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## DNA MARKERS – A PREDICTION CRITERION FOR YIELD AND QUALITY OF RAW MILK

**Abstract.** The aim of the study is to assess productive qualities of animals with different milk protein genes genotypes by origin, that is, by pedigree, taking into account the closest ancestors in female line phenotype. The studies were carried out in JSC “Head Breeding Enterprise “Elita” conditions of Vysokogorsky District of the Republic of Tatarstan on bull’s purebred and crossbred Holstein breeds sample. As a result of molecular genetic studies (AS-PCR and PCR-RFLP analysis), animals were divided into groups taking into account genotypes for alpha S1-casein (*CSN1S1*), beta-casein (*CSN2*), kappa-casein (*CSN3*), beta-lactoglobulin (*BLG*) and alpha-lactalbumin (*LALBA*) genes. In studied bulls’ samples, two genotypes *BB*, *BC* were identified by *CSN1S1*-gene; *AA*, *AB* for *CSN2*-gene and three genotypes *AA*, *AB*, *BB* for *CSN3*, *BLG*, *LALBA*-genes, respectively. Milk productivity signs (milk yield and fat mass fraction in milk) of the nearest female servicing bulls’ ancestors with different milk protein genes genotypes were studied. Studies have shown that bulls with *BC* genotypes of *CSN1S1*-gene (10494 kg and 4.05 %), *AA* of *CSN2*-gene (8846 kg and 3.92 %), *AB* (8940 kg) and *BB* (3.95 %) of *CSN3*-gene, *AA* and *AB* of *BLG*-gene (9379-9382 kg and 3.95 %), *AA* of *LALBA*-gene (9405 kg and 3.93 %) compared to other genotypes analogues. It should be noted that servicing bull’s origin information is of exceptional importance, since it cannot be assessed for milk production, and the only criterion for its preliminary breeding qualities assessment is data on nearest female ancestors’ productivity of analyzed bull.

**Key words:** servicing bull, gene, genotype, *CSN1S1*, *CSN2*, *CSN3*, *BLG*, *LALBA*.

**Introduction.** Genetic animals’ pedigree assessment is fundamental to predicting their breeding value. Incorrect animals’ pedigree assessment can lead to significant errors in prediction. At present, with DNA analysis advent, errors in determination of animal’s paternity and genotype are practically excluded, data obtained are used to breeding animals’ assessment by origin [1].

Genetic progress in animal husbandry can only be achieved as a result of combined use of traditional breeding methods and modern DNA technologies. At the same time, assessment of agricultural animals’ productivity genetic potential by molecular genetic markers is a modern, sought-after and rapidly developing direction in breeding [2].

Markers use of milk production quantitative and qualitative indicators in cattle breeding provide more rapid and preferable increase in cattle productivity [3-9].

Presented numerous evidence that presence of certain alleles and milk protein genes genotypes in cows genome, namely alpha S1-casein (*CSN1S1*), beta-casein (*CSN2*), kappa-casein (*CSN3*), beta-lactoglobulin (*BLG*) and alpha-lactalbumin (*LALBA*) genes affect milk yield, mass fraction in milk, milk protein amount, quality and technological properties of their milk [4, 10-19].

Full provision of high-quality and safe dairy raw materials to processing industry enterprises is guarantee of stable dairy products production, such as fermented milk, cheese, canned, functional, gero-dietic, etc. [20-23].

In this regard, the purpose of research was to conduct comparative study of Holstein breeding servicing bulls with different milk protein genes genotypes (*CSN1S1*, *CSN2*, *CSN3*, *BLG*, *LALBA*) by origin under Republic of Tatarstan conditions.

**Material and research methods.** The studies were conducted in JSC "Head Breeding Enterprise "Elita" of Vysokogorsky District of the Republic of Tatarstan. For PCR analysis, 70 blood samples were taken from 70 purebred and crossbred Holstein breeds of servicing bulls with different milk protein genes genotypes. The blood obtained from the jugular vein of animals was added to test tubes with 100 mM EDTA to a final concentration of 10 mM.

DNA was isolated from blood by combined alkaline method: 100 ml of blood was mixed with 1 ml of distilled water and centrifuged at 10 000 rpm for 10 minutes. Supernatant was discarded, and 50 ml of 0.2 M NaOH was added to precipitate and mixture was vortexed thoroughly at room temperature until suspension cleared. The obtained homogenate was kept in a thermostat at 60 °C for 10 min. An equal volume (50 ml) of 1M Tris-HCl (pH 8.0) was added to lysate and mixture was vortexed thoroughly at room temperature. 500 ml of 96 % ethanol was added to obtained homogenate and mixture was kept at -20 °C for 30 minutes. Nucleoprotein complex was precipitated by centrifugation at 12000 rpm for 10 minutes. Supernatant was discarded, and the precipitate was dried at 60 °C for 12 min with an open tube. 100 ml of 10 % ammonia was added to dried precipitate, mixture was vortexed thoroughly at room temperature and kept in a thermostat at 60 °C for 10 minutes, then re-vortexed and incubated in thermostat at 60 °C for 10 minutes. Obtained homogenate was kept in thermostat at 95 °C for 15 min with an open tube.

Animals genotyping by milk protein genes (*CSN1S1*, *CSN2* [24], *CSN3* [25], *BLG* [26], *LALBA* [27]) was performed by PCR-RFLP and AS-PCR methods. In the work, along with experimental materials, data from zootechnical and pedigree registration of this economy, as well as catalogs and pedigree certificates of servicing bulls were used.

Calculated parental index for each bull (PBI) by yield and milk content of their female ancestors according to the formula:

$$PIB = (2M + MM + MF) / 4,$$

where M – mothers, MM – mothers of mothers, MF – mothers of fathers.

Results obtained in course of scientific research are processed by biometric method.

**Results and discussion.** Studies have been carried out and an assessment has been made of purebred and crossbred Holstein breeds of servicing bulls with different genotypes for milk protein genes *CSN1S1*, *CSN2*, *CSN3*, *BLG*, *LALBA* by origin.

Characteristics of purebred and crossbreeds based on Holstein breed of servicing bulls with different *CSN1S1*-gene genotypes by origin are presented in table 1.

Table 1 – Characteristics of servicing bulls with different *CSN1S1*-gene genotypes for milk female ancestors productivity

Indicator		Bulls genotype at locus <i>CSN1S1</i> -gene		
		<i>BB</i>	<i>BC</i>	<i>CC</i>
Number of bulls		64	6	–
Mother	milk yield, kg	8574±219,7	10281±360,1	–
	fat, %	3,88±0,03	3,88±0,06	–
MM	milk yield, kg	7074±265,4	9708±517,6	–
	fat, %	3,87±0,04	3,92±0,14	–
MF	milk yield, kg	10538±381,7	11704±734,1	–
	fat, %	4,01±0,04	4,53±0,28	–
Parental bull index (PBI)	milk yield, kg	8690±217,3	10494±192,2	–
	fat, %	3,91±0,02	4,05±0,10	–
***P<0,001.				

Analysis of table 1 shows that bulls mothers with *BB* genotype of *CSN1S1*-gene (10281 kg) had the highest milk yield, which is higher than that of bulls with *BC* genotype of 1707 kg ( $P<0.001$ ). At the same time, bulls' mothers with *BB* and *BC* genotypes did not differ in fat mass fraction in milk.

Higher milk yield and fat content in milk were higher for mothers of mothers (MM) and mothers of fathers (MF) of bulls with *CSN1S1*-gene *BC* genotype (9708 kg and 11704 kg; 3.92 % and 4.53 %), which is higher than for analogues with *BB* genotype, respectively, by 2634 kg ( $P<0.001$ ) and 1166 kg; 0.05 % and 0.52 %.

Characteristics of purebred and crossbred according to Holstein breed of servicing bulls with different *CSN2*-gene genotypes by origin are presented in table 2.

Table 2 – Characteristics of servicing bulls with different *CSN2*-gene genotypes for milk female ancestors productivity

Indicator		Bulls genotype at locus <i>CSN2</i> -gene		
		<i>AA</i>	<i>AB</i>	<i>BB</i>
Number of bulls		61	9	–
Mother	milk yield, kg	8752±225,5	8363±654,2	–
	fat, %	3,89±0,03	3,80±0,04	–
MM	milk yield, kg	7201±262,5	8104±1226,4	–
	fat, %	3,89±0,05	3,74±0,04	–
MF	milk yield, kg	10678±394,9	10230±227,8	–
	fat, %	4,04±0,05	4,13±0,18	–
Parental bull index (PBI)	milk yield, kg	8846±225,8	8765±570,6	–
	fat, %	3,92±0,03	3,87±0,05	–
* $P<0,05$ .				

Analysis of table 2 shows that mothers of bulls with *CSN2*-gene of *AA* genotype (8752 kg and 3.89 %) had the highest indicators for milk yield and fat content in milk, which were higher than for bulls' mothers with *AB* genotype by 389 kg and 0.09 %, respectively.

Higher milk yield was characteristic for mothers of mothers (MM) of bulls with *AB* genotype (8104 kg), whereas mothers of bulls with *AA* genotype (3.89 %) were allocated for fat mass fraction in milk. At the same time, difference between mothers of mothers with *AA* and *AB* genotypes of *CSN2*-gene in terms of fat mass fraction was 0.15% ( $P<0.05$ ).

Higher milk yield is characteristic for mothers of fathers (MF) of bulls with *AA* genotype (10678 kg), and higher fat content in milk differed in analogues with *AB* genotype (4.13 %). At the same time, difference in these indicators was 448 kg and 0.09 %.

Characteristics of purebred and crossbred according to Holstein breed of servicing bulls with different genotypes of *CSN3*-gene by origin are presented in table 3.

Table 3 – Characteristics of servicing bulls with different *CSN3*-gene genotypes for milk female ancestors productivity

Indicator		Bulls genotype at locus <i>CSN3</i> -gene		
		<i>AA</i>	<i>AB</i>	<i>BB</i>
Number of bulls		48	18	4
Mother	milk yield, kg	8715±263,8	8665±413,2	8919±541,0
	fat, %	3,89±0,03	3,81±0,03	3,92±0,17
MM	milk yield, kg	7170±306,3***	7964±592,0***	5906±126,2
	fat, %	3,88±0,05	3,83±0,08	4,03±0,18
MF	milk yield, kg	10718±426,7	10464±812,0	10191±73,3
	fat, %	4,07±0,06*	4,01±0,09	3,93±0,01
Parental bull index (PBI)	milk yield, kg	8830±252,8	8940±464,4	8484±296,6
	fat, %	3,93±0,03	3,87±0,04	3,95±0,11
* $P<0,05$ , *** $P<0,001$ .				

Analysis of table 3 shows that mothers of bulls with *BB* genotype of *CSN3*-gene (8919 kg and 3.92 %) had the highest rates of milk yield and fat content in milk, which were higher than those of bulls with *AA* and *AB* genotypes by 204 -254 kg and 0.03-0.11 %, respectively.

Higher milk yield was typical for mothers of mothers (MM) of bulls with *AA* and *AB* genotypes (7170 kg and 7964 kg), whereas mothers of bulls with *BB* genotype (4.03%) were allocated by fat mass fraction in milk. At the same time, superiority of bulls' mothers with *AA* and *AB* genotypes of *CSN3*-gene over their analogues with *BB* genotype yields amounted to 1264–2058 kg ( $P < 0.001$ ).

A higher yield is characteristic for mothers of fathers (MF) of bulls with *BB* genotype (10191 kg), and higher fat fractions in milk differed in analogues with *AA* genotype (4.07 %). At the same time, difference in these indicators with peers with other genotypes was 273-527 kg and 0.06-0.14 % (differences between *AA* and *BB* genotypes in terms of fat mass fraction were significant, with  $P < 0.05$ ).

Characteristics of purebred and crossbred according to Holstein breed of servicing bulls with different genotypes of *BLG*-gene by origin are presented in table 4.

Table 4 – Characteristics of servicing bulls with different *BLG*-gene genotypes for milk female ancestors productivity

Indicator		Bulls genotype at locus <i>BLG</i> -gene		
		<i>AA</i>	<i>AB</i>	<i>BB</i>
Number of bulls		9	26	35
Mother	milk yield, kg	8956±449,7	9402±373,2**	8163±271,9
	fat, %	3,92±0,06	3,89±0,04	3,86±0,04
MM	milk yield, kg	8279±889,3	7466±435,1	6910±343,0
	fat, %	3,90±0,06	3,90±0,08	3,85±0,06
MF	milk yield, kg	11338±1080,5	11247±737,1	10017±367,1
	fat, %	4,07±0,14	4,11±0,09	4,00±0,06
Parental bull index (PBI)	milk yield, kg	9382±606,1	9379±403,9*	8313±226,9
	fat, %	3,95±0,06	3,95±0,05	3,89±0,03
* $P < 0,05$ , ** $P < 0,01$ .				

Analysis of table 4 shows that bulls mothers with *AB* genotype of *BLG*-gene (9402 kg) had the highest milk yield indicators, while bulls mother with *AA* genotype (3.92 %) differed in fat mass fraction in milk, which was higher than in bulls mothers with other genotypes by 446 kg and 1239 kg ( $P < 0.05$ ) and 0.03-0.06 %, respectively.

Higher milk yield was characteristic for mothers of mothers (MM) of bulls with *AA* genotype (8279 kg), whereas mothers of bulls with *AA* and *AB* genotypes (3.90 %) were distinguished by fat mass fraction in milk. Difference between mothers of mothers with other genotypes of *BLG*-gene was 813-1369 kg and 0.05 %, respectively.

Table 5 – Characteristics of servicing bulls with different *LALBA*-gene genotypes for milk female ancestors productivity

Indicator		Bulls genotype at locus <i>LALBA</i> -gene		
		<i>AA</i>	<i>AB</i>	<i>BB</i>
Number of bulls		23	35	12
Mother	milk yield, kg	8875±407,5	8605±308,1	8776±430,5
	fat, %	3,87±0,05	3,88±0,04	3,88±0,05
MM	milk yield, kg	8208±515,7	7219±353,7	6179±464,0**
	fat, %	3,87±0,09	3,86±0,06	3,91±0,05
MF	milk yield, kg	11660±803,4	10483±496,8	9583±319,0*
	fat, %	4,10±0,10	4,03±0,07	4,03±0,09
Parental bull index (PBI)	milk yield, kg	9405±459,3	8728±291,5	8328±282,3*
	fat, %	3,93±0,04	3,91±0,04	3,93±0,03
* $P < 0,05$ , ** $P < 0,01$ .				

Higher milk yield is characteristic for mothers of fathers (MF) of bulls with *AA* genotype (10338 kg), and higher fat content in milk differed in analogues with *AB* genotype (4.11 %). At the same time, the difference in these indicators was 91-1321 kg and 0.04-0.11 %, respectively.

Characteristics of purebred and crossbreeds according to Holstein breed of servicing bulls with different genotypes of *LALBA*-gene by origin are presented in table 5.

Analysis of table 5 shows that mothers of bulls with *AA* genotype of *LALBA*-gene (8875 kg) had the highest indicators of milk yield, which is higher than that of bulls' mothers with *AB* and *BB* genotypes of 99-270 kg. At the same time, bulls' mothers with different genotypes of *LALBA*-gene did not differ much in fat mass fraction in milk.

Mothers of mothers (MM) and mothers of fathers (MF) bulls with *AA* genotype of *LALBA*-gene 8208 kg and 11660 kg had higher rates for milk yield, which is higher than for analogues with *AB* and *BB* genotypes by 989-1177 kg and 2029-2077 kg, respectively ( $P < 0.05-0.01$ ). In terms of fat mass fraction in milk, the matter of mothers of bull mothers and mothers of bull fathers with *LALBA*-gene genotypes, respectively *BB* and *AA*, was superior. At the same time, the superiority in this indicator over animals with other genotypes was 0.04-0.05 % (for mothers of bull mothers) and 0.07 % (for mothers of bull fathers).

**Conclusion.** Bulls assessment on parent index revealed that bulls used with different genotypes of milk protein genes are not equivalent in origin. For example, an assessment of servicing bulls with different milk protein genes genotypes by origin showed that greatest data on PBI (parent bull index) were in milk yield and fat in bulls with *BC* genotypes of *CSN1S1*-gene (10494 kg and 4.05 %), *AA* of *CSN2*-gene (8846 kg and 3.92 %), *AB* (8940 kg) and *BB* (3.95 %) of the *CSN3*-gene, *AA* and *AB* of the *BLG*-gene (9379-9382 kg and 3.95 %), *AA* of the *LALBA*-gene (9405 kg and 3.93 %) compared with analogues of other genotypes.

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#### ДНҚ-МАРКЕРЫ – СҮТ ШИКІЗАТЫНЫҢ ШЫҒЫМЫ МЕН САПАСЫНЫҢ БОЛЖАМДЫ ӨЛШЕМІ

**Аннотация.** Зерттеудің мақсаты – әртүрлі генотипті сүт ақуыз гендері бар жануарлардың шығу тегіне, яғни асыл тұқымды, аналық ұрпақтарға жақын ата-бабаларының фенотипін ескере отырып бағалау. Зерттеу Татарстан Республикасы Высокогорский ауданының «Элита» асыл тұқымды мал өсіру кәсіпорны АҚ жағдайында, жергілікті голштин және таңдалған асыл тұқымды бұқаларға жүргізілді. Молекулалық-генетикалық зерттеулер нәтижесінде (АС-ПЦР және ПЦР-ПДРФ талдауы) жануарларды гендерінің генотиптерін ескере отырып мынадай топтарға бөлді: альфа S1-казеин (*CSN1S1*), бета-казеин (*CSN2*), каппа-казеин (*CSN3*), бета лактоглобулин (*BLG*) және альфа-лакталбумин (*LALBA*). Бұқалардың зерттелген үлгісінде *CSN1S1* геніне арналған *BB*, *BC* екі генотиптері; *CSN2* геніне арналған *AA*, *AB* және *CSN3*, *BLG*, *LALBA* гендері үшін үш *AA*, *AB*, *BB* генотиптері анықталды. Сүт ақуыз генінің әртүрлі генотиптері бар өндіруші бұқалардың ең жақын аналық ата-бабаларының сүт өнімділігінің белгілері (сүт өнімділігі және сүттегі майдың үлес салмағы) зерттелді. Зерттеулер көрсеткендей, *CSN1S1* генінің *BC* генотиптері бар бұқалар (10494 кг және 4,05%), *CSN2* гені *AA* (8846 кг және 3,92%), *AB* (8940 кг), *CSN3* генінің *BB* (3,95%), *BLG* генінің *AA* және *AB* (9379-9382 кг және 3,95%), *LALBA* генінің *AA* (9405 кг және 3,93%) бұқаның асыл тұқымдық индексі бойынша басқа генотиптердің аналогтарымен салыстырғанда жоғары бағаланды. Айта кету керек, өндіруші бұқаның шығу тегі туралы ақпарат өте маңызды, өйткені оны сүт өнімділігімен бағалау мүмкін емес және оның асыл тұқымдылығын алдын-ала бағалаудың жалғыз критерийі - талданатын бұқаның ең жақын аналық ата-бабаларының өнімділігі туралы мәліметтер.

**Түйін сөздер:** бұқа- өндіруші, ген, генотип, *CSN1S1*, *CSN2*, *CSN3*, *BLG*, *LALBA*.

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## **ДНК-МАРКЕРЫ – ПРОГНОЗНЫЙ КРИТЕРИЙ ВЫХОДА И КАЧЕСТВА МОЛОЧНОГО СЫРЬЯ**

**Аннотация.** Цель исследований заключается в оценке продуктивных качеств животных с разными генотипами генов белков молока по происхождению, то есть по родословной, с учётом фенотипа ближайших предков по женской линии. Исследования проводились в условиях АО «Головное племенное предприятие «Элита» Высокогорского района Республики Татарстан на выборке чистопородных и помесных по голштинской породе племенных быков. В результате молекулярно-генетических исследований (АС-ПЦР и ПЦР-ПДРФ-анализ) животных разделили на группы с учётом генотипов по генам альфа S1-казеина (*CSN1S1*), бета-казеина (*CSN2*), каппа-казеина (*CSN3*), бета-лактоглобулина (*BLG*) и альфа-лактальбумина (*LALBA*). В исследуемой выборке быков выявлены два генотипа *BB*, *BC* по гену *CSN1S1*; *AA*, *AB* по гену *CSN2* и три генотипа *AA*, *AB*, *BB* по генам *CSN3*, *BLG*, *LALBA*, соответственно. Изучены признаки молочной продуктивности (удой и массовая доля жира в молоке) ближайших женских предков быков-производителей с разными генотипами генов белков молока. Исследования показали, что более высокую оценку по происхождению, судя по родословному индексу быка, имели быки с генотипами *BC* гена *CSN1S1* (10494 кг и 4,05 %), *AA* гена *CSN2* (8846 кг и 3,92 %), *AB* (8940 кг) и *BB* (3,95 %) гена *CSN3*, *AA* и *AB* гена *BLG* (9379-9382 кг и 3,95%), *AA* гена *LALBA* (9405 кг и 3,93 %) по сравнению с аналогами других генотипов. Следует отметить, что информация о происхождении быка-производителя имеет исключительное значение, так как он сам не может быть оценён по молочной продуктивности, и единственным критерием его предварительной оценки племенных качеств являются данные продуктивности ближайших женских предков анализируемого быка.

**Ключевые слова:** бык-производитель, ген, генотип, *CSN1S1*, *CSN2*, *CSN3*, *BLG*, *LALBA*.

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