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ХАРАКТЕРИСТИКА ЛИНИЙ ПОРОДЫ КРУПНОГО РОГАТОГО СКОТА СИБИРЯЧКА ПО ГЕНАМ *CSN*3, *BLG, LALBA, LEP* И ИХ СВЯЗЬ С МОЛОЧНОЙ ПРОДУКТИВНОСТЬЮ

(✉) [1]**Гончаренко Г.М.**, [1]**Гришина Н.Б.**, [1]**Шишкина М.А.**, [1]**Хорошилова Т.С.**, [1]**Халина О.Л.**, [2]**Шукюрова А.М.**, [2]**Авадани Д.А.**
[1]*Сибирский федеральный научный центр агробиотехнологий Российской академии наук*
Новосибирская область, р.п. Краснообск, Россия
[2]*Новосибирский государственный аграрный университет*
Новосибирск, Россия
(✉) e-mail: gal.goncharenko@mail.ru

Представлены результаты исследований по продуктивности и генотипической структуре коров ведущих линий породы крупного рогатого скота Сибирячка, ассоциативным связям генотипов *CSN*3, *BLG, LALBA, LEP* с экономически важными признаками. Сравнительная оценка показала, что наиболее высокий удой имели коровы линии быка Рефлекшн Соверинга – 6851 кг, содержание жира – 4,05%, белка – 3,15%. Формируемые сибирские линии быков Франка 937, Урагана 27 и Курса 1949 уступают им по удою, содержанию жира и белка с показателями 5246–5504 кг, 3,92–3,94%; 3,10–3,12% соответственно. Выявлена генотипическая структура стада и ведущих линий. Линия быка Вис Бэк Айдиала характеризуется более высокой частотой $CSN3^{AA}$ и LEP^{TT} генотипов – на 18,2 и на 11,0% по сравнению с линией Рефлекшн Соверинга. По другим генотипам различия не достигают порога достоверности. Средний уровень гомозиготности по исследованным генам варьирует от 51,2 до 73,4%. Наиболее высокая гомозиготность отмечена по *CSN*3 гену в линии Вис Бэк Айдиала – 79,6%. Число эффективно действующих аллелей составляет 1,66–1,72; степень генетической изменчивости – 40,2–42,7%. Коровы с $CSN3^{AB}$ генотипом имели удой на 544,0 кг выше по сравнению с гомозиготными животными по аллелю А ($p < 0{,}05$). Наиболее высокий удой отмечен у животных BLG^{AA} – 6790,1 кг, что выше, чем у коров с альтернативным генотипом BLG^{BB}, на 947,2 кг ($p < 0{,}01$). Животные с LEP^{CC} генотипом превосходили по удою коров с LEP^{TT} на 718,7 кг. По гену *LALBA* приоритетных генотипов не выявлено. Также не установлена связь между генотипами и качественными показателями молока.

Ключевые слова: крупный рогатый скот, линия, генотип, гомозиготность, продуктивность

CHARACTERISTICS OF THE LINES OF THE SIBIRYACHKA CATTLE BREED BY GENES *CSN3, BLG, LALBA, LEP* AND THEIR RELATIONSHIP WITH DAIRY PRODUCTIVITY

(✉) [1]**Goncharenko G.M.**, [1]**Grishina N.B.**, [1]**Shishkina M.A.**, [1]**Khoroshilova T.S.**, [1]**Khalina O.L.**, [2]**Shukyurova A.M.**, [2]**Avadani D.A.**
[1]*Siberian Federal Scientific Center of Agrobiotechnologies of the Russian Academy of Sciences*
Krasnoobsk, Novosibirsk Region, Russia
[2]*Novosibirsk State Agrarian University*
Novosibirsk, Russia
(✉) e-mail: gal.goncharenko@mail.ru

The results of studies on productivity and genotypic structure of cows of the leading lines of Sibiryachka cattle breed, associative links of CSN3, BLG, LALBA, LEP genotypes with economically important traits are presented. Comparative evaluation showed that Reflection Sovering bull cows had the highest milk yield of 6851 kg, fat content of 4.05% and protein content of 3.15%. The Siberian bull lines Frank 937, Uragan 27 and Kursa 1949 which are being shaped are inferior to them in milk yield, fat and protein content with values of 5246-5504 kg, 3.92-3.94%; 3.10-3.12% respectively. The genotypic structure of the herd and the leading lines is identified. The Vis Back Aydiala bull line is characterized by a higher frequency of $CSN3^{AA}$ and LEP^{TT} genotypes by 18.2 and 11.0%, in comparison with the Reflection Sovering line. For other genotypes, the differences do not reach the confidence threshold. The average level of homozygosity for the genes studied varies from 51.2% to 73.4%. The highest homozygosity was found for the CSN3 gene in the Vis Back Aydiala line at 79.6%. The number of effectively acting alleles is 1.66-1.72; the degree of genetic variability is 40.2-42.7%. The cows with $CSN3^{AB}$ genotype had 544.0 kg higher milk yield than homozygous animals for the A allele ($p < 0.05$). The highest milk yield was observed in BLG^{AA} animals - 6790.1 kg, which is 947.2 kg higher than in cows with the alternative BLG^{BB} genotype ($p < 0.01$). Animals with the LEP^{CC} genotype outperformed LEP^{TT} cows in milk yield by 718.7 kg. No priority genotypes were identified for the LALBA gene. Also, no connection has been established between genotypes and the quality indicators of milk.

Keywords: cattle, line, genotype, frequency, homozygosity, productivity.

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INTRODUCTION

The productivity of the black-and-white breed of cattle on farms in the Siberian region is increasing largely due to the use of bull breeders with high genetic potential, effective breeding and pedigree work, and an increased level of feeding that meets the needs of the animal. The new cattle breed Sibiryachka (patent No. 9498 of 08.02.2018) has good dairy productivity and extensive longevity [1]. To further improve the breed, it is advisable to use molecular genetic markers along with traditional breeding methods to ensure earlier prediction of genetic potential, efficient selection and selection of animals.

Currently, there is a steady trend in animal breeding towards the spread of the Holstein breed through the intensive use of imported breeding products. This leads not only to changes in phenotypic traits, but also to the impoverishment of the gene pool of local breeds and the risk of increased homozygosity [2, 3]. It should be noted that changes in genotypic structure, monitoring of homozygosity, inbredness in a number of generations on the example of a single herd have not been sufficiently studied. Polymorphic structural genes detected by PCR-RFLP method serve not only as potential genetic markers of economically important animal traits, but can also be used to characterize the ongoing changes in breeding and genetic parameters in the herd.

The most informative dairy cattle genes include CSN3, BLG, LALBA, LEP, where CSN3 occupies a special place, due to the proven influence of its B allele on increased protein content in milk and better cheese suitability [4–6].

Another equally important milk protein, beta-lactoglobulin (β-LG), belongs to the serum proteins of ruminants and is characterized by acid resistance with an optimum pH of 6.5. The β-LG content in goat and cattle milk is about 4 g/l, or 13 and 11% of total protein, respectively, and in serum up to 50% of all serum proteins

[7]. Thirteen allelic variants of the BLG gene have been identified and confirmed. The A and B alleles are the most common in cattle and are more widely studied [8]. A number of studies have shown an association of BLG^{BB} with fat and protein content in milk, and BLG^{AA} with high milk yield in cows [9, 10].

Leptin gene and its derivatives proteins of the hormone leptin are produced by adipose tissue cells and play an important role in the regulation of energy metabolism, affecting the synthesis of adipose tissue in animals and humans [11, 12]. In beef cattle breeding, the LEP gene is considered as a potential marker of fat accumulation in the carcass of an animal, associated with meat quality, its marbling [13, 14]. In dairy cattle breeding, an associative association of some genotypes with dairy performance and milk quality composition has been revealed[1] [15].

Lactalbumin alpha (LALBA) is an important mammalian serum protein encoded by the LALBA gene. A study [16] shows that α-lactalbumin is a protein that regulates lactose production in milk in almost all mammals. It plays a functional role in changing the volume of synthesized milk, so it is of interest for evaluation and prediction of milk productivity of cows.

The purpose of the study was to identify the genetic structure of the Sibiryachka cow herd and individual genealogical lines using CSN3, BLG, LALBA, LEP genes, to determine homozygosity of other breeding and genetic parameters as well as desirable genotypes of dairy productivity.

MATERIAL AND METHODS

The object of the study was Sibiryachka cows from Kirzinsky herd (Novosibirsk region) with milk yield of 6376 kg, fat and protein content in milk of 4.11 and 3.13% respectively. The service period was 135 days and calf yield was 81%.

Molecular genetic studies of cows were performed in the laboratory of biotechnology at the Siberian Research Institute of Animal Husbandry (SibNIPTIZh) of the Siberian Federal Scientific Centre of AgroBioTechnologies of the Russian Academy of Sciences. Genomic DNA was isolated from blood using the "Ampli-Prime DNA-sorb-B" clinical extraction kit according to the prescription of the manufacturer "NextBio" LLC (Moscow).

The quality and concentration of isolated DNA and identification of PCR-RFLP results were assessed in agarose gel by horizontal electrophoresis using an E-Box-CX5.TS-20.M gel documentation system in transmitted ultraviolet light by ethidium bromide fluorescence.

The polymorphisms of the CSN3, BLG genes were detected using the PCR-RFLP methodology developed at the All-Russian Research Institute of Animal Breeding[2]. LALBA gene polymorphism was determined using the PCR-RFLP methodology described in [17]. LEP genotyping of animals was performed according to the method [18].

The data were statistically processed using Microsoft Exel computer programs and generally accepted methods [19]. The compliance of the actual distribution of genotype frequencies with the theoretically expected frequency distribution was checked using the $\chi 2$ criterion [20].

RESULTS AND DISCUSSION

The genealogical structure of the Siberian herd is represented mainly by Holstein lines, among which the line of the bull Vis Back Aydial 1013415 accounts for 60,7% of cows, Reflection Sovering 198998 - 21,6%. The formed Siberian lines of bulls Frank 937, Uragan 27, Kurs 1949 are currently few in number and considerably inferior in productivity to Holstein lines (see Table 1). The highest milk yield (6851 kg) by the first lactation was noted in cows of the Reflection Sovering line, which was 1347-1605 kg higher than in the animals of Frank

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937, Uragan 27, Kurs 1949 lines (p <0,001). The milk yield of cows of the Vis Back Aydial 1013415 bull line is slightly lower - 5995 kg, but it is higher than that of the animals of formed lines by 491-749 kg (p <0,5; p <0,001). In addition, cows of Holstein lines have milk with 0,11-0,13% higher fat content than that of Siberian lines (p <0,001). No significant differences were found in protein content between the lines.

Analysis of the genotypic structure of the Siberyachka breed herd and the leading lines for the genes CSN3, BLG, LALBA, LEP revealed a mostly similar genotype ratio, except for $CSN3^{AA}$ and LEP^{TT}, whose frequency in the Vis Back Aydial line is 18,2 and 11,0% higher than in the Reflection Sovering line (p <0,05) (see Table 2).

It should be emphasized that the ratio of CSN3 genotypes largely corresponds to the polymorphism of these genes in black-and-white breeds. As shown by the studies[3] [21, 22], the homozygous $CSN3^{AA}$ genotype was detected in 55.2-73.2%, the heterozygous $CSN3^{AB}$ genotype had 26.8-38.9% of animals, and the homozygous $CSN3^{BB}$ genotype accounted for 5.6-10.2%. Similar frequency of CSN3 genotypes was observed in Simmental cows: CS-

Табл. 1. Молочная продуктивность коров основных линий
Table 1. Milk productivity of cows of the main lines

Line	Heads	First lactation		
		Milk yield, kg	Fat, %	Protein, %
Vis Back Aydial 1013415	334	5995 ± 55	3,97 ± 0,01	3,12 ± 0,002
Reflection Sovering 198998	119	6851 ± 98	4,05 ± 0,02	3,15 ± 0,006
Montwick Chiftein 95679	38	6221 ± 78	4,04 ± 0,02	3,14 ± 0,006
Frank 937	25	5504 ± 147	3,92 ± 0,01	3,11 ± 0,004
Uragan 27	22	5366 ± 178	3,94 ± 0,01	3,12 ± 0,004
Kurs 1949	12	5246 ± 235	3,92 ± 0,02	3,10 ± 0,01

Табл. 2. Генотипическая характеристика коров черно-пестрой породы СПК «Кирзинский» по гену *CSN3* с учетом линейной принадлежности
Table 2. Genotypic characteristics of black-and-white cows of the "Kirzinsky" APC by the *CSN3* gene with respect to linear affiliation

Genotype	Line			
	Vis Back Aydial (*n* = 78)	Reflection Sovering (*n* = 46)	By herd	χ2
$CSN3^{AA}$	76,9 ± 4,77	58,7 ± 7,26	71,0 ± 3,96	
$CSN3^{AB}$	23,1 ± 4,77	34,8 ± 7,02	26,7 ± 3,86	0,020
$CSN3^{BB}$	0 ± 0,00	6,5 ± 3,64	2,3 ± 1,31	
BLG^{AA}	29,5 ± 5,16	43,5 ± 7,31	35,9 ± 4,19	
BLG^{AB}	46,2 ± 5,64	39,1 ± 7,20	43,5 ± 4,33	1,555
BLG^{BB}	24,4 ± 4,86	17,4 ± 5,59	20,6 ± 3,53	
LEP^{CC}	35,9 ± 5,43	54,4 ± 7,34	48,9 ± 4,37	
LEP^{CT}	48,7 ± 5,66	41,3 ± 7,26	45,0 ± 4,35	1,367
LEP^{TT}	15,4 ± 4,09	4,4 ± 3,01	6,1 ± 2,09	
$LALBA^{AA}$	52,6 ± 5,65	43,5 ± 7,31	41,2 ± 4,30	
$LALBA^{AB}$	41,0 ± 5,57	52,2 ± 7,37	48,1 ± 4,36	0,555
$LALBA^{BB}$	6,4 ± 2,77	4,35 ± 3,01	10,7 ± 2,70	

N3^{AA} = 0.626, CSN3^{AB} = 0.306, and CSN3^{BB} = 0.068 [23]. In general, the low frequency of the desirable CSN3^{BB} genotype, whose associative link with protein content in milk and higher cheese suitability has been proved by many authors [3-5, 24], draws attention in the herd as a whole.

Our studies showed a BLG^{AA} frequency of 35.9%, a heterozygote frequency of 43.5, and a homozygous BLG^{BB} genotype of 20.6%. Variation in genotype frequencies was noted in the lines, but no significant differences were found due to insufficient sample size. The BLG genotypic structure of the black-and-white breed that we have identified is consistent with the findings of a number of authors. Studies [25-27] claim that about half of the animals are heterozygotes, with homozygotes with the BLG^{A} allele accounting for 24-27% according to various sources.

There is conflicting data on the frequency of LEP genotypes. In Holstein cows, the genotype frequency is as follows: LEP^{CC} - 10%, LEP^{CT} - 62, LEP^{TT} - 28% (see footnote 1). The distribution of genotypes in Holmogor cows of the Tatarstan type is somewhat different: LEP^{CC} - 25%, LEP^{CT} - 55,5, LEP^{TT} - 19,5% [15]. According to our data, the lowest share of cows is occupied by animals with the LEP^{TT} genotype - 6.1%, in the Reflection Sovering line - 4.4%. The LEP^{CC} homozygous genotype was detected in half of the animals in the herd as a whole, slightly less in animals belonging to the Vis Back Aydial line - 35.9%.

Analysis of LALBA gene genotype frequencies in black-and-white cows shows a low frequency of LALBA^{BB} genotype, ranging from 3 to 20%, whereas the frequency of LALBA^{AA} and LALBA^{AB} genotypes is up to 50% [28, 29].

Based on genotype frequencies, breeding and genetic parameters were calculated: Ca - homozygosity, SH - homozygosity coefficient, Na - number of effective alleles, V - degree of genetic variability in the population. The average level of homozygosity for the studied genes ranged from 51.2 to 73.4%, with the highest homozygosity being recorded for CSN3 in the Vis Back Aydial line at 79.6%. The number of effective alleles and degree of genetic variability in the lines are approximately at the same level: 1.66-1.72 and 40.2-42.7% (see Table 3).

In studies on the use of genetic markers in breeding, the relationship between genotypes and performance is of particular interest. We found that heterozygous CSN3^{AB} cows had the highest milk yield. The surplus was 544.0 kg compared to cows with the homozygous CSN3^{AA} genotype ($p < 0.05$). Literature data on this issue is ambiguous.

The priority of the CSN3^{AB} genotype for milk yield was established in the work [30]. Given the low frequency of the CSN3^{BB} genotype in the herd, these animals were not considered in the productivity analysis (see Table 4).

Among cows with BLG genotypes, the highest milk yield was observed in BLG^{AA} animals - 6790.1 kg, which is 947.2 kg higher than in cows with the alternative BLG^{BB} genotype ($p < 0.01$). The association of this gene with milk production in black-and-white cows was found to be somewhat different. Cows with BLG^{AB}

Табл. 3. Селекционно-генетические параметры стада Сибирячка
Table 3. Breeding and genetic parameters of the Sibiryachka herd

Line	*n*	*Ca*, %				*SH*	N_a	*V*
		CSN3	*BLG*	*LALBA*	*LEP*			
Vis Back Aydial	78	79,6	50,2	52,2	60,6	0,100	1,66	40,2
Reflection Sovering	46	63,6	53,4	62,6	57,6	0,064	1,72	42,7
By herd	127	73,4	51,2	55,0	59,4	0,08	1,67	40,6

[3]Lihodeevkaya O.E., Lihodeevkiy G.A., Gorelik O.V. et al. Effect of genetic and paratypil factors on milk production in cattle // III International scientific conference: agritech-iii-2020: agribusiness, environmental engineering and biotechnologies. Volgograd, Krasnoyarsk, 2020. 82009 p.

Табл. 4. Продуктивность коров черно-пестрой породы в зависимости от носительства генотипа *CSN3* (первая лактация)

Table 4. Productivity of black-and-white cows depending on the carriage of the *CSN3* genotype (first lactation)

Genotype	*n*	Rate for the first 305 days of lactation		
		Milk yield, kg	Fat, %	Protein, %
*CSN3^AA^*	70	6197,8 ± 164,80	4,00 ± 0,03	3,12 ± 0,01
*CSN3^AB^*	21	6741,8 ± 207,70	4,03 ± 0,04	3,14 ± 0,02
*BLG^AA^*	29	6790,1 ± 255,28	4,04 ± 0,04	3,13 ± 0,01
*BLG^AB^*	40	6319,1 ± 209,88	4,01 ± 0,04	3,11 ± 0,01
*BLG^BB^*	24	5842,9 ± 265,35	3,98 ± 0,03	3,12 ± 0,01
*LALBA^AA^*	46	6404,2 ± 224,33	3,99 ± 0,03	3,11 ± 0,01
*LALBA^AB^*	41	6248,8 ± 197,70	4,04 ± 0,04	3,13 ± 0,01
*LALBA^BB^*	6	6519,2 ± 333,01	3,92 ± 0,03	3,12 ± 0,02
*LEP^CC^*	39	6726,3 ± 193,73	4,06 ± 0,04	3,13 ± 0,01
*LEP^CT^*	40	6086,8 ± 232,53	3,98 ± 0,03	3,12 ± 0,01
*LEP^TT^*	14	6007,6 ± 300,98	3,94 ± 0,02	3,10 ± 0,01

had an advantage over their counterparts with BLG^{AA} and BLG^{BB} genotypes by 295 and 178 kg of milk, in fat and protein content in milk by 0.09 and 0.05 % respectively [9].

When analyzing the association of LEP genotypes with milk production of cows in the analyzed herd, the milk yield of LEP^{CC} animals was found to be 718.7 kg higher than that of LEP^{TT} cows ($p < 0.05$). Similar results in prioritizing LEP^{CC} genotype for milk yield were obtained in the work [15].

No association between genotypes and milk performance was found in the LALBA gene. There is also no association between genotypes and milk quality parameters.

CONCLUSIONS

1. A comparative evaluation showed that the highest milk yield was achieved by cows of the Reflection Sovering bull line - 6851 kg, fat content was 4.05%, protein content - 3.15%. The Siberian lines Frank 937, Uragan 27 and Kurs 1949 were inferior to them in milk yield, fat and protein content: 5246-5504 kg, 3.92-3.94%, 3.10-3.12% respectively.

2. The frequency of CSN3, BLG, LALBA, LEP genotypes of the Sibiryachka breed is generally consistent with the black- and- white breed. A low frequency of $CSN3^{BB}$ genotype - 2.3% and a high $CSN3^{AA}$ genotype - 71.0% was detected. The ratio of genotypes in the BLG gene: $BLGA^{AA}$: BLG^{AB}: BLG^{BB} is 35.9: 43.5: 20.6%. In the LALBA and LEP genes, homozygous genotypes $LALB^{AB}$ and LEP^{TT} account for 6.1 and 10.7% respectively, while the occurrence of the other two genotypes is 41.2-48.9%. In the leading lines of the Sibiryachka breed the CSN3, BLG, LALBA, LEP found a relatively equal ratio of genotypes, except for $CSN3^{AA}$ and LEP^{TT}, whose frequency in the Vis Back Aydial line is 18.2 and 11.0% higher than in the Reflection Sovering line ($p < 0.05$).

3. The average level of homozygosity for the genes studied ranges from 51.2 to 73.4%, with the highest homozygosity observed for CSN3 in the Vis Back Aydial line at 79.6%. The number of effective alleles and degree of genetic variability in the lines are approximately at the same level: Na - 1.66-1.72, V - 40.2-42.7%.

4. Cows of the Sibiryachka breed with the heterozygous $CSN3^{AB}$ genotype had a 544.0 kg higher milk yield compared to cows with the homozygous $CSN3^{AA}$ genotype ($p < 0.05$). Cows with BLG genotypes had the highest milk yield of 6,790.1 kg in BLG^{AA} cows, which was 947.2 kg higher than in cows with the alter-

native BLGB genotype ($p < 0.01$). Cows with the LEP^{CC} genotype also had a 718.7 kg higher milk yield than cows with LEP^{TT} ($p < 0.05$).

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ИНФОРМАЦИЯ ОБ АВТОРАХ

(✉) **Гончаренко Г.М.,** доктор биологических наук, главный научный сотрудник; **адрес для переписки:** Россия, 630501, Новосибирская область, р.п. Краснообск, а/я 463; e-mail: gal.goncharenko@mail.ru

Гришина Н.Б., кандидат биологических наук, старший научный сотрудник

Шишкина М.А., кандидат сельскохозяйственных наук, старший научный сотрудник

Хорошилова Т.С., научный сотрудник

Халина О.Л., младший научный сотрудник

Шукюрова А.М., младший научный сотрудник

Авадани Д.А., младший научный сотрудник

AUTHOR INFORMATION

(✉) **Galina M. Goncharenko,** Doctor of Science in Biology, Head Researcher; **address:** PO Box 463, Krasnoobsk, Novosibirsk Region, 630501, Russia; e-mail: gal.goncharenko@mail.ru

Natalya B. Grishina, Candidate of Science in Biology, Senior Researcher

Mariya A. Shishkina, Candidate of Science in Agriculture, Senior Researcher

Tatyana S. Khoroshilova, Researcher

Olga L. Khalina, Junior Researcher

A.M. Shukyurova, Junior Researcher

D.A. Avadani, Junior Researcher

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