Bulgarian Journal of Agricultural Science, 25 (No 2) 2019, 375–379

Polymorphisms of CAPN1, CAST, GDF5, TG5 and GH genes in Russian Hereford cattle

Kinispay Dzhulamanov, Nikolay Gerasimov*, Marina Dubovskova, Asemgul Baktygalieva

Federal Research Centre of Biological Systems and Agro-technologies of the Russian Academy of Sciences, 460000 Orenburg, Russia
**Corresponding author:* nick.gerasimov@rambler.ru

Abstract

Dzhulamanov, K., Gerasimov, N., Dubovskova, M., & Baktygalieva, A. (2019). Polymorphisms of CAPN1, CAST, GDF5, TG5 and GH genes in Russian Hereford cattle. *Bulgarian Journal of Agricultural Science*, *25*(2), 375–379

The allelic and genetic polymorphisms of genes of calpain 1 (CAPN1), calpastatin (CAST), growth differentiation factor 5 (GDF5), thyroglobulin 5 (TG5) and growth hormone (GH) was examined in Russian population of Hereford cattle. The studies were carried out on purebred Hereford animals (sires, cows, bull-calves, heifers) from Chelyabinsk Region and Stavropol Territory, Russia. Maximum frequency of the genotype GG was observed at the locus of CAPN1 gene, amounting to 0.632. The study of single nucleotide polymorphisms (SNPs) in CAST gene showed a high prevalence of animals with CC genotype – 0.700. The genetic variability in GDF5 gene locus indicates the predominance of animals with AA genotype in studied population. The allelic polymorphism in thyroglobulin gene (TG5) is characterized by a high concentration of the CC (0.741) homozygous genotypes. The homozygotes concentration in growth hormone gene (GH) is in the range of 0.202-0.494, with the maximum value in LL variant and minimal in VV genotype. The maximum heterozygosity, which varied between 0.289-0.303, was observed for CAPN1 and GH genes across genetic markers studied in Hereford population. Minimum effective number of alleles (1.120) was established in GDF5 gene. A relatively high effective number of alleles was fixed in GH gene (1.843). The lowest genetic diversity was observed in Russian population. These results can be used to improve the breeding and selection work with Hereford cattle.

Keywords: alleles; genetic diversity; genotype; Hereford cattle; Russian population

Introduction

For a long time, breeding and selection work with Hereford cattle were carried out at the expense of internal resources in Russia. In this regard, the breed has a unique genetic structure (Dzhulamanov et al., 2015). High acclimatization ability and unpretentiousness to feeding and maintenance conditions promoted wide distribution of the breed in various climatic zones of Russia. The diversity of rearing areas had a modifying effect on the phenotype of separate groups, differing in body conformation type and weight growth (Ernst et al., 2010).

Recently, the genealogical structure of Hereford in Russia was significantly influenced by imported genetic material (Dzhulamanov and Dubovskova, 2012). Intensive use of animal leaders from world gene pool in herds' reproduction by artificial insemination and embryo transplantation contributed to the enlargement of the exteriors and increase the heaviness of individuals in the Hereford population (Dubovskova et al., 2016). In addition, agricultural enterprises were very active in importing heifers, mainly of Canadian origin, for the stocking of their own mature herds (Morgan et al., 2013). Thus, there was an enrichment of the interbreed gene pool in Russian Hereford. Beef cattle breeding provides for further international

exchange of genetic resources in order to intensively use the best achievements of the world gene pool of thin our country.

The DNA markers using in allele fund characterization in different cattle breeds has become possible with the development of methods in molecular genetic analysis (Miroshnikov et al., 2017). Nowadays, genetic markers are increasingly used in population genetics. It can perform as tools to evaluate the level of inbreeding, to characterize the genetic structure of populations, to establish an effective population size, to assess the effective direction of the gene flow between populations. Monitoring of population structure based on genetic markers is of great practical importance in selection and breeding work when studying their association with traits of meat productivity (Sedih et al., 2014).

The aim of the study is to analyze the single nucleotide polymorphisms of DNA markers in Hereford cattle of the Russian population.

Materials and Methods

The research was carried out on a sample of pure bred animals (sires, cows, bull-calves, heifers) of Hereford breed from LLC "Agrofirma Kalininskaya", OJSC "Pticefabrika Chelyabinskaya", Chelyabinsk Region, and OJSC "Belokopanskoe", Stavropol Territory, Russia.

Blood samples were taken from jugular vein of experimental animals to determine single nucleotide polymorphisms in CAPN1, CAST, GDF5, TG5, GH genes. Blood was injected into test tubes with 600 µl ethylenediaminetetra-acetic acid (EDTA) to obtain a volume of 10 ml.

Genomic DNA was extracted from whole blood using the reagent kit "DIAtom^{tm} DNA Prep 200" (IsoGene Lab, Moscow). A GenePak^{tm} PCR Core kit (IsoGene Lab, Moscow) and an Encyclo PCR kit (Evrogen, Moscow) were used to conduct the polymerase chain reaction. Primers were synthesized in LLC Research and Production Firm "Lytech". The nucleotide sequence of the primers is presented in Table 1.

Table 1. Specific single nucleotides

Gene	Primer sequences
CAPN1	F: 5'-AGCAGCCCACCATCAGAGAAA-3' R: 5'-TCAGCTGGTTCGGCAGAT-3'
CAST	F: 5'-TGGGGCCCAATGACGCCATCGATG-3' R: 5'-GGTGGAGCAGCACTTCTGATCACC-3'
GDF5	F: 5'-TGTCCGATGCTGACAGAAAGG-3' R: 5'-GAGTGAGGTTAATCCCAGATACCA-3'
TG5	F: 5'-GTGAAAATCTTGTGGAGGCTGTA-3' R: 5'-GGGGATGACTACGAGTATGACTG-3'
GH	F: 5'-ATCCACACCCCCTCCACACAGT-3' R: 5'-CATTTTCCACCCTCCCCTACAG-3'

The genetic characters of the cattle CAPN1, CAST, GDF5, TG5, GH genes, including genotypic and allelic frequencies, observed heterozygosity (H_o) and expected heterozygosity (H_e), effective allele numbers (N_e), Hardy-Weinberg equilibriums were calculated for each of single nucleotide polymorphisms (SNPs) according to Nei (1978).

Frequencies of different genotypes were estimated according to the following statistical model:

$$p = n/N,$$

where p – genotypic frequencies, n – number of individuals with a specific genotype, N – total number of individuals.

Frequencies of different alleles were estimated according to the following statistical equation:

$$P_A = (2nAA + nAB) \div 2N;$$
$$q_B = (2nBB + nAB) \div 2N,$$

where P_A – allelic "A" frequencies, q_B – allelic "B" frequencies, N – total number of alleles.

Standard error of frequencies was estimated according to the following statistical model:

$$S_p - \sqrt{\frac{p(1-p)}{N}},$$

where S_p – standard error of frequencies, p – genotypic and allelic frequencies, N – number of individuals.

Selander's index of heterozygote excess were estimated according to Selander (1970):

$$D = \frac{H_{obs} - H_{exp}}{H_{exp}}.$$

The expected genotypic frequencies in the studied population were calculated according to the Hardy-Weinberg equilibrium.

Statistical data was processed using the program STATISTICA 6.0. The reliability level of the obtained results was determined by the criteria χ^2.

Results and Discussion

The genetic characteristics of Hereford cattle population according to CAPN1, CAST, GDF5, TG5 and GH are presented in Table 2.

Numerous studies have shown the relationship of polymorphism in the CAPN1 gene with the meat tenderness (Page et al., 2002; White et al., 2005; Casas et al., 2006; Van Eenennaam et al., 2007; Gill et al., 2009). DNA diagnostics at the locus of CAPN1 gene established the maximum frequency of the genotype GG in Hereford population, amount-

Table 2. Genetic and allelic frequencies of CAPN1, CAST, GDF5, TG5, GH genes SNPs in Hereford population

Genotype	Number of individuals	Genetic frequencies		Allelic frequencies	χ^2
		observed	expected		
CAPN1 (n = 114)					
GG	72	0.632±0.045	0.603	G = 0.776±0.039 C = 0.224±0.039	0.028
CG	33	0.289±0.042	0.347		
CC	9	0.079±0.025	0.050		
CAST (n = 60)					
CC	42	0.700±0.059	0.640	C = 0.800±0.052 G = 0.200±0.052	0.141
CG	12	0.200±0.052	0.320		
GG	6	0.100±0.039	0.040		
GDF5 (n = 44)					
AA	41	0.932±0.038	0.890	A = 0.943±0.030 C = 0.057±0.030	0.621
AC	1	0.023±0.023	0.107		
CC	2	0.045±0.031	0.003		
TG5 (n = 81)					
CC	60	0.741±0.049	0.715	C = 0.846±0.055 T = 0.154±0.055	0.038
CT	17	0.210±0.045	0.261		
TT	4	0.049±0.024	0.024		
GH (n = 89)					
VV	18	0.202±0.043	0.125	V = 0.354±0.051 L = 0.646±0.051	0.113
LV	27	0.303±0.049	0.457		
LL	44	0.494±0.053	0.417		

ing to 0.632. A homozygous CC variant has found in 7.9% of animals, and heterozygous CG – in 28.9% of individuals. The G allele frequency reaches 0.776 that exceeds the distribution of C allele by 0.552. Analysis of single nucleotide polymorphisms in Calpain1 gene in beef cattle showed the variability of the G-allele concentration in the range of 0.663-0.730 (Kosyan et al., 2012).

The calpastatin enzyme encoded by the CAST gene is an inhibitor of μ-calpain and is also responsible for the formation of meat tenderness (Koohmaraie, 1996). The study of single nucleotide polymorphisms in CAST gene showed a high prevalence of animals with CC genotype – 0.700, exceeding the concentration of GG homozygotes by 60%. At the same time, 20% of individuals in Hereford breed are carriers of heterozygous variant. The C-allele frequency in CAST gene is 0.800 in cattle population. Similar alleles distribution (C = 0.72 and G = 0.28) in beef cattle was studied by Van Eenennaam et al. (2007).

Growth differentiation factor 5 (GDF5) has a crucial role in bones, ligaments and tendons morphogenesis (Chhabra et al., 2003; Coleman and Tuan, 2003; Nakamura et al., 2003; Chen et al., 2006). The genetic variability in GDF5 gene locus indicates the predominance of animals with AA genotype in studied population. The occurrence of homozygous CC variant was only 0.045, and heterozygous genotype was found only in 4.5% of individuals. This genetic distribution was associated with a rather large difference in the A and C allele concentrations – 0.943 and 0.057, respectively.

The TG5 gene controls the thyroglobulin production, which has a significant effect on fat metabolism. In this regard, the gene is considered as a marker of meat marbling (Panier et al., 2010). The allelic polymorphism in thyroglobulin gene (TG5) is characterized by a high concentration of the CC (0.741) homozygous and the average heterozygous CT (0.210) genotypes. Individuals-carriers of the homozygous TT variant make up only 4.9% of the population. At the same time, the minimal frequency was fixed in T-allele of the TG5 gene (0.154). This is consistent with studies of polymorphisms in thyroglobulin gene carried out by Surundayeva (2016) in Hereford cattle.

Growth hormone in cattle has an impact on the intensity of live weight gain, body conformation type, milk productivity and animal health (Woychic et al., 1982; Gordon et al., 1983; Sumantran et al., 1992; Ho and Hoffman, 1993; Lincoln et al., 1995; Ge et al., 2003). The study of genetic variability by growth hormone gene (GH) showed that the homozygotes concentration is in the range of 0.202-0.494, with the maximum value in LL variant and minimal in VV genotype. The frequency of V allele reached 0.354 in the studied group of animals and the same index of L allele was at the level of 0.646. The minor allele frequency in growth hormone gene ranged from 0.000 to 0.054 in Hereford, An-

Table 3. Standard measures of genetic diversity for Hereford cattle

Gene	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)	Heterozygote excesses (D)	Effective number of alleles (N_e)
CAPN1	0.289	0.347	-0.166	1.532
CAST	0.200	0.320	-0.375	1.471
GDF5	0.023	0.107	-0.788	1.120
TG5	0.210	0.261	-0.196	1.353
GH	0.303	0.457	-0.337	1.843

gus, Charolaise, Holstein, Simmental, Limousines in the studies carried out by Yoon et al. (2003). At the same time, the L allele concentration ranged from 0.520 to 0.867 in beef cattle (Lee et al., 1996).

The degree of genetic diversity in beef cattle population determines the effectiveness of directed selection by meat productivity markers, forming herds with high breeding value by selection and mating the individuals with favorable genotype. The maximum heterozygosity, which varied between 0.289-0.303, was observed for CAPN1 and GH genes across genetic markers studied in Hereford population (Table 3). The minimum heterozygosity was detected in GDF5 gene. It should be noted the heterozygotes deficiency for all studied genetic markers, and the highest shortage was observed in GDF5 gene. Liu et al. (2010) have established a wide range of heterozygosity variability (0.046-0.499) for the GDF5 gene in the context of breeds. Shi et al. (2011) noted that the heterozygosity for the CAPN1 gene was within 0.4735-0.4957 in Chinese populations of beef cattle.

Data analysis showed that a decrease in the number of effective alleles is noted with a decrease in heterozygosity. As a result, genetic diversity is reduced in population. In our study, the minimum effective number of alleles (1.120) was established in GDF5 gene. A relatively high effective number of alleles was fixed in hormone somatotropin gene (1.843).

Conclusion

The obtained results point to the lowest genetic diversity of Russian population and can be used to improve the breeding and selection work with Hereford cattle. The use of CAPN 1, CAST, GDF5, TG5, GH gene polymorphisms directly for marker-assisted selection should be estimated in a large number of Hereford individuals from the Russian population and further studies are needed to confirm the associations with productive traits and to define more accurate the molecular mechanisms of expression in phenotype by these genes.

Acknowledgements

This research work was carried out according the task № 0761-2019-0012.

References

Casas, E., White, S. N., & Wheeler, T. L. (2006). Effects of calpastatin and micro-calpain markes in beef cattle on tenderness traits. *Journal of Animal Science*, 520-525.

Chhabra, A., Tsou, D., Clark, R. T., Gaschen, V., Hunziker, E. B., & Mikic, B. (2003). GDF-5 deficiency in mice delays Achilles tendon healing. *Journal of Orthopaedic Research*, *21*(5), 826-835.

Chen, X., Zankl, A., Niroomand, F., Liu, Z., Katus, H. A., Jahn, L., & Tiefenbacher, C. (2006). Upregulation of ID protein by growth and differentiation factor 5 (GDF5) through a smad-dependent and MAPK-independent pathway in HUVSMC. *Journal of Molecular and Cellular Cardiology*, *41*(1), 26-33.

Coleman, C. M., & Tuan, R. S. (2003). Growth/differentiation factor 5 enhances chondrocyte maturation. *Developmental Dynamics*, *228*(2), 208-216.

Dubovskova, M. P., Vorozheikin, A. M., Gerasimov, N. P., & Kolpakov, V. I. (2016). Improvement of Hereford breed. *The Herald of Beef Cattle Breeding*, *3*(95), 26-33 (Ru).

Dzhulamanov, K. M., & Dubovskova, M. P. (2012). Breeding resources of Hereford cattle. *The Herald of Beef Cattle Breeding*, *3*(77). 21-26 (Ru).

Dzhulamanov, K. M., Kolpakov, V. I., & Gerasimov, N. P. (2015). Immunogenetic characteristic of Hereford in JSC "Polotsky". *The Herald of Beef Cattle Breeding*, *2*(90), 24-26 (Ru).

Ernst, L. K., Mazurovskiy, L. Z., & Gerasimov, N. P. (2010). Use of intrabreed reserves in selection of beef cattle. *Agricultural Biology*, *6*, 35-40 (Ru).

Ge, W., Davis, M. E., Hines, H. C., Irvin, K. M., & Simmen, R. C. M. (2003). Association of single nucleotide polymorphisms in the growth hormone and growth hormone receptor genes with blood serum insulin-like growth factor I concentration and growth traits in Angus cattle. *Journal of Animal Science*, *81*(3), 641-648.

Gordon, D. F., Quick, D. P., Erwin, C. R., Donelson, J. E., & Maurer, R. A. (1983). Nucleotide sequence of the bovine growth hormone chromosomal gene. *Molecular and Cellular Endocrinology*, *33*(1), 81-95.

Ho, K. K., & Hoffman, D. M. (1993). Aging and growth hormone. *Hormone Research in Paediatrics*, *40*(1-3), 80-86.

Gill, J. L., Bishop, S. C., McCorquodale, C., Williams, J. L., & Wiener, P. (2009). Association of selected SNP with carcass and taste panel assessed meat quality traits in a commercial population of Aberdeen Angus-sired beef cattle. *Genetics Selection Evolution*, *41*(36).

Koohmaraie, M. (1996). Biochemical factors regulating the toughening and tenderization processes of meat. *Meat Science*, *43*, 193-201.

Kosyan, D. B., Surundaeva, L. G, Mayevskaya, L. A., Rusakova, E. A., & Kvan, O. V. (2012). Using PCR for genotyping cattle CAPN1 the gene using genetic markers. *Vestnik of the Orenburg State University*, *6*(142), 26-30 (Ru).

Lee, B. K., Lin, G. F., Crooker, B. A., Murtaugh, M. P., Hansen, L. B., & Chester-Jones, H. (1996). Association of somatotropin (BST) gene polymorphism at the 5th exon with selection for milk yield in Holstein cows. *Domestic Animal Endocrinology*, *13*(4), 373-381.

Lincoln, D. T., Sinowatz, F., El-Hifnawi, E., Hughes, R. L., & Waters, M. (1995). Evidence of a direct role for growth hormone (GH) in mammary gland proliferation and lactation. *Anatomia, Histologia, Embryologia*, *24*(2), 107-115.

Liu, Y. F., Zan, L. S., Li, K., Zhao, S. P., Xin, Y. P., Lin, Q., Tian, W. Q. & Wang, Z. W. (2010). A novel polymorphism of GDF5 gene and its association with body measurement traits in Bos taurus and Bos indicus breeds. *Molecular Biology Reports*, *37*(1), 429-434.

Miroshnikov, S. A., Kosyan, D. B., Surundaeva, L. G., & Rusakova, E. A. (2017). Assessment of relationship between polymorphism of CAPN1 gene with hematological parameters and characteristics of nonspecific immunity of cattle. *Modern problems of science and education*, 6, 258 (Ru).

Morgan, G. A., Miroshnikov, S. A., Mazurovskiy, L. Z., & Gerasimov, N. P. (2013). Canadian genetic for Russian beef cattle breeding. *The Herald of Beef Cattle Breeding*, *5*(83), 6-9.

Nakamura, T., Yamamoto, M., Tamura, M., & Izumi, Y. (2003). Effects of growth/differentiation factor-5 on human periodontal ligament cells. *Journal of Periodontal Research*, *38*(6), 597-605.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, *89*(3), 583-590.

Page, B. T., Casas, E., Heaton, M. P., Cullen, N. G., Hyndman, D. L., Morris, C. A., Crawford, A. M., Wheeler, T. L., Koohmaraie, M., Keele, J. W. & Smith, T. P. L. (2002). Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. *Journal of Animal Science*, *80*(12), 3077-3085.

Pannier, L., Mullen, A. M., Hamill, R. M., Stapleton, P. C., & Sweeney, T. (2010). Association analysis of single nucleotide polymorphisms in DGAT1, TG and FABP4 genes and intramuscular fat in crossbred Bos taurus cattle. *Meat Science*, *85*(3), 515-518.

Sedih, T. A., Gladir`, E. A., Dolmatova, I. Yu., Volkova, V. V., Gizatullin, R. S., & Zinov`eva, N. A. (2014). Microsatellite polymorphism of different ecological and genetic Hereford cattle generations. *Agricultural Bulletin of Stavropol Region*, *3*(15), 121-128 (Ru).

Selander, R. K. (1970). Behavior and genetic variation in natural populations. *American Zoologist*, *10*(1), 53-66.

Shi, M., Gao, X., Ren, H., Yuan, Z., Wu, H., Li, J., Zhang, L., Gao, H. & Xu, S. (2011). Association analysis of CAPN1 gene variants with carcass and meat quality traits in Chinese native cattle. *African Journal of Biotechnology*, *10*(75), 17367-17371.

Sumantran, V. N., Tsai, M. L., & Schwartz, J. (1992). Growth hormone induces c-fos and c-jun expression in cells with varying requirements for differentiation. *Endocrinology*, *130*(4), 2016-2024.

Surundayeva, L. G. (2016). Allelic polymorphism of thyroglobulin gene in beef cattle. *The Herald of Beef Cattle Breeding*, *3*(95), 47-53 (Ru).

Van Eenennaam, A. L., Li, J., Thallman, R. M., Quaas, R. L., Dikeman, M. E., Gill, C. A., Franke, D. E. & Thomas, M. G. (2007). Validation of commercial DNA tests for quantitative beef quality traits. *Journal of Animal Science*, *85*(4), 891-900.

White, S. N., Casas, E., Wheeler, T. L., Shackelford, S. D., Koohmaraie, M., Riley, D. G., Chase Jr, C.C., Johnson, D.D., Keele, J.W. & Smith, T. P. L. (2005). A new single nucleotide polymorphism in CAPN1 extends the current tenderness marker test to include cattle of Bos indicus, Bos taurus, and crossbred descent. *Journal of Animal Science*, *83*(9), 2001-2008.

Woychik, R. P., Camper, S. A., Lyons, R. H., Horowitz, S., Goodwin, E. C., & Rottman, F. M. (1982). Cloning and nucleotide sequencing of the bovine growth hormone gene. *Nucleic Acids Research*, *10*(22), 7197-7210.

Yoon, D. H., Kim, T. H., Lee, K. H., Park, E. W., Lee, H. K., Cheong, I. C., & Hong, K. C. (2003). A missense mutation in exon 5 of the bovine growth hormone gene. *Journal of Animal Science and Technology*, *45*(1), 13-22.

Received: 27.04.2018; *Accepted*: 17.06.2018