used in assessing the young animals by their own productivity, did not give significant results, however, it showed tendencies along which scientific research can be directed to create new biotechnological methods in breeding.

Acknowledgments
The studies were carried out in accordance with the research plan for 2019-2021. FSSI FRC BST RAS (No. 0761-2019-0012)

References
[1] Yadav B R, Mitra A 1999 One Day Symposium on Emerging DNA Technologies for the Next Millennium CCMB/CDFD, Hyderabad, 23 February, Abstract 9
[2] Kayumov F G et al 2008 The first breeding plant for breeding the "Bredy meat" type of Simmental Bull. of meat cattle breed. 1(61) 117–9
[3] Kanatpaev S M et al 2013 What do we know about meat Simmentals? Niva Trans-Urals. 2(102) 78–9
[4] Tyulebaev S D 1994 Economic-useful features of Simmental, Hereford cattle and hybrids of Simmental with meat breeds (Cand. Dissertation thesis) (Orenburg)
[5] Tyulebaev S D et al 2011 Domestic meat breed of intensive type – a new direction in beef cattle breeding in Russia Probl. of boil. of product. animals 3 20–6
[6] Ankeny R A 2003 Sequencing the genome from nematode to human: changing methods, changing science Endeavour 27(2) 87–92
[7] Terletsky V P, Tyshchenko V I, Novikova I I et al 2016 An efficient method for genetic certification of bacillus subtilis strains, prospective producers of biopreparations Microbiol. 85(1) 71–6
[8] Singh U, Deb R et al 2014 Molecular markers and their applications in cattle genetic research: A review Biomarkers and Genomic Med. 649–58
[9] Pintos D and Corva P M 2011 Association between molecular markers for beef tenderness and growth traits in Argentinian angus cattle Anim. Genet. 42 329–32
[10] Tait R G, Shackelford S D, Wheeler T L et al 2014 CAPN1, CAST, and DGAT1 genetic effects on preweaning performance, carcass quality traits, and residual variance of tenderness in a beef cattle population selected for haplotype and allele equalization J. Anim. Sci. 92 5382–93
[11] Koohmaraie M 1996 Biochemical Factors Regulating the Toughening Tenderization Processes of Meat Meat Sci. 43(S1) 193–201
[12] Miroshnikov S, Kharlamov A, Zavyalov O et al 2015 Method of sampling beef cattle hair for assessment of elemental profile Pakistan Journal of Nutrition 14(9) 632-636
[13] Georges M, Charlier C and Hayes B 2018 Harnessing genomic information for livestock improvement Nature Rev. Genet. 20(3) 135–56 DOI: 10.1038/s41576-018-0082-2
[14] Kolosova M A, Getmantseva L V, Bakoev S Y et al 2019 Associations of mtDNA haplotypes with productive traits in pigs Rendiconti Lincei. Sci. Fisiche e Naturali DOI: 10.1007/s12210-019-00853-1.