Assessment on milk productivity and milk quality in cattle with different genotypes by *HSP70.1* gene

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**Abstract.** The aim of research was to study the milk productivity and milk quality of cows and
the closest female ancestors of stud bulls with different genotypes of the chaperone protein
gene (*HSP70.1*). The genotypes of a chaperone protein gene in stock of cattle were
determined by PCR-RFLP. It was established that among cows of the Kholmogory breed of the Tatarstan
type, the highest milk yields, mass fraction, and the amount of fat and protein in milk were in
animals with the *HSP70.1/CC* genotype compared to the analogues of the *HSP70.1/C--* genotype.
Whereas in the closest female ancestors of bulls of the Holstein black-motley breed,
individuals with the *HSP70.1/C--* and *HSP70.1/C-- C--* genotypes stood out favorably by milk
yield and mass fraction of fat in the milk, respectively.

1. **Introduction**

A heat stress is a condition in which an animal is unable to distribute a sufficient amount of heat
regardless of whether it is generated or consumed by the body to maintain heat balance. A number of
physiological and behavioral reactions lead to physiological disorders that can negatively affect the
productivity and reproductive capacity of live-stock animals [1-3].

Numerous researches of the influence of genetic factors on milk yield and reproductive quality
under conditions of heat stress have indicated a negative relationship between these indicators [4, 5].
The presented research results indicate that the intensive selection of dairy cows, in particular for milk
yield, probably led to an increase in susceptibility to heat stress in these individuals [4, 6].

Research conducted on Holstein cows in the United States confirms the negative impact of heat
stress on dairy stock productivity. It is also noted that multiple cows are more susceptible to heat stress
and are manifested by a decrease in milk yield up to 1 kg per day. Along with this, the selection of
animals’ heat resistance is difficult due to the negative correlation of this indicator with milk
productivity. However, it is recommended that heat resistance be used as a selection criterion, especially for dairy cattle kept and raised in hot natural climatic conditions [7].

Heat shock proteins (HSPs) are the proteins that are expressed under stress conditions; they play a special role in the animal’s organism thermoregulation and adaptation processes to extreme heat stress conditions. The corresponding gene (HSP70.1) is responsible for the synthesis of chaperone proteins in cattle stock [8-15].

A research shows that the HSP70 gene affects the milk yield level of cows, and also affects the quality of their milk [2, 4]. Along with this, research has shown that polymorphic variants of the HSP70 gene are associated with weight during weaning of beef breed calves [16]. The HSP70 is also considered as an ideal biological marker for measuring thermal stress in animals; its polymorphism can affect the stress resistance in bulls and their fertility rates [17]. The results showing that mutations in the promoter region of the HSP70.1 gene possibly negatively affect the spermatogenesis, embryonic mortality, gestation course [18, 19] and qualitative indicators of sperm are also presented [11].

For Russian dairy enterprises, an increase in the volume of high-quality and safe dairy raw materials is necessary. These principles are important in the production of a variety of dairy products, especially canned, functional, gerodietetic, etc. [20-26].

There is no available information on the relationship of the HSP70.1 gene polymorphism with the milk yield and milk quality of milk cattle of the Republic of Tatarstan and the Russian Federation, therefore, the research to determine the effect of the HSP70.1 gene polymorphism on milk yield and cow milk quality are highly relevant.

The research purpose is to study the milk productivity and quality of milk of cows and the closest female ancestors of stud bulls with different genotypes of the chaperone protein gene (HSP70.1).

2. Materials and methods

The researches were conducted on samples from 58 stud bulls of the Holstein black-and-white breed and 139 cows of the Kholmogorsk breed of the Tatarstan type, owned by the JSC Elite Head Breeding Enterprise and the SKHP Tatarstan LLC of the Vysokogorsky and Baltasinsky districts of the Republic of Tatarstan, respectively. Blood samples were taken in cattle stock for PCR diagnostics of the HSP70.1 gene. Whole blood from animals was added to test tubes with 100 mM of EDTA to a final concentration of 10 mM.

The combined alkaline method was used to extract DNA from whole canned blood of bulls and cows.

Nested PCR protocol for a HSP70.1 gene locus of 96 bp in length amplification performed using the appropriate reagents of LLC SibEnzyme (Russia) production and two pairs of specific primers: 5'-GTCGCCAGGAAACCAGAGAC-3' (20 n.), 5'-GGAAACCCCTACCGCAGGAG-3' (20 n.) and 5'-GTTCTGGGAGGAGAGCATTTCAAG-3' (23 n.), 5'-CTGCCATGTCCGGGAATATTCAA-GG-3' (24 n.) Subsequently, the RFLP analysis was performed, including processing of amplification products of 10 MspR91 restriction endonucleases units in 1 × "O" (LLC "SibEnzyme", Russia) buffer at 37 °C during the night [11, 13, 15].

The analysis of results of the whole fragments and PCR-RFLP products was carried out using a set of reagents for gel electrophoresis, produced by the Federal State Budgetary Institution of Science Central Research Institute of Epidemiology of Rospotrebnadzor (Russia). The results were recorded using the gel-documenting system GelDoc X + (Bio-Rad, USA).

The frequency of genotypes and alleles occurrence according to the HSP70.1 gene, as well as the parental bull index for the closest female ancestors’ productivity, were determined by generally accepted formulas.

The milk yield of cows was determined as a result of weekly control milking operations, that is, for full and incomplete lactation, for 305 days and at least 240 days.

The mass fraction of fat and protein in milk was determined on a LACTAN 1-4 milk analyzer (Russia).

The results obtained in the course of scientific research are processed by the biometric method.
3. Results and discussion
As a result of testing the well-known nested PCR protocol to identify allelic variants and genotypes for the HSP70.1 gene in cattle stock, two pairs of primers were tested. During nested PCR, a 96 bp region of the HSP70.1 gene in cattle stock was amplified; later, after the HSP70.1-RFLP-MspR9I step, the HSP70.1/CC = 49/47 bp, HSP70.1/C– = 96/49/47, HSP70.1/”–” = 96 bp genotype profiles were detected (figure 1).

Figure 1. Electrophoregram of the MspR9I-PCR-RFLP-identification of C and “–” cattle chaperone protein gene alleles results.
Designations: M) 50 bp + 100 bp + 1 kb DNA markers (SibEnzyme); 1, 2) MspR9I-PCR-RFLP profile of the HSP70.1/”–” genotype (96 bp); 3, 4), 8), 9), 10), 11), 12), 13) MspR9I-PCR-RFLP-profile of the HSP70.1/C– genotype (96/49/47 bp); 5, 6) MspR9I-PCR-RFLP-profile of the HSP70.1/CC genotype (49/47 bp); 7), 14) whole PCR fragment of the chaperone protein gene (96 bp).

A distribution among the bulls of the Holstein black-motley breed and cows of the Kholmogory breed of the Tatarstan type according to the genotypes and the chaperone protein gene (HSP70.1) was the following: HSP70.1/CC (50.0%), HSP70.1/C– (31.0%), HSP70.1/”–” (19.0%) genotypes, HSP70.1/C (0.66), HSP70.1/”–” (0.34) alleles and HSP70.1/CC (59.0%), HSP70.1/C– (40.3%), HSP70.1/”–” (0.7%) genotypes, HSP70.1/C (0.79), HSP70.1/”–” (0.21%) alleles, respectively (table 1).

The research results obtained by foreign scientists on the occurrence of HSP70.1/C and HSP70.1/”–” alleles of chaperone protein genes showed that the desired HSP70.1/C allelic variant prevailed in most cattle breeds and populations. Thus, the occurrence of the HSP70.1/C allele among cows of the Holstein breed was the following: of Indonesian (0.63-0.70) [13], German was (0.78) [15], Italian (0.812) [27], Turkish (0.8382) [28] origin, among the crossbred (Holstein × Sahiwal) cows [29] and bulls of Indian origin [11], the occurrence was as follows (0.695 and 0.52).

Table 1. The occurrence of alleles and genotypes of the HSP70.1 gene in cattle stock.

| Indicator | Genotype frequency | Allele frequency |
|-----------|--------------------|-----------------|
|           | HSP70.1/CC | HSP70.1/C– | HSP70.1/”–” | n | % | n | % | n | % |
| bulls, n=58 | 29 | 50.0 | 18 | 31.0 | 11 | 19.0 | 0.66 | 0.34 |
| cows, n=139 | 82 | 59.0 | 56 | 40.3 | 1 | 0.7 | 0.79 | 0.21 |
To assess the stud bulls by origin with different genotypes of the chaperone protein gene, we used the indices of breeding evaluation of bulls, which included the productivity of the closest female ancestors (table 2).

Table 2. Characteristics of stud bulls with different genotypes of the HSP70.1 gene for milk production of female ancestors.

| Indicator          | Genotype of bulls by the chaperone protein gene locus |
|--------------------|-----------------------------------------------------|
|                    | HSP70.1/CC | HSP70.1/C- | HSP70.1/“-” |
| Number of bulls    | 29         | 18         | 11          |
| BD                 | milk yield, kg | 8749±319.9 | 8638±367.0 | 8494±489.1 |
|                    | fat, %    | 3.86±0.03 | 3.88±0.05 | 3.86±0.05 |
| DoBD               | milk yield, kg | 6895±382.3* | 8102±440.9 | 6495±487.6* |
|                    | fat, %    | 3.83±0.06 | 3.86±0.08 | 3.89±0.06 |
| BD                 | milk yield, kg | 9814±467.3 | 11444±723.3 | 11051±954.0 |
|                    | fat, %    | 3.97±0.05 | 3.98±0.08 | 4.14±0.15 |
| Bull brood index   | milk yield, kg | 8552±294.3 | 9205±395.6 | 8633±488.8 |
|                    | fat, %    | 3.88±0.03 | 3.90±0.04 | 3.94±0.04 |

* - P<0.05, a difference between the highest and the given indicator.

The research has also shown that the highest milk yields were for bull dams (BD) with the HSP70.1/CC and HSP70.1/C- genotypes (8749 kg and 8638 kg), in which these indicators were slightly higher than in stud bull dams with the HSP70.1/“-” genotype for 114-255 kg of milk. While the mass fraction of fat in milk was minimally superior to stud bull dams with the HSP70.1/C- genotype (3.88%), the difference in their benefit compared to analogues of other genotypes of the HSP70.1 gene was 0.02% of fat.

The highest milk yields were observed for dams of bull dams (DoBD) bearing both the HSP70.1/C- genotype (8102 kg), while the dams of stud bull dams with the HSP70.1/“-” genotype (3.89%) differed in the mass fraction of fat in milk. The intergroup differences in these values of dams of cows with peers of other genotypes of the HSP70.1 gene were respectively 1207-1607 kg (P <0.05) of milk and 0.03-0.06% of fat.

The highest milk yields were found in dams of stud bull sires (DoBS) with the HSP70.1/C- and HSP70.1/“-” genotypes (11444 kg and 11051 kg), while dams of stud bull sires favorably stood out with a mass fraction of fat in milk with the HSP70.1/“-” genotype. The intergroup difference in these values of dams of bull sires with analogues of other genotypes of the HSP70.1 gene was 1237-1630 kg of milk and 0.16-0.17% of fat, respectively.

We also assessed the milk productivity (milk yield for lactation, mass fraction and amount of fat in milk, mass fraction and amount of protein in milk) of cows of the Kholmogory breed of the Tatarstan type with different genotypes for the HSP70.1 gene (table 3).

Table 3. Milk productivity of cows with different genotypes of the HSP70.1 gene.

| Indicator         | Cow genotype |
|-------------------|--------------|
|                   | HSP70.1/CC   | HSP70.1/C-  |
| n                 | 82           | 56          |
| milk yield, kg    | 6992±122.9   | 6425±180.6**|
| fat, %            | 3.83±0.01    | 3.79±0.01** |
| milk fat, kg      | 267.8±4.57   | 243.5±6.92**|
| protein, %        | 3.19±0.01    | 3.18±0.01   |
| milk protein, kg  | 223.0±3.88   | 204.3±5.70**|

** - P<0.01, a difference between the highest and the given indicator.
The average milk yield of cows for lactation in groups of animals with the HSP70.1/CC and HSP70.1/C− genotypes was 6992 and 6425 kg, respectively. Cows with the HSP70.1/CC genotype were 567 kg more superior to their peers with the HSP70.1/C− genotype (P < 0.01).

The mass fraction of fat in milk ranged from 3.79% (HSP70.1/C− genotype) to 3.83% (HSP70.1/CC genotype). By mass fraction of fat in milk, cows with the HSP70.1/CC genotype were superior to analogues with the HSP70.1/C− genotype by 0.04% (P < 0.01). A higher amount of fat in milk for lactation was obtained from animals with the HSP70.1/CC genotype, which is 24.3 kg (P < 0.01) more of milk fat than from cows with the HSP70.1/C− genotype. Moreover, the highest amount of milk fat was in cows with the HSP70.1/CC genotype and amounted to 267.8 kg.

The mass fraction of protein in milk ranged from 3.18% (HSP70.1/C− genotype) to 3.19% (HSP70.1/CC genotype), with a slight difference. Data were also obtained that from cows with the HSP70.1/C− genotype less milk protein was obtained for lactation by 18.7 kg (P < 0.01) than from cows with the HSP70.1/CC genotype. At the same time, the greatest amount of milk protein was among peers with the HSP70.1/CC genotype and amounted to 223.0 kg.

4. Conclusion
The frequency of the HSP70.1/C and HSP70.1/“−” alleles occurrence in the chaperone protein gene among the livestock of stud bulls of the Holstein black-and-white breed and cows of the Kholmogory breed of the Tatarstan type averaged 0.66-0.79 and 0.21-0.34, respectively. These results on the HSP70.1 gene alleles occurrence are consistent with published data.

An assessment of stud bulls by origin with different genotypes of the HSP70.1 gene showed that bulls with the HSP70.1/C− (9205 kg) and HSP70.1/“−” genotypes had the highest brood index for milk yield and mass fraction of fat in milk (3.94%), respectively.

An assessment of cows by milk productivity and milk quality with different genotypes of the HSP70.1 gene allowed determining that cows with the HSP70.1/CC genotype significantly exceeded peers of the HSP70.1/C− genotype by 567 kg of milk, by 0.04% and 24.3 kg of milk fat and 18.7 kg of milk protein.

References
[1] Das R, Sailo L, Verma N, Bharti P and Saikia J 2016 Impact of heat stress on health and performance of dairy animals: A review Vet. World 9 260-268 doi: 10.14202/vetworld.260-268
[2] Nardone A, Ronchi B, Lacetera N, Ranieri M S and Bernabucci U 2010 Effects of climate changes on animal production and sustainability of livestock systems Livest. Sci. 130 57-69 doi: 10.1016/j.livsci.2010.02.011
[3] West J W 2003 Effects of heat-stress on production in dairy cattle J. Dairy Sci. 86 2131-2144 doi: 10.3168/jds.S0022-0302(03)73803-X
[4] Aguilar I, Misztal I and Tsuruta S 2009 Genetic components of heat stress for dairy cattle with multiple lactation J. Dairy Sci. 92 5702-5711 doi: 10.3168/jds.2008.1928
[5] Rosenkranz C Jr, Banksa A, Reitera S and Looperb M 2010 Calving traits of crossbred Brahman cows are associated with heat shock protein 70 genetic polymorphisms Anim. Reprod. Sci. 119 178-182 doi: 10.1016/j.anireprosci.2010.02.005
[6] Ravagnolo O and Misztal I 2002 Studies on genetics of heat tolerance in dairy cattle with reduced weather information via cluster analysis J. Dairy Sci. 85 1586-1589 doi: 10.3168/jds.S0022-0302(02)74228-8
[7] Bernabucci U, Bifani S, Buggiotti L, Vitali, A, Lacetera N and Nardone A 2014 The effects of heat stress in Italian Holstein dairy cattle J. Dairy Sci. 97 471-486 doi: 10.3168/jds.2013-6611
[8] Adamowicz T, Pers E and Lechniak D 2005 A new SNP in the 3′-UTR of the hsp 70-1 gene in Bos Taurus and Bos indicus Biochemical Genetics 43 623-627 doi: 10.1007/s10528-005-9119-2
[9] Ajayi O O, Peters S O, Donato M D, Sowande S O, Mujibi F D N, Morenikeji O B, Thomas B N, Adeleke M A and Imumorin I G 2018 Computational genome-wide identification of heat shock protein genes in the bovine genome [version 1; peer review: 2 approved, 1 approved with reservations] F1000Research 7 1504 doi: 10.21688/f1000research.16058.1

[10] Bellagi R, Martin B, Chassaing C, Najar T and Pomies D 2017 Evaluation of heat stress on Tarentaise and Holstein cow performance in the Mediterranean climate International Journal of Biometeorology 61(8) 1371-1379 doi: 10.1007/s00484-017-1314-4

[11] Deb R, Sajjanar B, Singh U, Kumar S, Sengar G S, Alex R, Das A K, Tyagi S, Raja T V, Alyehodi R R, Singh R, Bhanuprakash V and Prakash B 2016 Cytosine deletion at AP2-box region of HSP70 promoter and its influence on semen quality traits in crossbred bulls Journal of Genetics 95(4) 1035-1038 doi: 10.1007/s12041-016-0727-x

[12] Kerekopppa R P, Rao A, Basavaraju M, Geetha G R, Krishnamurthy L, Rao T V L N, Das D N and Mukund K 2015 Molecular characterization of the HSPA1A gene by singlestrand conformation polymorphism and sequence analysis in Holstein-Friesian crossbred and Deoni cattle raised in India Turkish J. of Vet. and Anim. Sci. 39 128-133 doi: 10.3906/vet-1212-3

[13] Mariana E, Sumantri C, Astuti D A, Anggраeni A and Gunawan A 2020 Association of HSP70 gene with milk yield and milk quality of Friesian Holstein in Indonesia IOP Conf. Ser.: Earth Environ. Sci. 425 012045 doi: 10.1088/1755-1315/425/1/012045

[14] Mkize L S and Zishiri O T 2020 Novel single nucleotide polymorphisms in the heat shock protein 70.1 gene in South African Nguni crossbred cattle Trop. Anim. Health prod. 52 893-901 doi: 10.1007/s11250-019-02088-6

[15] Schwerin M, Sanftleben H and Grupe S 2003 Genetic predisposition for productive life is associated with functional inactivation of a AP2-binding site in the promoter of the stress protein 70.1-encoding gene in cattle Archives Animal Breeding 46(2) 177-185 doi: 10.5194/aab-46-177-2003

[16] Starkey L, Looper M L, Banks A, Reiter S and Rosenkrans Jr C 2007 Identification of polymorphisms in the promoter region of the bovine heat shock protein gene and associations with bull calf weaning weight American Society of Animal Science Southern Section Meeting 85 (2) 42

[17] Habib H N, Hassan A F and Khudaler B Y 2017 Molecular detection of polymorphism of heat shock protein 70 (Hsp70) in the semen of Iraqi Holstein bulls Asian J. Anim. Sci. 11(3) 132-139 doi: 10.3923/ajas.2017.132.139

[18] Huang S Y, Chen, M Y, Lin E C, Tsou H L, Kuo Y H, Ju C C and Lee W C 2002 Effects of single nucleotide polymorphisms in the 5′ flanking region of heat shock protein 70.2 gene on semen quality in boars Anim. Reprod. Sci. 70 99-109 doi: 10.1016/S0378-4320(01)00202-0

[19] River R M and Hansen P J 2001 Evaluation of heat stress on high temperatures in the physiological range Reproduction 121(1) 107-115 doi: 10.1530/rep.0.1210107

[20] Donnik I M, Vafin R R, Galstyan A G, Krivonogova A S, Shaeva A Y, Gilmanov Kh Kh, Karimova R G, Tyulkin S V and Kuźmak J 2018 Genetic identification of bovine leukaemia virus Foods and Raw Materials 6 (2) 314-324 doi: 10.21603/2308-4057-2018-2-314-324

[21] Galstyan A G, Petrov A N, Radaeva I A, Sarukhanyan O O, Kurzanov A N and Storozhuk A P 2016 Scientific bases and technological principles of the production of gerodietetic canned milk Problems of Nutrition [in Russian – Voprosy’ pitaniya] 85(5) 114-119

[22] Petrov A N, Galstyan A G, Radaeva I A, Turovskaya S N, Illarionova E E, Semipyatniy V K, Khurshudyan S A, Dubuske L M and Krikunova L N 2017 Indicators of quality of canned milk: Russian and international priorities Foods and Raw Materials 5(2) 151-161 doi: 10.21603/2308-4057-2017-2-151-161

[23] Turovskaya S N, Galstyan A G, Petrov A N, Radaeva I A, Illarionova E E, Semipyatniy V K and Khurshudyan S A 2018 Safety of canned milk as an integrated criterion of their technology effectiveness. Russian experience Food systems 1(2) 29-54 doi: 10.21323/2618-
9771-2018-1-2-29-54

[24] Tyul’kin S V, Akhmetov T M, Valiullina E F and Vafin R R 2013 Polymorphism of somatotropin, prolactin, leptin, and thyreoglobulin genes in bulls Russ J Genet Appl Res 3 222-224 doi: 10.1134/S2079059713030118

[25] Tyulkin S V, Vafin R R, Zagidullina L R, Akhmetov T M, Petrov A N and Diel F 2018 Technological properties of milk of cows with different genotypes of kappa-casein and beta-lactoglobulin Foods and Raw Materials 6(1) 154-162 doi: 10.21603/2308-4057-2018-1-154-162

[26] Tyulkin S V, Vafin R R, Gilmanov Kh Kh, Rzhanova I V, Galstyan A G, Bigaeva A V, Khurshudyan S A and Nurmukhanbetova D E 2019 DNA markers – a prediction criterion for yield and quality of raw milk News of National Academy of Sciences of the Republic of Kazakhstan 6 (438) 177-183 doi: 10.32014/2019.2518-170x.168

[27] Basirico L, Morera P, Primi V, Lacetera N, Nardone A and Bernabucci U 2011 Cellular thermotolerance is associated with heat shock protein 70.1 genetic polymorphisms in Holstein lactating cows Cell Stress and Chaperones 16 441-448 doi: 10.1007/s12192-011-0257-7

[28] Oner Y, Keskin A, Ustuner H, Soysal D and Karakas V 2017 Genetic diversity of the 3’ and 5’ untranslated regions of the HSP70.1 gene between native Turkish and Holstein Friesian cattle breeds S. Afr. J. Anim. Sci. 47(4) 424-439 doi: 10.4314/sajas.v47i4.2

[29] Deb R, Sajjanar B, Singh U, Kumar S, Brahmane M P, Singh R, Sengar G and Sharma A 2013 Promoter variants at AP2 box region of Hsp70.1 affect thermal stress response and milk production traits in Frieswal cross bred cattle Gene 532(2) 230-235 doi: 10.1016/j.gene.2013.09.037