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EXPRESSION OF CANDIDATE GENES OF PROTEIN METABOLISM IN SIMMENTALS OF KAZAKHSTANI SELECTION

This research work describes the polymorphism of kappa-casein (CSN3), beta-lactoglobulin (LGB) and prolactin (bPRL) in cows of Simmental breed at farm «Galitskoye» in Pavlodar region. Frequency distribution of alleles and genotypes of the studied genes were conducted. It is found that the occurrence frequency of A and B alleles according to kappa-casein (CSN3) gene was 0,51 and 0,49, according to beta-lactoglobulin (LGB) had 0,36 and 0,64, prolactin (bPRL) reached 0,68 and 0,32. Results of the study about genes' influence on milk production showed that milk yield per lactation of cows in the group of animals with kappa-casein genotype was AA – 5356 kg, AB – 5387 kg, BB – 5517 kg. The study noted the superiority of AA alleles of beta-lactoglobulin genotypes and prolactin on the studied indices, the advantage in milk yield, protein content and milk fat according to kappa-casein genotype was observed in cows with BB genotype. Research results stated that cows with BB genotype according to kappa-casein and AA alleles were more productive and had the highest yield of milk protein according to beta-lactoglobulin and prolactin.

Keywords: Simmental breed, milk production, gene expression, DNA – polymorphism, candidate genes, kappa-casein gene (CSN3), beta-lactoglobulin (LGB), prolactin (bPRL).

Introduction

Currently in Kazakhstan the processing industry requirements for milk procurement quality and its suitability for processing has increased. There was

a necessity of modern selection methods based on the use of genetic markers to maintain the competitiveness and to improve technological properties of milk.

The use of DNA-markers in breeding provides the possibility of determining their genetic potential. Animals' genotyping allows to conduct selection purposefully on identifying and fixing in a population of valuable genotypes associated with milk quality.

Polymorphic genes of milk proteins of beta-lactoglobulin and kappa-casein are directly related to milk quality, its technological properties and suitability for protein containing products development [1].

The identification of preferred variants of kappa-casein and beta-lactoglobulin allows to carry out selection directly on genotype in addition to the traditional selection of animals for fat content in milk and the level of milk yield. The advantage of DNA technology is that it is possible to determine the genotype of the animal by beta-lactoglobulin and kappa-casein genes, which is an important factor in accelerating breeding process.

The livestock, bred in Kazakhstan differs in genetic characteristics. It is necessary to determine the genetic value of the animal in order to use it effectively. The identification of preferred variants of genes associated with animals productive traits is relevant because it provides an opportunity to conduct a selection at the DNA level along with the traditional selection methods.

As potential and promising markers of milk productivity, the alleles of milk protein genes and hormones involved in the regulation of lactation are primarily considered [2].

Kappa-casein (CSN3) is one of the few genes that provides optimal technological properties of milk in cheese production, that's why the gene is considered as one of the key markers of breeding value of cattle. Kappa casein gene (CSN3) is situated on the 6th chromosome in representatives of *Bos taurus* L. species. Allelic variants A and B are the most common ones of the two alleles of the gene described. The milk of cows with BB genotype has a higher protein content and by the action of rennet it coagulates earlier than milk of cows with AA genotype.

Beta-lactoglobulin (LGB) represents a very valuable component of milk, necessary for the growth of young animals, therefore, is the main protein of whey and located on 11th chromosome. Beta-lactoglobulin is responsible for high protein content in milk and the rate of biological value of milk. LGB^B variant is associated with a high content of casein in milk, high fat percentage. LGB^A variant is characterized by a high content of whey protein. The variant of B beta-lactoglobulin is the main one, as it is widespread in most species.

Prolactin (bPRL) – one of the most versatile hormones of the pituitary gland in terms of its biological activity. It is involved in the differentiation of mammary epithelial cells, initiation and maintenance of lactation, regulation of synthesis of milk proteins and fat. Prolactin is a potential genetic marker of milk production traits in cattle and located on the 23rd chromosome. Prolactin has a direct effect on lactogenic function, the volume and quality of produced milk.

Marking signs of milk production according to several DNA markers is considered to be more effective, however, the studies of dairy cattle on a set of kappa-casein, beta-lactoglobulin, prolactin genes in the north-east of Kazakhstan have not been conducted.

In this regard, the aim of our research was to determine the gene polymorphism of kappa-casein, beta-lactoglobulin and prolactin, in the study of relationship of genotypes of kappa-casein, beta-lactoglobulin and prolactin with milk productivity and technological properties of milk in cows of Simmental breed at farm «Galitskoye» in Pavlodar region.

Materials and research methods

Work on allocation of gene polymorphism was performed in 2015 in a certified laboratory of DNA technology «Biotechnology of animals» on the basis of Pavlodar State University named after S.Toraigrov. The laboratory is certified by the National Center of Expertise and Certification, certificate number 370.

Research object was purebred cows of Simmental breed. The studies to identify the relationship of genotypes with milk productivity were carried out at farm «Galitskoye» in Pavlodar region.

While conducting the experiments the following indicators were studied: milk yield, protein content in milk, milk protein yield for 305 days.

The blood samples were selected for carrying out DNA diagnosis in animals in the amount of 123 cows. Blood was obtained from animals' jugular vein, was put into tubes containing 100 mM EDTA to a final concentration of 10 mM.

DNA was isolated from animals' blood with the use of «DNA-sorb-B» kit of reagents for DNA extraction from clinical specimens (LLC «InterLabService», Russia).

Evaluation of polymorphism of kappa casein gene.

The following primers (Kirilenko S.D., 1995) were used to amplify a fragment of exon IV kappa-casein gene:

BOSAS A: - 5' ATG TGC TGA GCA GGT ATC CTA GTT ATG G - 3'

BOSAS B: - 5' CCA AAA GTA GAG TGC AAC AAC ACT GG - 3'

PCR was carried out on a programmable thermal cycler «Tertsik» (Russia) in a reaction volume of 25 mcl containing 60 mM Tris-HCl (pH 8.5), 1.5 mM MgCl₂,

25 mM KCl, 10 mM меркаптоэталол; 0.1 mM Triton X-100; 0.2 mM dNTP, 1 unit of Taq DNA polymerase, 0.5 mM of each primer BOSAS.

Amplification was carried out as follows: 94 °C – 1 min – denaturation, 62 °C – 1 min – annealing of primers, 72 °C – 1.5 min – synthesis (total 35 cycles); storage – 4 °C.

For RFLP identification of genotypes of kappa-casein gene 20 µl of PCR sample (883 pairs of nucleotide) was treated with 10 units of PstI endonuclease in 1 × buffer «O» (made by «SibEnzyme» company, Russia) at 37 °C overnight.

To visualize DNA fragments the samples were put into sample wells of 2.5 % agarose gel containing ethidium bromide (0.5 µg / ml) and horizontal electrophoresis was performed at 15 V / cm for 50 min in 1 × TBE buffer.

After using electrophoresis, the gel is viewed in UV transilluminator. The presence of four fragments of length 106, 306, 471, 777 pairs of nucleotide corresponded to genotype k-Cn^{AB}, two fragments of 106 and 777 pairs of nucleotide – to genotype k-Cn^{BB}, three fragments of length 106, 306 and 471 pairs of nucleotide corresponded to genotype k-Cn^{AA}.

Evaluation of beta-lactoglobulin gene polymorphism. PCR was carried out on a programmable thermal cycler «Tertsik» (Russia) in a volume of 20 µl containing buffer (60 mM Tris-HCl (pH 8.5), 1.5 mM MgCl₂, 25 mM KCl, 10 mM меркаптоэталол; 0.1 mM Triton X-100), 0.2 mM dNTP, 0.2 µl of Taq DNA polymerase, 0.5 mM of primer BLGP3: 5' – GTC CTT GTG CTG GAC ACC GAC TAC A – 3', 0.5 mM of primer BLGP4: 5' – CAG GAC ACC GGC TCC CGG TAT ATG A – 3' to amplify a fragment of beta-lactoglobulin gene at length of 262 pairs of nucleotide, 1 µl of DNA sample in the following way: ×1:94 °C – 4 min; ×38:94 °C – 10 sec, 60 °C – 10 sec, 72 °C – 10 sec; ×1:72 °C – 5 min; storage: 4 °C.

To determine the beta-lactoglobulin gene polymorphism according to variants A and B 20 µl of PCR sample was treated with 5 units of Hae III restriction endonuclease in 1 × buffer «C» (made by «SibEnzyme» company, Russia) at 37 °C overnight.

To visualize DNA fragments the samples were put into sample wells of 2.5 % agarose gel containing ethidium bromide (0.5 µg / ml) and horizontal electrophoresis was performed at 15 V / cm for 40 min in 1 × TBE buffer.

After using electrophoresis, the gel is viewed in UV transilluminator at 310 nm wavelength. The identification of genotypes was determined by quantitative and qualitative characteristics of PCR RFLP.

PCR-RFLP analysis of the cattle on BLG gene variants showed that the presence of the four fragments 153/109/79/74 pairs of nucleotide corresponded

to genotype AB, three fragments 109/79/74 – to genotype BB, the two fragments at length of 153/109 pairs of nucleotide corresponded to AA genotype.

Evaluation of prolactin gene polymorphism. Amplification of exon III fragment of prolactin gene was performed by PCR-RFLP method with the following primers (MitraA. et al., 1995):

PRL 1 5'-CGA GTC CTT ATG AGC TTG ATT CTT-3'

PRL 2 5'-GCCTTCCAGAAG TCGTTTGTTTTTC-3'.

To conduct PCR reaction 5 mcl of genomic DNA in a volume of 25 mcl was taken, 2.5 mcl of 10 x PCR buffer (67 mM Tris-HCl, pH 8.8; 16,6 mM (NH₄)₂SO₄, 0,1 % of Tween-20), 2,5 mM MgCl₂, 2,0 mcl of dNTP, 0,5 mcM of each primer, 1.0 unit of thermo stable Taq DNA polymerase.

PCR was carried out on a programmable thermal cyclers «Tertsik» (Russia) in a reaction volume of 25 microliters.

Amplification of DNA fragments of prolactin gene was carried out as follows: 95 °C – 30 sec. – denaturation, 59 °C – 30 sec. – primer annealing 59 °C – 30 sec. – synthesis (total 35 cycles); the final synthesis – 72 °C – 10 minutes; storage – 4 °C.

Prepared by amplifying prolactin gene fragment was incubated at 37 °C with the restriction enzyme RsaI overnight.

The length of obtained restriction fragments was determined after using the gel electrophoresis in 4% agarose gel and UV transilluminator.

PCR-RFLP analysis of prolactin gene variants showed that the presence of a non-restriction fragment of 156 pairs of nucleotide corresponded to genotype PRL^{AA}, two fragments of 82 and 74 pairs of nucleotide to genotype PRL^{BB}, three fragments of 156, 82, 74 of nucleotide corresponded to PRL^{AB} genotype.

The frequency of appearance of kappa-casein, beta-lactoglobulin, prolactin genotypes was determined by the formula:

$p = n/N$, p – frequency of genotyping, n – number of animals, having a certain genotype, N – number of animals.

Statistical calculations were performed with the help of «Pastprogram» computer program.

Results and discussion

According to the research of genotypes of cows at farm «Galitskoye» for the locus of kappa-casein, beta-lactoglobulin, prolactin genes, we obtained the following data. The frequency of alleles A and B from genes of 123 cows on kappa casein gene (CSN3) 0,51 and 0,49, on beta-lactoglobulin (LGB) 0,36 and 0,64, prolactin gene (bPRL) 0,68 and 0,32 (see table 1).

Table 1 – Frequency of alleles and genetic structure of Kazakhstani Simmentals according to candidate genes of protein metabolism

Gene	Quantity of animals	Genotype	Frequency of genotypes, %	Allele	Frequency of alleles	Ho	X ²
(LGB)	14	AA	12,28	A	0,36	15,104	0,19
	55	AB	48,25			52,78	
	45	BB	39,47	B	0,64	46,11	
(bPRL)	32	AA	41,55	A	0,68	35,08	2,63
	40	AB	51,96			33,67	
	5	BB	6,49	B	0,32	8,08	
CSN3	28	AA	27,2	A	0,51	26,2	0,06
	50	AB	48,5			49,5	
	25	BB	24,3	B	0,49	24,3	

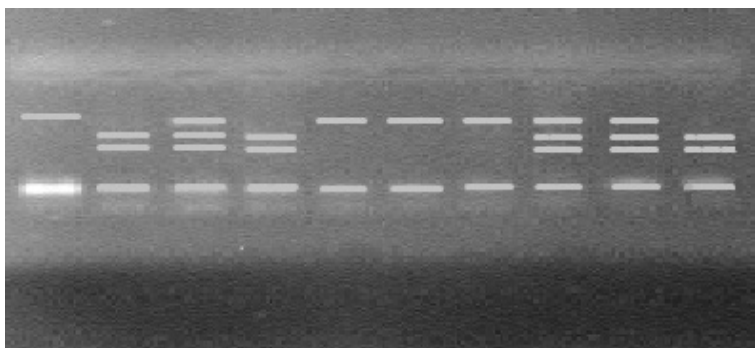


Figure 1 – The kappa-casein gene. Four fragments of 106, 306, 471, 777 pairs of nucleotide correspond to the genotype k-Cn^{AB}, two fragments of 106 and 777 pairs of nucleotide correspond to the genotype k-Cn^{BB}, three fragments of length 106, 306 and 471 pairs of nucleotide correspond to the genotype k-Cn^{AA}

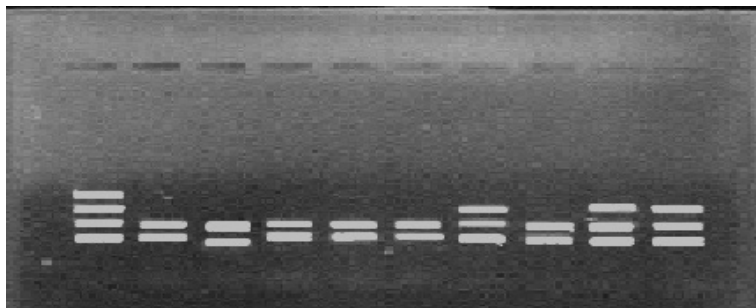


Figure 2 – Beta lactoglobulin gene. Four fragments with the length of 153/109/79/74 pairs of nucleotide correspond to the genotype AB , three fragments of 109/79/74 to genotype BB , two fragments of 153/109 pairs of nucleotide correspond to the genotype AA

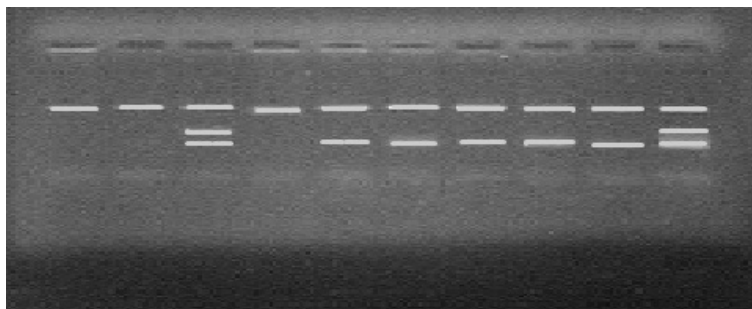


Figure 3 – Prolactin gene. Non-digested fragment of 156 pairs of nucleotide correspond to the genotype PRL^{AA} , two fragments of 82 and 74 pairs of nucleotide to the genotype PRL^{BB} , three fragments with the length of 156, 82, 74 pairs of nucleotide correspond to the genotype PRL^{AB}

According to kappa-casein gene (CSN3) the frequency of homozygous genotype AA was 27,2 %, the heterozygous genotype AB – 48,5 %, homozygous genotype BB – 24,3 %.

According to beta lactoglobulin gene (LGB) with AA genotype there was 12,28 %, AB genotype was detected in 48,25%, and BB genotype was found in 39,47 %.

According to prolactin gene (bPRL) 41,55 % of cows were assigned to AA genotype, 51,96 % to AB genotype and 6,49 % to BB genotype.

The studies showed a significant prevalence of animals with heterozygous genotype AB (CSN3 CSN3 – 48,5 %, LGB – 48,25 %, bPRL – 51,96 %). Genotype AA is less desirable in milk production and was detected in cows according to genes CSN3 – 27,2 %, LGB – 12,28 %, bPRL – 41,55 %. The frequency of BB genotype was average for the genes CSN3 – 24,3 %, LGB – 39,47 %. bPRL gene showed a low level and amounted to 6,49 %.

Table 2 provides the data on the study of genotypes polymorphism at phenotypic expression of milk productivity traits of Simmentals of Kazakhstani selection.

Table 2 – Effect of candidate genes of protein metabolism on milk productivity of cows within 305 lactation days.

Studied gene	Genotype	n	Yield for 305 days, kg			Protein, %			Milk protein, kg
			max	min	M±m	max	min	M±m	
Kappa casein (CSN3)	AA	28	8598	3522	5356,7±219,65	3,8	2,8	3,20±0,03	171,4
	AB	50	8292	3346	5387,8±248,32	3,8	2,5	3,25±0,05	175,1
	BB	25	7452	3731	5517,1±256,17	4,8	2,6	3,26±0,06	177,6
Beta-lactoglobulin (LGB)	AA	14	9032	3558	5571,2±461,71	3,8	2,5	3,24±0,03	180,5
	AB	57	9056	3377	5316,6±155,98	3,9	2,5	3,20±0,05	170,1
	BB	44	8598	2938	5357,2±213,89	4,8	2,6	3,26±0,03	174,6
Prolactin (bPRL)	AA	32	8017	3550	5741,1±282,60	4,4	2,5	3,23±0,08	185,4
	AB	40	9032	3524	5457,6±204,18	4,2	2,6	3,22±0,02	175,7
	BB	5	6755	3089	4743,6±97,36	3,5	2,8	3,16±0,06	149,9

The average milk yield per lactation in the group of animals amounted with AA kappa-casein genotype – 5356 kg, AB – 5387 kg, BB – 5517 kg. The milk yield of cows with BB kappa-casein genotype exceeded up to 130–161 kg of milk in comparison with cows of the same age. According to protein content in milk from cows with homozygous BB kappa-casein genotype insignificantly exceeded to 0.01–0.06 % from animals of another group. On average, 177.6 kg of milk protein was obtained per lactation, which is 2,5–6,2 kg more than from cows with AB and AA genotype. The study of genotype expression according to milk yield showed that wider variability was observed in AA (8598 kg–3522 kg), whereas according to high content of protein in milk with BB genotype (4,8 %–2,6 %).

The highest milk yield from cows with beta-lactoglobulin genotype was observed in AA genotype – 5571.2 kg. Their milk yield exceeded the milk yield of cows with AB genotype in 254.6 kg and BB genotype – 214 kg of milk. An advantage was observed in cows with BB genotype according to protein content in milk – 3.26 %. The highest yield of milk protein was obtained from cows with AA genotype – 180.5 kg, in comparison with AB and BB genotype the studied

index was higher up to 10.4 kg and 5.9 kg. The expression on the studied gene showed that wider variability according to milk yield was observed in cows with AB genotype (9056 кг–3377 kg) and according to high content of protein in milk in cows with BB genotype (4,8 %–2,6 %).

According to prolactin gene expression research the greatest quantity of milk was obtained from cows with AA genotype – 5741.1 kg of milk. Cows with AA genotype kept the advantage on milk yield, output of milk fat and protein. The lag of cows with BB genotype on yield and milk protein yield in comparison with cows of AA and AB genotypes was noted. The maximum yield and protein was obtained from cows with AA genotype (9032 kg–4.4 %), the minimum result of yield was shown in cows with BB genotype (3089 kg) and the minimum result of protein in cows with AA genotype (2.5 %).

Thus, according to our gene expression studies was found that cows with BB genotype on kappa casein and AA were more productive and had the highest quantity of milk protein on beta-lactoglobulin and prolactin.

The development of animal husbandry at the present stage is impossible without introduction of new biotechnological methods for assessing animal productivity features based directly on the analysis of genetic information. In this context, the development and implementation of DNA diagnostics is an important task.

It is known, the level of productivity of animals is caused by both genetic and environmental factors. The majority of economically useful features of farm animals related to polygenic features, i.e. their level is determined by several loci scattered throughout the whole genome [3].

Many scientific studies [3, 4] are aimed at identifying genes associated with economically useful features of animals, representing economic interest. However, traditional animal breeding is often used in practice, which is based on the phenotypic feature manifestation; in this regard, the assessment of true genetic potential of animals may be underestimated. Therefore, the use of DNA technology is required to improve accuracy in animals' potential assessment and selection effectiveness regardless of sex or age.

According to L. V. Getmantseva the gene expression is ultimately manifested by phenotypic line of quantitative and qualitative characteristics. This path is controlled by the operation of complex, heterogeneous mechanisms. The problem of multiple variants of genotype relationship is that the phenotype remains relevant for molecular - genetic analysis used in animal breeding. This approach contributes to understanding genotype relationship with phenotypic manifestations of monogenic mutations and to determine the genetic basis of economically useful features of animals, characterized by polygenic type of inheritance [5].

In J. Dekkers studies has been shown that genetically determined variability of features varies considerably from one to another, as well as for the majority of phenotypic features included in the analysis more than 50 % of genetic variation fall within genomic regions with small phenotypic effects, the order of magnitude of which corresponds to the polygenic nature of inheritance [6].

Genes polymorphism associated with milk productivity parameters allows selecting animals, taking into account valuable genotypes in relation to economically useful features.

J. Domagala, M. Sady, T. Grega, D. Najgebauer-Lejko proved close relationship between the polymorphism of milk proteins and its technological properties [7].

The range of candidate genes associated with milk productivity features established for cattle include genes of main milk proteins, genes of hormones stimulating expression, as well as genes that regulate the lipids and proteins exchange in the body. Among them a special place is occupied by the genes of kappa-casein, beta-lactoglobulin and prolactin.

The study of kappa-casein gene polymorphism in Simmentals at farm «Galitskoye» found that appearance frequency of allele B was relatively high and was detected in 48 % of cows. Analysis of DNA testing results on kappa-casein gene locus has shown that the highest frequency of desired homozygous BB genotype was detected in 25 % of Simmental cows, while according to other researchers this figure varies between 12–20 % in other breeds and populations.

According to A.V. Perchun this figure is counted in cows of black-and-white breed (12 %) and red steppe breed (20 %). Our results taken from Simmentals has exceeded the figure of cows of black-and-white and red steppe breeds by 13 % and 5 % [8].

The results of studies of beta-lactoglobulin genotype indicate higher production rates in cows with genotypes AA and BB. The highest quantity of milk and milk protein was obtained from cows with AA genotype. The cows with BB genotype have higher protein content in milk.

While studying the prolactin genotype was found that the highest rates of milk yield had cows with AA genotype, similar results were also obtained in studies of G.M. Japaridze, according to this data Holstein cows of Canadian selection with AA genotype had a high milk yield, had high protein content in milk [9].

I. V. Lazebnaya [10] notes that AA genotype is less desirable in milk production, in our studies AA genotype was detected in a small number of cows according to kappa-casein at 27 %, beta lactoglobulin at 12 % and prolactin levels in 41 % of Simmental cows at the studied farm.

Conclusions

The results of these studies suggest the influence of candidate genes of protein metabolism of kappa-casein, beta-lactoglobulin and prolactin on milk productivity and technological quality of milk of Simmentals at farm «Galitskoye».

On the basis of the conducted researches we have found that when breeding and developing animals of the Simmental breed with the aim of improving the level and quality of milk received from them at Kazakhstani farms, it is useful to consider the obtained results as an additional criterion in conducting selection and breeding work with the use of DNA markers at selection.

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ҚАЗАҚСТАНДЫҚ СЕЛЕКЦИЯ СИММЕНТАЛДАРЫНДАҒЫ АҚУЫЗ АЛМАСУЫНА КАНДИДАТ ГЕНДЕРДІҢ ЭКСПРЕССИЯСЫ

Бұл зерттеу жұмысында Павлодар облысындағы «Галицкое» ЖШС-дағы симментал тұқымды сиырларындағы каппа-казеин (CSN3), бета-лактоглобулин (LGB) және пролактин (bPRL) полиморфизмі сипатталған. Зерттелетін гендердің аллельдері мен генотиптерінің жиілік таралуы жүргізілді. Каппа-казеин гені (CSN3) бойынша А және В аллельдерінің пайда болу жиілігі 0,51 және 0,49, бета-лактоглобулин (LGB) бойынша 0,36 және 0,64, пролактин (bPRL) бойынша 0,68 және 0,32 болғаны анықталды. Гендердің сүт өнімділігіне әсерін зерттеу нәтижелері каппа-казеин генотипі бар сиырлар тобындағы сиырларда лактация үшін сауу АА – 5356 кг, АВ – 5387 кг, ВВ – 5517 кг құрағанын көрсетті. Зерттеу барысында зерттелетін көрсеткіштер бойынша бета-лактоглобулин және пролактин генотиптерінің АА аллельдерінің артықшылығы атап өтілді, Каппа-казеин генотипі бойынша сауу, ақуыз және сүт майының артықшылығы ВВ генотипі бар сиырларда байқалды. Зерттеу нәтижелері каппа-казеин және АА аллельдері бойынша ВВ генотипі бар сиырлардың өнімділігі жсоғары және ең жсоғары бета-лактоглобулин мен пролактин бойынша сүт ақуызының жсоғары өнімділігімен ерекшеленді.

Кілтті сөздер: симментал тұқымы, сүт өнімділігі, ген экспрессиясы, ДНҚ полиморфизмі, кандидат гендер, Каппа-казеин гені (CSN3), бета-лактоглобулин (LGB), пролактин (bPRL).

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ЭКСПРЕССИЯ ГЕНОВ-КАНДИДАТОВ БЕЛКОВОГО ОБМЕНА У СИММЕНТАЛОВ КАЗАХСТАНСКОЙ СЕЛЕКЦИИ

В данной исследовательской работе описан полиморфизм каппа-казеина (CSN3), бета-лактоглобулина (LGB) и пролактина (bPRL) у коров симментальской породы на ферме «Галицкое» в Павлодарской области. Проведено частотное распределение аллелей и генотипов исследуемых генов. Установлено, что частота встречаемости аллелей А и В по гену каппа-казеина (CSN3) составила 0,51 и 0,49, по бета-лактоглобулину (LGB) – 0,36 и 0,64, по пролактину (bPRL) – 0,68 и 0,32. Результаты исследования влияния генов на молочную продуктивность показали, что удой за лактацию у коров в группе животных с каппа-казеиновым генотипом составил AA – 5356 кг, AB – 5387 кг, BB – 5517 кг. В ходе исследования отмечено превосходство аллелей AA генотипов бета-лактоглобулина и пролактина по изучаемым показателям, преимущество по удою, содержанию белка и молочного жира по генотипу каппа-казеина наблюдалось у коров с генотипом BB. Результаты исследований показали, что коровы с генотипом BB по аллелям каппа-казеина и AA были более продуктивными и имели самый высокий выход молочного белка по бета-лактоглобулину и пролактину.

Ключевые слова: симментальская порода, молочная продуктивность, экспрессия генов, ДНҚ-полиморфизм, гены-кандидаты, ген каппа-казеина (CSN3), бета-лактоглобулин (LGB), пролактин (bPRL).

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