Use of genetic markers of meat productivity in breeding of Hereford breed bulls

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Abstract. The aim of the study was to assess the genetic potential in Canadian Hereford sires using DNA markers, identify complex genotypes and assess their impact on the growth, development and meat productivity of its offspring. Groups of sons were formed taking into account the complex genotypes of sires: group 1 \((n = 28)\) – sons of bulls carrying in their genome a complex of genotypes with desired alleles; group 2 \((n = 30)\) – sons of bulls with a complex of genotypes that lack the desired alleles. The offspring from bulls-carriers of the “desirable” alleles in complex CAPN1, GH, Lep, TG5 genes that meet the exterior requirements exceeded their peers in live weight \((P < 0.05)\), carcass weight \((P < 0.05)\) and muscle tissue \((P < 0.05)\). The maximum conversion rate of feed protein into product protein was also established in the group of sons from selected bulls. Thus, animal selection for body conformation type is advisable to combine with the herd genotyping for a complex of genotypes associated with different economically useful traits when creating highly efficient population of beef cattle.

1. Introduction

The experience of developed countries shows that the improvement of the genetic potential in beef cattle and the creation of competitive beef production are possible only through the use of molecular genetic methods. Thus, the leading National Associations of the Angus breed [¹], Hereford [²], Simmental and others [³] widely using DNA testing to predict and evaluate the expected productivity of animals continuously monitor its effectiveness [⁴].

For Russia, the question of using the already known different QTL-MAS models or choosing one’s own genomic assessment method adapted to the world genetic process is extremely relevant. The present study will be directed to the solution of individual tasks in this strategic direction for livestock breeding.

Calpain (CAPN1) is one of the genes associated with the marbling of meat, which determines its softness. It is proved that in the decomposition of muscle tissue that occurs after slaughter of an animal, the protein of the Calpain family (Calpain) takes an active part. Its mechanism of action lies in the fact that the Calpain system based on calcium-dependent cysteine protease and due to the decomposition of the skeletal muscle Z-discs and weakening of the bonds between muscle fibers creates conditions for the even distribution of intramuscular fat between the fibers, which provides a softness, juiciness of meat, its marbling [⁵-⁹].
Somatotropin (GH) is produced by the anterior lobe of hypophysis, is one of the most important regulators of the somatic growth in animals. It is established that the gene controlling the synthesis of Somatotropin regulates the growth of the animal, and also plays a key role in metabolic processes (carbohydrate, fat processes) [10-12].

Thyreoglobulin (TG5) influences lipid metabolism, participates in the formation of fat cells and forms the so-called “marbling” of muscle tissue [13].

Leptin (LEP) is a hormone produced by the cells of adipose tissue, plays an important role in metabolism, in particular, in the accumulation of fat in the body. In beef cattle breeding, polymorphism of the leptin gene is an important genetic factor affecting slaughter yield and meat quality [14-16].

The purpose of the study is to assess the genetic potential in the Canadian selection servicing bulls of the Hereford breed using DNA markers, identify complex genotypes and assess their impact on the growth, development and meat productivity of the offspring.

2. Materials and methods
Object of study. Large Hereford breed sires of Canadian origin (n = 18). Bull calves (n = 58) got from servicing bulls with a different complex of genotypes for the studied SNP markers. Animal attendance and experimental studies were carried out in accordance with Russian Regulations instructions, 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, D.C. 1966). In performing research, efforts were made to minimize the suffering of animals and reduce the number of samples used.

To assess the genetic potential of large Hereford servicing bulls of Canadian selection (n = 18), the gene polymorphism of Calpain (CAPN1), Somatotropin (GH), Thyreoglobulin (TG5), Leptin (LEP) was studied in the herds of stud farms of the APC (Kolkhoz) “Rodina” and OJSC “Belokopanskoye” on the Stavropol Territory. Taking into account the complex genotypes of servicing bulls, groups of sons were formed, which were raised at the experimental fattening station in accordance with the requirements for the technology of specialized beef cattle breeding: group 1 (n = 28) – sons of bulls carrying in their genome a complex of genotypes with desired alleles; group 2 (n = 30) – sons of bulls with a complex of genotypes that lack the desired alleles.

Evaluation of meat productivity, synthesis of meat components were determined by the results of control slaughter, 3 heads from each group, after 24 h period of fasting. Obtained during slaughter of young stock bio substrates (longest back muscle, fat, minced meat) were subjected to chemical and biochemical analyses.

To determine the single nucleotide polymorphism primers by genes were used: Calpain (CAPN1), Somatotropin (GH), Thyreoglobulin (TG5), and Leptin (LEP). Genotyping was carried out on the basis of DNA isolated from blood using the “DIAtomtmDNAPrep” reagent kit for DNA isolation (IsoGeneLab, Moscow). The DNA yield was 3-5μg/100μl with an OD of 260/280 from 1.6 to 2.0. For carrying out PCR, “GenePakPCRCore” kits were used (IsoGeneLab, Moscow). Genotyping for GH, TG5, LEP genes was performed by PCR-RFLP method using primers: GH- (F: 5’-gct-gct-gct-gct-cct-cct -3’ and R: 5’-ggg-gcg-gea-cct-cat-gac-cct -3’), TG5- (F: 5’-ggg-gat-gac-tac-gag-tat-gac-tg-3’ and R: 5’-tgt-gaa-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’). To identify the studied genes, gel electrophoresis was used with visualization under ultraviolet light of PCR-RFLP products obtained using endonucleases: GH – AluI, TG5 – BstX2I, LEP – BstMBI; Calpain – PCR-RV using the “CAPN1” reagent kit, which is used to determine one binary SNP mutation of C316G gene in genome samples of Bovine DNA using allele specific probes.

The statistical analysis of results was conducted using the statistical software package of Statistics 10.0 (“Stat. Soft. Inc.” USA). Comparison of results was carried out using the parametric method of Student’s criterion. Parameter of P ≤ 0.05 was taken as a limit of significance.
3. Results

An analysis of the results in genotyping of large Hereford servicing bulls getting Canadian selection revealed features of the gene polymorphism (CAPN1, GH, TG5, LEP) that control meat productivity (Table 1).

CAPN1 gene polymorphism in the studied population of large Canadian selection bulls is represented by two alleles (C and G) with a very low (0.06) occurrence frequency of the allele C and a high frequency (0.94) of the allele G. This provided a clear advantage of the homozygous (GG) genotype over the homozygous (CC) variant: 94.0% vs. 6.0% in the absence of a heterozygous (CG) genotype.

Table 1. CAPN1, GH, TG5, LEP gene polymorphism in Hereford servicing bulls of Canadian origin \( (n = 18) \)

| Gene | genotypes | Frequency of occurrence | alleles | \( \chi^2 \) |
|------|------------|-------------------------|---------|-----------|
|      | n | %     |         |       |
| CAPN1 | CC | 0.06 | 1 | 6.0 | C | 0.06 | 14.45 |
|      | CG | 0   | 0 | 0   | G | 0.94 |       |
|      | GG | 0.94 | 17 | 94.0 |     |       |
| GH   | LV | 0.28 | 5 | 28.0 | V | 0.36 | 2.02 |
|      | LL | 0.50 | 9 | 50.0 | L | 0.64 |       |
| TG5  | CC | 0.78 | 14 | 78.0 |     |       |
|      | CT | 0.17 | 3 | 17.0 | T | 0.14 | 1.76 |
|      | TT | 0.05 | 1 | 5.0  |     |       |
| LEP  | CT | 0.33 | 6 | 33.0 | C | 0.61 | 0.51 |
|      | TT | 0.56 | 10 | 56.0 | T | 0.39 |       |

The polymorphism of Somatotropin allelic profile (GH) in the studied bull population is represented by two alleles (V and L) with occurrence frequency of 0.36 and 0.64, respectively. The distribution among the studied bulls of the homozygous (VV) and heterozygous (LV) genotypes in the Somatotropin (GH) was relatively the same (22.0 and 28.0%) with a clear advantage (50.0%) of the homozygous (LL) genotype.

A study of thyreoglobulin (TG5) gene polymorphism represented by two alleles (C and T), significant variability in their frequency of occurrence, which was 0.14 (T allele) and 0.86 (C allele) was found that is reflected in the occurrence frequency of homozygous (CC, TT) and heterozygous (CT) genotypes: 78.0; 5.0 and 17.0%, respectively.

Allelic Leptin profile (LEP) in the studied beef livestock population is represented by two alleles (C and T). A distinctive feature in the polymorphism of this gene was a high (0.61) occurrence frequency of the C allele, a low (0.39) frequency of the T allele. This provided significant variability in the occurrence frequency of homozygous (CC, TT) and heterozygous (CT) genotypes: 33.0; 11.0 and 56.0%, respectively.

Since the complex marking of a selective significant trait for several genes is more effective, one of the objectives in this study was to determine and to compare the genetic structure of Canadian selection large bulls using the complex genotypes of the CAPN1, GH, TG5, LEP genes. Nine complex genotypes with different occurrence frequency of marker alleles were identified (Table 2).

Complex genotypes, the allelic profile of which is represented by eight desired alleles (CAPN1\(^{CC}\) GH\(^{VV}\) TG5\(^{TT}\) LEP\(^{TT}\)), by six (GH\(^{VV}\) TG5\(^{TT}\) LEP\(^{TT}\)), by five (GH\(^{VV}\) TG5\(^{TT}\) LEP \(^{TC}\) and GH\(^{VV}\) TG5\(^{TT}\) LEP\(^{TT}\)) were found in four servicing bulls, the progeny of which was combined into the 1st experimental group. Five bulls (27.8%) were carriers of the complex genotype (CAPN1\(^{GG}\) GH\(^{LL}\) TG5\(^{CC}\) LEP\(^{CC}\)), in the allelic spectrum of which there were no desired alleles. Their sons represented the 2nd experimental group.
Table 2. Occurrence of complex CAPN1, GH, TG5, LEP genotypes in population of Hereford bulls of Canadian origin (n = 18)

| Number in order | Genotype       | Number of heads | %   |
|-----------------|----------------|-----------------|-----|
| 1               | CC VV TT TT    | 1               | 5.6 |
| 2               | GG VV TT TT    | 1               | 5.6 |
| 3               | GG VV TC TT    | 1               | 5.6 |
| 4               | GG VV TT CT    | 1               | 5.6 |
| 5               | GG LL TT TT    | 2               | 11.1|
| 6               | GG LV TC TT    | 3               | 16.6|
| 7               | GG LV TC CC    | 2               | 11.1|
| 8               | GG LL CC TC    | 2               | 11.1|
| 9               | GG LL CC CC    | 5               | 27.8|

In sons, depending on the presence of desirable alleles in the genotype, the features of growth, development, formation of meat productivity, quality of meat products were studied. Comparative analysis of live weight, average daily gain indices revealed the superiority in bull calves of the 1st group (Table 3).

Table 3. Live weight of bull calves, kg

| Age period, months | Group | I                  | II                |
|--------------------|-------|--------------------|------------------|
|                    |       | 29.7±0.22          | 30.7±0.87        |
| 3                  |       | 99.7±3.56          | 94.3±3.58        |
| 7                  |       | 231.6±9.05         | 206.3±7.46       |
| 8                  |       | 265.3±8.70         | 244.5±7.88       |
| 12                 |       | 371.3±10.26        | 348.6±7.96       |
| 15                 |       | 471.8±9.93         | 434.5±9.68       |

So, in the early period of ontogenesis (the first 3 months) bull calves of the 1st group surpassed their herd mates of the 2nd group in live weight by 5.4 kg (5.3%, P<0.05). The revealed regularity remained the same in subsequent age periods: at the age of 7 months, the advantage was 23.5 kg (12.3%, P<0.05), during the weaning period, at the age of 8 months, it was 20.8 kg (8.5%, P>0.05), and it was most clearly manifested at 15 months of age (37.3 kg, P<0.05).

An analysis of the average daily gains dynamics, a factor that most fully reflects the productive qualities of young stock, established a common regularity for all animals: an increase in average daily gains up to 7 months of age. However, a large value of this index was typical for bull calves of the 1st group: 1089.5±52.81 vs. 925.6±42.82, with a difference of 163.9 g (17.7%, P<0.05).

After weaning as a result of stress, a decrease in the growth rate in all animals occurred. However, this decrease was more noticeable in bull calves of the 2nd group: 806.1±40.24 vs. 883.4±49.87 g. A distinctive feature of bull calves in the 1st group, sons from bull carriers of complex genotypes with desired alleles, was a high growth rate during all the observed periods compared to herd mates in the 2nd group, sons from bull carriers of complex genotypes without desired alleles, with superiority in all age periods: by 23.3 g (2.7%) from 8 to 12 months, by 83.6 g (9.4%) from birth to 15 months, by 160.3 g (14.5%) from 12 to 15 months.

Analysis of the control slaughter results revealed that for the main traits (carcass weight, slaughter weight, slaughter yield) the advantage was behind the bull calves of the 1st group that has made 14.1; 12.4 and 1.7%, respectively, with a lower content of adipose tissue by 3.6% (Table 4).
Table 4. Results of the bull calves control slaughter

| Index                      | Group     |
|----------------------------|-----------|
|                            | I         | II        |
| Detachable weight, kg      | 475.0±7.58| 434.0±9.18|
| Pre-slaughter weight, kg    | 457.8±6.44| 421.5±9.27|
| Carcass weight, kg         | 258.7±5.66| 226.7±5.39|
| Carcass yield, %           | 56.5±0.35 | 53.8±1.37 |
| Weight of internal crude fat, kg | 15.9±1.03 | 16.5±0.35 |
| Yield of internal crude fat, % | 3.5±0.17  | 3.9±0.18  |
| Slaughter weight, kg       | 274.6±6.63| 243.2±5.54|
| Slaughter yield, %         | 60.0±0.51 | 57.7±1.32 |

Analysis of the morphological carcass composition, which is an important index of the meat value as a food product, revealed that the absolute amount of flesh part was greater in the carcasses of bull calves in the 1st group, by 13.9 kg (14.9%, P < 0.05) (Table 5).

Table 5. Morphological composition of bull calves half carcasses (X±Sx)

| Index                             | Group     |
|-----------------------------------|-----------|
|                                   | I         | II        |
| Chilled half carcass weight, kg    | 129.7±3.06| 113.7±4.30|
| Flesh, total, kg                  | 106.7±2.66| 92.8±3.09 |
| Including: adipose tissue, kg     | 13.1±0.20 | 14.9±0.36 |
| Muscle tissue, kg                 | 93.6±2.62 | 77.9±5.09 |
| Flesh yield, %                    | 82.3±2.06 | 81.6±5.28 |
| Adipose tissue yield, %           | 10.1±0.15 | 13.1±0.32 |
| Muscle tissue yield, %            | 72.2±2.02 | 68.5±4.48 |
| Bony tissue, kg                   | 18.3±0.43 | 16.8±1.21 |
| Bones yield, %                    | 14.1±0.33 | 14.8±1.06 |
| Tendons and cartilages, kg        | 4.6±0.28  | 4.1±0.15  |
| Tendons and cartilages yield, %   | 3.6±0.02  | 3.6±0.13  |
| The ratio of edible and inedible carcass parts | 4.65      | 4.44      |

The process of muscle tissue accumulation in these animals was more pronounced: 15.7 kg (20.1%, P < 0.05). The maximum ratio of edible and inedible parts of the carcass was observed in bull calves of the 1st group, by 13.9 kg (14.9%, P < 0.05) (Table 5).

A comparative analysis of the meat energy value suggests that in the average sample of minced meat in bull calves of the 1st group, there were 0.87% more protein and 2.48% less fat in comparison with meat obtained from the bull calves of the 2nd group. Analysis of the flesh chemical composition indicates a more favorable ratio of protein and fat in the bull calves meat of the 1st group: 1:0.70 vs. 1:0.87 in the 2nd group.

The established differences in the accumulation of nutrients in the bull calves carcasses of different origin had a significant impact on protein and energy conversion ratios (Table 6).

Bull calves of the 1st group differed in a relatively lower value of the feed energy conversion ration differing from their herd mates by 0.38%. On the contrary, the feed protein conversion ratio in sons from the bull carriers of the desired alleles was higher relative to the bull calves in the 2nd group: 9.88% versus 9.8%. The revealed regularity indicates that the greater protein deposition in the body of bull calves from the 1st group contributed to a higher protein transformation into the meat parts of their carcasses.
Table 6. Bio conversion of protein and feed energy to dietary protein and edible parts of bull calves carcass

| Index                                      | Group I   | Group II  |
|--------------------------------------------|-----------|-----------|
| Protein consumption per 1 kg of gain in live weight, g | 1025.6    | 1005.4    |
| Energy consumption per 1 kg of gain, mJ     | 87.55     | 86.72     |
| Content in carcass flesh, kg                |           |           |
| protein                                    | 46.39     | 38.91     |
| fat                                        | 43.18     | 44.85     |
| Yield per 1 kg of pre-slaughter live weight: |           |           |
| protein, g                                 | 101.33    | 92.31     |
| fat, g                                     | 94.32     | 106.41    |
| energy, mJ                                  | 6.11      | 6.37      |
| Feed protein conversion ratio, %            | 9.88      | 9.18      |
| Feed energy conversion ratio, %             | 6.97      | 7.35      |

It should be noted that the youngsters of both experimental groups were characterized by a harmonious and proportional constitution, typical for meat cattle production direction. However, indices of body weight, the dynamics of average daily gains, the values of constitution indices, the quality of meat products, as well as the intensity of feed protein conversion into meat parts of carcasses indicate that the bull calves of the 1st group are more in line with the requirements for the model Hereford breed animals of Canadian selection.

Studies conducted, analysis of the results indicate that with the greatest completeness, the genetic uniqueness of breeding animals is revealed by their genotyping for several genes that took part in the formation of productivity traits and product quality. In particular, the high prepotencies in large servicing bulls of Canadian selection, in the genome of which there are complexes of genotypes with a high concentration of alleles that mark economically useful traits, was a fundamental factor in the steady genetic inheritance contributing to the formation of desirable traits in the offspring.

4. Discussion

Breeding work with the Stavropol population of Herefords is aimed at the formation of large framed herds. Currently, the mature herd of the population has 2500 heads. At the present stage, selection for exterior traits is complemented by animals breeding with regard to molecular genetic markers associated with meat productivity and quality of beef in cattle. First of all, the identification and selection of desirable genotypes carriers is conducted in a group of sires, which are intensively used in herd reproduction. The population genotyping for MAS selection apply the number of DNA markers including CAPN1, GH, Lep, TG5. Single nucleotide polymorphisms in these genes have association with growth and development in animals, marbling and tenderness formation in beef.

SNP in C2141G position of 5th exon in growth hormone gene results to substitution of coding amino-acid leucine to valine. This substitution has a reliable effect on growth traits and meat productivity in beef cattle [11]. The individuals with GHTT genotype had a significant superiority for average daily gain compared with its peers.

Polymorphic variants of CAPN316 in exon 9 of the CAPN1 gene, located on chromosome 29 in cattle, are non-synonymous substitutions in the nucleotide sequence C-G, which results in the coding of alanine instead of glycine [17]. The role of this gene in formation of meat tenderness slaughter is noted. There are also studies that report the superiority of CC-genotype carriers by weight of the hind-quarter in half-carcass [6].

At the same time, MAS-selection, which includes only one gene associated with a small number of economically useful traits, shows low efficiency. In order to optimize breeding programs with beef
cattle, aimed at improving the herds for a complex of traits, it is advisable to carry out the selection for several genes [18].

There were 7-8 variants of paired genotypes for the bGH and RORC genes with different frequencies out of nine possible variants when identified beef cattle populations [19]. At the same time, the pattern of distribution of the complex bGH / RORC genotypes differed greatly among the populations.

In our researches, a total of 18 Hereford sires were genotyping by CAPN1, GH, Lep, TG5 genes. They were carries of 9 complex genotypes out of 81 possible variants. The highest frequency of "desirable" alleles was found in four bulls. Their intensive use in herd reproduction allowed to get 28 calves-descendant that meet the requirements for body type and exterior. Evaluation of growth and development in their sons showed a significant superiority (P<0.05) for live weight at 15 months of age compared with peers obtained from bulls with a low frequency of "desirable" alleles. In addition, the sires selection with regard to the "desirable" alleles in CAPN1, GH, Lep, TG5 genes contributed to production the descendants with more massive carcasses (P<0.05), which contained 13.9 kg (P<0.05) more muscle tissue. At the same time, the process of fat deposition was more intense in group II.

Breeding work with composite population of MARC II was also aimed at increasing the frequency of “desirable” alleles in the studied herd through the selection of sires, taking into account genetic markers. A significant effect was established when studying the association of 9 combined genotypes CSN1S1 × TG with fat thickness (P <0.06) and meat tenderness (P <0.04) in the composite MARC II population [20].

The association of polymorphism in CAPN1 / CAST genes combination with the formation of the body type of animals was revealed in the study of the exterior of Angus cattle. The carriers of the genotype CG/GG and CG/CG in CAPN1316 and CAST282 genes had a higher score for the exterior development, and also there was a positive correlation between the number of alleles G with the harmonious body conformation (r = 0.78; P < 0.05). At the same time, animals with combined CC/CC genotype were characterized by more massive hip limbs [21].

5. Conclusion
The breeding work with Stavropol population of Herefords is aimed at the animals selection for exterior type with regard to the genetic markers associated with meat productivity and beef quality. The offspring from bulls-carriers of the “desirable” alleles in complex CAPN1, GH, Lep, TG5 genes that meet the exterior requirements exceeded their peers in live weight (P < 0.05), carcass weight (P < 0.05) and muscle tissue (P < 0.05). The maximum conversion rate of feed protein into product protein was also established in the group of sons from selected bulls. Thus, animal selection for body conformation type is advisable to combine with the herd genotyping for a complex of genotypes associated with different economically useful traits when creating highly efficient population of beef cattle.

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