The use of modern biotechnology of reproduction to improve the Hereford gene pool

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Abstract. The gene pool improvement of North Caucasian population is carried out through artificial insemination of herds with sperm from sires carrying the desired alleles of genes associated with meat productivity and beef quality. The frequency of homozygotes in CAPN1 gene was found in Stavropol population of Hereford sires: CC – 6 %, GG – 88 % and more. This indicator was 50.0 % in the LL genotype of somatotropin gene (GH), which is 22-28 % more than that in hetero and homozygotes LV and VV. The frequency of CC genotype was 61 % higher in TG5 DNA-marker than that of the heterozygous CT form. The use of modern biotechnological methods of herd reproduction made it possible to reduce the degree of homozygosity in CAPN1 and TG5 genes by 28.8 % and by 18.1 % in the next generation. The homozygosity degree in CAPN1 and TG5 genes were less by 28.8 % and 18.1 % in progeny than that in sires. However, the number of effective alleles was higher by 0.53 % and 0.41 % for CAPN1 and TG5 SNP markers in progeny bulls. The observed heterozygosity varied in the range of 0.281-0.344 for gene markers CAPN1, GH and TG5 in the sire group. A high genetic similarity was established between sires and progeny – an average of 0.973. This indicator was 0.999 and 0.970 for GH and TG5 gene-markers. The largest genetic distance was determined in CAPN1 gene – 0.044. Thus, the high plasticity of the gene pool in North Caucasian Herefords population has been proved under the influence of a targeted mating system of parental pairs based on the improvement of herd reproduction biotechnology. The progeny bulls from sires-carriers of the desired alleles had an advantage over peers in growth rate at the age of 3-7 months and 12-15 months, it amounted to 163.9 g (17.7 %; P <0.05) and 160.3 g (14.5 %; P <0.05) respectively.

1. Introduction

The intensive use of economic meat traits in Hereford breed involves assessing the polymorphisms of DNA markers that determine the development and inheritance of such traits as animal growth, slaughter yield and meat quality, its tenderness, juiciness and marbling. The analysis of domestic and foreign information data made it possible to determine CAPN1, GH, TG5, LEP genes as potential markers of these traits and raise the question of the nature of their inheritance [1, 2].

The most important tasks are the assessment and selection of intramuscular fat accumulation and animal growth at the genetic level at an early age, which provides an analysis of polymorphism and the identification of desired alleles. The informational content of DNA markers allows selection at a young age according to the traits that will develop in the older age, while being characterized by the polygenic nature of inheritance. Genetic improvement of bulls can be carried out by selecting sires and...
The polymorphism of 2 nucleotides in \( \mu \)-calpain following by amino acid substitution (glycine / alanine) provides a uniform distribution of intramuscular fat between the fibers, which leads to higher tenderness, juiciness of meat (more than 30%) compared to glycine allele [5]. Further, the desired genotype of sires is extended to the entire population through an improved selection to mature herd. Modern biotechnological methods of reproduction (artificial insemination and embryo transplantation) allow the gene pool changing in population at an accelerated rate, which contributes to the intensification of selection work in breeding herds.

The adipocytes formation, lipid metabolism, and, as a result, the development of meat marbling and tenderness are associated with the action of thyroglobulin (TG5) [6, 7]. Leptin gene was mapped on 4 bovine chromosomes, and it is involved in fat accumulation of the body, the regulation of body weight and feeding behavior, affects growth and constitution. This hormone consists of 3 exons (2 are translated into protein) and 2 introns [8, 9].

Analysis of the single nucleotide polymorphism of gene-markers allows us to determine the desired forms and determine the allelic frequency for use in the selection process.

**The aim of the research** The aim of the study was to determine the effectiveness of using modern biotechnological methods of reproduction to improve the gene pool of the North Caucasian population of Hereford cattles.

### 2. Material and methods

The studies were carried out on Hereford sires of Canadian selection (n = 18) and their bulls-progeny (n = 58) in the breeding factories of the APC (collective farm) Rodina and Belokopanskoye OJSC in the Stavropol Territory. The management system for animals and research were conducted according to instructions and guidelines of the 1987 Russian Regulations (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, D.C. 1996). During the study, the authors took all efforts to minimize the suffering of animals and to reduce the number of used samples.

#### 2.1. Scheme of the experiment

The polymorphisms of calpain – CAPN1, somatotropin – GH, thyroglobulin – TG5, and leptin – LEP genes were studied in Hereford sires of Canadian selection (n=18). Two experimental groups of progeny were formed as a result of genotyping. The I group (n = 28) was completed with bulls progeny of sires with the desired genotypes with marker alleles: (CAPN1<sup>CC</sup>GH<sup>VV</sup>TG5<sup>TT</sup>LEP<sup>TT</sup>); (GH<sup>VV</sup>TG5<sup>TT</sup>LEP<sup>TT</sup> и GH<sup>VV</sup>TG5<sup>TT</sup>LEP<sup>TT</sup>). The II group consisted of sons from sires without desired alleles in genotype. During the experiment, the live weight and average daily gain were studied in young animals.

#### 2.2. Equipment and technical tools

Genomic DNA was extracted from the whole blood using the reagent kit "DIAtomtm DNA Prep 200" (IsoGene Lab, Moscow). DNA yield was 3-5 µg / 100 µl with OD 260/280 ranged from 1.6 to 2.0. A GenePaktm PCR Core kit (IsoGene Lab, Moscow) was used to conduct the polymerase chain reaction.

Genotyping for GH, TG5, LEP genes was carried out by PCR-RFLP using primers: GH- (F: 5'-gct-gct-cct-gag- cct-tcg -3' and R: 5'- gcg-gcg-gca-ctt-cat-gac-cct -3'), TG5- (F:5'-ggg-gat-gac-tac- gag-tat-gac-tg-3' and R:5'gtg-aaa-atc-ttc-tgg-agg-ctg-ta-3'), LEP- (F:5'-tgt-ctt-acg-tgg-agg-ctg-tgc-cca- gct-3' and R:5'-agg-gtt-ttc-gtg-tca-tcc-tgg-acc-ttt-cg-3').

Gel electrophoresis was used to identify the studied genes with ultraviolet visualization of PCR-RFLP products obtained using endonucleases: GH – AluI, TG5 – BstX2I, LEP – BstMBI. Real-time PCR was used to identify the specific sequence of CAPN1 gene with “CAPN1-detect” reagent kit designed to determine one binary SNP C316G mutation in genomic DNA samples using allele-specific probes.
Polymorphism in CAPN1, GH, TG5, LEP genes were determined at the Laboratory of Immunogenetics and DNA-technologies of the Federal State Budget Scientific Institution «North Caucasian Federal Agricultural Research Center» (Stavropol, Russia).

Statistical analysis was carried out using the statistical software package Statistica 10.0 (Stat Soft Inc., USA). Comparison of the results was carried out using the Student criterion of the parametric method.

3. Results and discussion

Canadian sires are the large and tall animals, their live weight was greater than requirements of elite-record class by 130 kg (15 %), hip height exceeded the measurements of their peers by 16 cm (9.2 %), their body volume was 0.60-0.87m³. However, the issue of inheritance of the valuable qualities of sires by progeny, taking into account gene markers of productivity (CAPN1, GH, TG5, LEP), is relevant.

The frequency of the homozygous CC allele in CAPN1 gene was 6 %, which is 88 % less than the frequency of GG allele. The LL genotype of somatotropin (GH) gene was characterized by a higher concentration (50 %) compared with heterozygous LV and homozygous VV by 22–28 %.

The CC genotype of TG5 gene had a higher frequency (78 %) than that of CT and TT genotypes. The differences were 61 % and 73 %, respectively. The frequencies of effective alleles have different occurrence ranged from 4.5 % (TT) in animals of Hereford breed of Siberia to 17 % (CT) in the herds of Stavropol region [10]. Carriers of such alleles had marbling score 20 % higher compared to the cattle with other polymorphic variants in Aberdeen-Angus and Shorthorn breeds in Australia populations [11, 12].

The genotypic frequency of CT heterozygotes in leptin gene was 56 %, which is 23 % and 45 % more than the allelic homozygous forms of CC and TT. The studies of leptin gene loci revealed a frequency of CC homozygotes – 56 % and TC heterozygotes – 38 % in herds of Aberdeen Angus cattle [13].

The genetic variability of sires and their progeny by SNP-markers was determined taking into account the values of genetic constants: degrees of homo- and heterozygosity (Ca and H), level of polymorphism (Na), Selender coefficient, heterozygosity test (Tables 1, 2).

Consolidation of the population is more pronounced in sires according to the studied gene-markers. Thus, the degree of homozygosity in bulls-progeny was less by 28.8 % and by 18.1 %, respectively in CAPN1 and TG5 genes. This is probably due to the effect of the maternal genotype. The number of effective alleles were higher by 0.53 and 0.41 % for CAPN1 and TG5 SNP-markers in bulls-progeny compared with sires. Moreover, they characterized by the smallest number of effective alleles in CAPN1 and LEP gene-markers (1,119 and 1,244), but the largest in GH and TG5 (1,855 and 1,314). The number of effective alleles in CAPN1 and GH genes were 1,650 and 1,855 in progeny.

Table 1. Genetic variability by SNP-markers CAPN1 C316G, GH C2141G, TG5 C422T, LEP A80V, LEP Y7F in Hereford sires

| Indicator                          | CAPN1 C316G | GH C2141G | TG5 C422T | LEP A80V | LEP Y7F |
|------------------------------------|-------------|-----------|-----------|----------|---------|
| Observed heterozygosity (H_{obs})  | 0           | 0.389     | 0.167     | 0        | 0       |
| Expected heterozygosity (H_{ex})   | 0.106       | 0.461     | 0.239     | 0.196    | 0.196   |
| Level of homozygosity, % (Ca)      | 89.4        | 53.9      | 76.1      | 80.4     | 80.4    |
| Number of effective alleles (Na)   | 1.119       | 1.855     | 1.314     | 1.244    | 1.244   |
| The value of excess heterozygotes (Selender coefficient) | -1.0 | -0.156 | -0.431 | -1.0 | -1.0 |
| Heterozygosity test                | H_{obs}< H_{ex} | H_{obs}< H_{ex} | H_{obs}< H_{ex} | H_{obs}< H_{ex} | H_{obs}< H_{ex} |
Table 2. Genetic variability by SNP-markers CAPN1 C316G, GH C2141G, TG5 C422T in bulls-progeny

| Indicator                                      | CAPN1 C316G | GH C2141G | TG5 C422T |
|-----------------------------------------------|-------------|----------|-----------|
| Observed heterozygosity (H<sub>obs</sub>)      | 0.344       | 0.344    | 0.281     |
| Expected heterozygosity (H<sub>ex</sub>)       | 0.394       | 0.461    | 0.420     |
| Level of homozygosity, % (Ca)                  | 60.6        | 53.9     | 58.0      |
| Number of effective alleles (Na)               | 1.650       | 1.855    | 1.724     |
| The value of excess heterozygotes (Selender coefficient) | -0.127     | -0.254   | -0.331    |
| Heterozygosity test                            | H<sub>obs</sub>< H<sub>ex</sub> | H<sub>obs</sub>< H<sub>ex</sub> | H<sub>obs</sub>< H<sub>ex</sub> |

The level of observed and expected heterozygosity indicates a different nature of its distribution in the studied genes in population. Thus, the observed heterozygosity varied from 0 for the CAPN1 and LEP genes to 0.389 for the GH gene in sires. The observed heterozygosity varied in the range of 0.281-0.344 for gene-markers CAPN1, GH and TG5 in the group of sires.

The heterozygosity test is a measure of the frequencies deviation of heterozygous genotypes and theoretically expected heterozygotes according with Hardy-Weinberg proportion. This test was negative in all gene-markers.

Indicators of genetic distances and similarities were calculated based on the allelic frequencies in CAPN1, GH, and TG5 genes (Table 3). A high genetic similarity (an average of 0.973) between sires and progeny was established. This indicator was 0.999 and 0.970 for gene-markers GH and TG5 respectively. The largest genetic distance (0.044) was determined in CAPN1 gene-marker.

Table 3. Genetic distance between sires and progeny taking into account CAPN1, GH, TG5 genes

| Gene | similarity | distance |
|------|-----------|----------|
| CAPN1| 0.957     | 0.044    |
| GH   | 0.999     | 0.001    |
| TG5  | 0.970     | 0.030    |
| Average | 0.973 | 0.027 |

The desired genotypes were determined in 22.2 % of sires. Their progeny (I group) had an advantage for growth rate over analogues without valuable substitutions (II group) in studied genes in all age periods (Fig. 1, 2).

The average daily gain in I group of bull-calves was higher than that of the peers from II group – by 163.9 g (17.7 %; P <0.05) in the age period from 3 to 7 months, the advantage reached 160.3 g (14.5 %; P <0.05) in the age period from 12 to 15 months.

Thus, the genetic characteristics of sires and their progeny made it possible to identify the desired genotypes with a high genetic potential of productivity.
Figure 1. Variability of average daily gain in bull-calves from I group.

Figure 2. Variability of average daily gain in bull-calves from II group.

4. Conclusion
An analysis of the genetic structure of sires made it possible to establish the presence and frequencies of the desired alleles in gene-markers of meat productivity. The variability in allelic profiles in CAPN1, GH, TG5, and LEP genes indicate a different degree of herd consolidation, the presence of efficiently acting alleles, and observed and expected heterozygosity. The desired alleles were found in 22.2 % of sires, their progeny exceeded peers from other bulls for growth rate. Probably, the regulatory effect of the desired alleles of gene-markers of productivity has provided more favorable growth and development of bull-calves compared to peers.

The obtained data will help to optimize the selection process to identify the predicted (expected) productivity.

Acknowledgments
This work was supported by the State Task of the Federal Research Centre of Biological Systems and Agro-technologies of the Russian Academy of Sciences No. 0761-2019-0012.

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