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THE ANALYSIS OF POLYMORPHISM OF KAPPA-CASEIN, B-LACTOGLOBULIN AND PROLACTIN GENES AMONG YAKUTIAN CATTLE AND ITS INFLUENCE ON MILK PRODUCTION

Research article

Abstract

DNA samples of cows of Simmental and Kholmogory breeds served as the material for the research. Release of DNA was carried out by a standard method of phenol–chloroform extraction. Polymorphisms of rs43703015 of the CSN3 gene, rs109625649 of the LGB gene and rs134028641 of the PRL gene are studied by means of PCR-RFLP analysis. In all studied selections, the A allele prevails on the CSN3 gene. The frequency of the A allele prevailed over the frequency of the B allele on the PRL gene. The animals with the greatest milk yield and fat content of milk on the CSN3 gene had the genotype including the A allele. Depending on the LGB genotype, there was established that the largest size of milk yields have individuals with the AA and AB genotype in selections of the Simmental breed of both selections. In selection of the Kholmogory breed - with the B genotype. Adaptation process is fraught with a serious pressure, that, undoubtedly, affects the efficiency; while long-acting it leads to disorder of physiological functions. Therefore, realization of genetic potential of the delivered cattle to Yakutia will require time.

Keywords: gene, kappa-casein, beta-lactoglobulin, prolactin, genotypes, polymorphism.

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АНАЛИЗ ПОЛИМОРФИЗМА ГЕНОВ КАППА-КАЗЕИНА, В-ЛАКТОГЛОБУЛИНА И ПРОЛАКТИНА У КРУПНОГО РОГАТОГО СКОТА ЯКУТИИ И ИХ ВЛИЯНИЕ НА МОЛОЧНУЮ ПРОДУКТИВНОСТЬ

Научная статья

Аннотация

Материалом исследования служили образцы ДНК коров симментальской и холмогорской пород. Выделение ДНК проводилось стандартным методом фенольно-хлороформной экстракции. С помощью ПЦР ПДРФ анализа изучены полиморфизмы rs43703015 гена CSN3, rs109625649 гена LGB и rs134028641 гена PRL.

Во всех изученных выборках, по гену CSN3 преобладает аллель А. По гену LGB, во всех выборках преобладал гетерозиготный генотип АВ. По гену PRL частота аллели А преобладала над частотой аллели В. Наибольшим удоем и содержанием жира в молоке, по гену CSN3 обладали животные с генотипом несущим в себе аллель А. В зависимости от генотипа LGB, установили, что в выборках симментальской породы обоих селекций, наибольшей величиной удоев, обладают особи с генотипом AA и AB. В выборке холмогорской породы - с генотипом BB. В зависимости от гена PRL, более высокими надоями обладали особи несущие в своем генотипе аллель A.

Процесс адаптации сопряжен с серьезной нагрузкой, что, несомненно, сказывается на продуктивности, а при длительном действии приводит к расстройству физиологических функций. Следовательно, для реализации генетического потенциала завезенного скота в Якутию потребуется время.

Ключевые слова: ген, каппа-казеин, бета-лактоглобулин, пролактин, генотипы, полиморфизм.

1. Introduction

In today's conditions of milk cattle breeding the most important factor of ensuring necessary level of milk production of cows is the creation and use of animals with a high productive genetic potential. Animal selection questions with using of molecular genetic methods in different years were studied by many researchers (Denocourt et al., 1990; Medrano&Aguilar-Cordova,1990; Mitra et al.,2015) The most widespread potential DNA markers of cattle production signs are genes: kappa-casein (CSN3), beta-lactoglobulin (bLGB) and prolactin (bPRL).

The Sakha (Yakutia) Republic is one of dynamically developing territorial entity of the Far East region of the Russian Federation. Exactly here unique experience of farming in extreme climatic conditions of the North and permafrost is accumulated. The climate of Yakutia is sharply continental and differs with long winter and short summer. The difference of temperatures of the coldest and warmest months is 70-75 °C.

Selective breeding in extreme conditions of Yakutia is aggravated with a series of objective factors of the habitat and technology of animal husbandry: severe climatic conditions, duration of winter stabling, total energy deficit and biological inferiority of feeding of animals in winter keeping (Romanova et al., 2017). In this connection, the milk yield doesn't correspond to genetic potential. According to Rosstat, the milk yield of one cow is 2142 kg in the farms of all categories across the Republic of Sakha (Yakutia) that there is slightly more than a half of the average milk yield from one cow across Russia which is 4021 kg.

Indigenous Yakutian purebred cattle raised till 1929 in all territory of the Republic. According to C.B. Atlasova (1992), in 1928 there were 555193 heads of the Yakutian cattle (Atlasov, 1992). For mass improvement of the Yakutian cattle in the southern and central regions of the Republic it was planned to use Kholmogory and Simmental breeds by the plan of pedigree division into districts. In the Central Yakutia the purebred Yakutian cattle disappeared by the beginning of the 60th years (Ivanova, 1997).

As a result of accumulation crossbreeding of the indigenous Yakutian cattle with Simmental breed, the most northern population of the Simmental cattle was framed in the republic (Chugunov, 1981). The Simmental breed of the Austrian selection to Yakutia began to deliver since 2007. The cattle of the Austrian selection differs from local Simmental with higher body weight and meat-and-dairy orientation. Genetic potential of the Austrian Simmental breed is very high; under favorable conditions and feeding, the milk yield of cows of this selection can reach to 6707 kg with fat content of 4,13% and a protein mass fraction of 3,4%. The Kholmogory breed raised in Yakutia was also received as a result of accumulation crossbreeding of the Yakutian aboriginal cattle with Kholmogory breed.

For objective appraisal of polygenic characters it is necessary to consider a polymorphic impact of many QTL genes. The main protein components of milk are caseins – 2,6%, and beta-lactoglobulin – 0,3%; hormone prolactin takes part in lactation onset and control.

The researches of milk-yield genes polymorphism, prevalence of single locus alleles, their combinability in genotypes and populations, the influence of extreme environmental conditions to these genes offer new possibilities in the field of a comprehension of a number of theoretical and practical problems of farm animals genetics, and the roles of ecological conditions in the formation of species gene pool.

The object is an assessment gene pool by polymorphic variants of kappa-casein (CSN3), β-lactoglobulin (BLG), prolactin (PRL) genes and their influence on milk production of cattle raised in Yakutia.

2. Methods and materials

As the material of the research there were used DNA samples received from venous blood of Simmental breed cows of the Austrian selection of LLC "Agrofirma Nemyugyu" of the Khangalassky District: brought from Austria (selection 1; n=39), raised in Yakutia (selection 2; n=37); Simmental breed of local selection: agricultural production cooperative "Nayakhy" of the Ust-Aldansky District (selection 3; n=42), agricultural production cooperative "Lena" of the Ust-Aldansky District (selection 4; n=50) and Kholmogory breed: farm household "Daiyyna" of the Namsky District (selection 5; n=18), LLC "Kladovaya Olekmy" of the Olyokma District (selection 6; n=20).

DNA extraction from peripheral blood lymphocytes was carried out by a standard method of phenol–chloroform extraction. From each cow it was defined polymorphisms of rs43703015 of the CSN3 gene, rs109625649 of the LGB gene and rs134028641 of the PRL gene. Specific primers (CJSC Sintol, Russia) were used for carrying out the PCR method:

The following annealing temperatures to carry out the PCR (30-35 cycles) were used: CSN3 - 58 °, bLGB - 55 °, PRL - 59 °. The CSN3 gene amplification was segregated by endonuclease HindIII, the bLGB gene – HaeIII, and the PRL gene – RsaI ("SibEnzim"). Restriction fragments length and number were determined by an electrophoresis in 2,5% agarose gel in UV light by gel documentation system.

3. Results

In the analyzed samples, the A allele considerably prevails in the CSN3 gene, the heterozygous individuals prevail in the LGB gene, and the individuals with the homozygous AA genotype prevail in the PRL gene. It corresponds to results of the genetic analysis of the studied groups of cattle (tab. 1).

Table 1. Genotypes and alleles of genes frequency among cattle raised in Yakutia

Breed	Sample	n	Genotype, %			Frequency of alleles		χ2
			AA	AB	BB	A	B	
Kappa Casein								
Simmental Austrian selection	1	39	67	33	0	0,833	0,167	1,401
	2	37	54	38	8	0,730	0,270	0,000
Simmental local selection	3	42	53	45	2	0,750	0,250	1,229
	4	50	32	56	12	0,600	0,400	1,389
Kholmogory	5	18	56	33	11	0,722	0,278	2,600
	6	20	45	50	5	0,700	0,300	2,500
Beta-lactoglobulin								
Simmental Austrian selection	1	39	13	59	28	0,423	0,577	1,720
	2	37	19	57	24	0,473	0,527	0,725
Simmental local selection	3	42	4	48	48	0,286	0,714	0,910
	4	50	18	46	36	0,410	0,590	0,225
Kholmogory	5	18	44	39	17	0,611	0,389	3,26
	6	20	0	40	60	0,200	0,800	6,25
Prolactin								
Simmental Austrian selection	1	39	54	44	2	0,756	0,244	0,812
	2	37	73	27	0	0,865	0,135	1,536
Simmental local selection	3	42	55	45	0	0,774	0,226	3,227
	4	50	78	20	2	0,880	0,120	0,091
Kholmogory	5	18	83	17	0	0,917	0,083	1,280
	6	20	84	15	1	0,775	0,225	0,000

In all studied selections, the A allele and the genotypes of CSN3 AA and CSN3 AB prevail on the kappa-casein gene (0,600 - 0,833). Based on the genetic profile of the kappa-casein gene, the animals born in Yakutia differ from an initial profile of brought-in cows. On the beta-lactoglobulin gene, in all selections, except No. 5 (the Kholmogory breed of farm household "Daiyyna"), the prevalence of the heterozygous individuals of LGBAB was higher than the homozygous LGBAA and LGBBB, and varied on 40-59% depending on breed. Frequency of the B allele was (0,527-0,800). In the prolactin gene, the A allele frequency (0,756-0,917) accurately (p<l0,001) prevailed over the B allele frequency (0,083-0,244). Among the studied animals, a widespread genotype was PRLAA (54-84%).

While studying of the distribution of the CSN3/LGB/PRL gene genotypes as a whole, 19 complex genotypes was defined among all studied selections of cattle. The complex genotype of CSN3 AA LGBAB PRLAA (19-26%) meets more often over the Simmental breed of the Austrian selection of both selections and the Kholmogory breed selection of farm household "Daiyyna" ; the genotype of CSN3AB LGBBB PRLAA (12-15%) mostly meets over the Simmental breed of agricultural production cooperative "Lena" local selection and the Kholmogory breed of LLC "Kladovaya Olekmy"; the genotype of CSN3AA LGBAB PRLAB (18%) - over the Simmental breed of local selection of SHPK "Nayakhy".

The results of the analysis of cow milk production of various breeds and selections depending on the CSN3 gene genotype are presented in table 2.

Table 2. Cow milk production depending on the kappa-casein genotype

Genotype	By first lactation			Third lactation		
	yield of milk, kg	fat content of milk, %	milk fat, kg	yield of milk, kg	fat content of milk, %	milk fat, kg
Simmental breed of Austrian breeding, brought from Austria						
CSN3AA (n=13)	1393 ±84	3,7 ±0,05	52,2 ±3,43	3786,8 ±276,30	3,9 ±0,07	147,1 ±9,92
CSN3AB (n=4)	1202 ±162	3,8 ±0,1	45,6 ±7,1	3985,2 ±629,52	4,1 ±0,13	164,9 ±27,38
Simmental breed of Austrian breeding, born in Yakutia						
CSN3AA (n=16)	2915 ±136	3,7 ±0,02	107±5,02	3680 ±170,7	3,8 ±0,02	140,9 ±6,74
CSN3AB (n=13)	2965 ±165	3,8 ±0,03	111,6±6,3	3628,2 ±367,0	3,9 ±0,03	140,2 ±13,97
CSN3BB (n=3)	2673 ±481	3,7 ±0,05	99,7±17,4	2884	3,8	109,6
Simmental breed of local breeding SHPK «Najahy»						
CSN3AA (n=9)	1562 ±76	3,8 ±0,03	59,2 ±2,6	1369,7 ±863,1	3,9 ±0,04	52,8 ±7,93
CSN3AB (n=8)	1607 ±165	3,8 ±0,03	61,6 ±6,75	1549,0 ±751	3,9 ±0,1	60,0 ±29,4
Simmental breed of local breeding SPK «Lena»						
CSN3AA (n=12)	1876 ±76	3,9 ±0,05	73,4 ±2,95	2059 ±107,8	3,9 ±0,04	80,6 ±4,61
CSN3AB (n=22)	1750 ±39	4,0 ±0,06	70,3 ±1,8	2035,6 ±59,62	4,0 ±0,04	81,2 ±2,15
CSN3BB (n=4)	1829 ±100	4,1 ±0,34	74,8 ±2,75	2001,0	4,1	82,4
Kholmogory OOO «Kladovaja Olekmy»						
CSN3AA (n=9)	2286±124	3,43±0,02	78,52±4,3	2650 ±445	3,66 ±0,68	94,5±9,70
CSN3AB (n=10)	2379±111	3,44±0,02	81,72±3,6	3276 ±482	3,50±0,58	113±23,2
CSN3BB (n=1)	2727	3,5	95,45	2292	3,70	84,80

The results of the analysis of cow milk production of various breeds and selections depending on the kappa-casein genotype showed that individuals with the CSN3AA genotype have the highest milk yield among brought-in Simmental breed of the Austrian selection and the indigenous selection (APC Lena); individuals with the heterozygous genotype of CSN3AB - among indigenous Simmental breed cows (APC "Nayakhy") and Kholmogory breed cows.

According to the researches of Zinnatov F.F. (2011), it is noticed that individuals with the genotype of CSN3AB of black-and-white breed cows had the highest milk yield and fat content of milk (Zinnatova, 2011). On the basis of the researches of Ibragimova G. R. and Valitov F.R. (2012), cows with the CSN3BB genotype had the highest milk yield, and the smallest - with the CSN3AA genotype. The author notices that cows with the CSN3BB genotype had the highest fat content of milk, and cows with the genotype of CSN3AA (Ibragimova& Valitov, 2012) had the biggest protein content of milk.

Average values of first lactation milk yield and all lactations milk yield depending on a locus genotype of the β-lactoglobulin are given in table 3.

Table 3. Cow milk production depending on the beta-lactoglobulin gene genotype

Genotype	By first lactation			Third lactation		
	yield of milk, kg	fat content of milk, %	milk fat, kg	yield of milk, kg	fat content of milk, %	milk fat, kg
Simmental breed of Austrian breeding, brought from Austria						
LGBAA (n=4)	1199 ±95,0	3,8 ±0,1	45,4 ±4,3	2909,5 ±225,6	4,0 ±0,05	114,8 ±8,35
LGBAB (n=11)	1463 ±89,6	3,78 ±0,06	55,4±3,57	3938,45±298,78	3,90 ±0,09	154,07 ±13,03
LGBBB (n=2)	975 ±163,9	3,5 ±0,09	33,6 ±5,00	4002,3±1063,59	3,9 ±0,05	157,0 ±40,62
Simmental breed of Austrian breeding, born in Yakutia						
LGBAA (n=7)	2947 ±157	3,7 ±0,03	108,6±5,6	3646,3 ±273,64	3,9 ±0,03	141,0 ±10,74
LGBAB (n=17)	2839 ±113	3,7 ±0,02	105 ±4,05	3768,8 ±212,9	3,8 ±0,02	144,2 ±8,28
LGBBB (n=8)	3040 ±297	3,8 ±0,03	114±11,41	3021,7±215,93	3,8 ±0,05	115,9 ±9,58
Simmental breed of local breeding SHPK «Najahy»						
LGBAA (n=2)	1403 ±67,9	3,9 ±0,18	54,7 ±0,17	1369,7 ±863,1	3,9 ±0,14	52,8 ±31,7
LGBAB (n=6)	1711 ±238	3,8 ±0,05	65,6 ±9,44	2140,3	3,9	83,5
LGBBB (n=10)	1557 ±60,2	3,8 ±0,02	59,0 ±2,38	1253,3 ±198,6	3,9 ±0,1	48,3 ±8,53
Simmental breed of local breeding SPK «Lena»						
LGBAA (n=9)	1913 ±73	4,1 ±0,15	77,6 ±3,1	2033,3 ±138,06	4,0 ±0,08	80,6 ±5,9
LGBAB (n=13)	1787 ±51,8	4,0 ±0,09	71,7 ±2,14	2171,1 ±84,18	4,0 ±0,05	86,5 ±3,05
LGBBB (n=16)	1740 ±55,2	3,9 ±0,06	68,5 ±2,05	1957,3 ±56,01	4,0 ±0,04	77,4 ±1,84
Kholmogory OOO «Kladovaja Olekmy»						
LGBAB (n=8)	2474 ±151	3,43 ±0,02	84,74±5,2	2610 ±390	3,62 ±0,6	78,4±3,09
LGBBB (n=12)	2275 ±92,6	3,45 ±0,02	95± 19,8	3208 ±689	3,4 ±0,65	107± 19,3

According to the analysis of cow milk production of various breeds and selections depending on the beta-lactoglobulin genotype, it is established that individuals with the genotype of LGBAA and LGBAB have the greatest milk yield among the Simmental breed of both selections. Individuals with LGBBB genotype have the greatest milk yield among the Kholmogory breed selection (LLC "Kladovaya Olekmy"), but, at the same time, individuals with the genotype of LGBAA weren't revealed in this selection. In the researches of Dyman O. P. (2015) it is noticed that cows of the Ukrainian black-and-white breed, which are carriers of the LGBAA genotype, are characterized by higher rates of milk yielding; at the same time, in comparison with genotypes of LGBAA and LGBAB, higher fat content of milk (%) were from homozygotes of LGBBB (Dyman&Plivachuk, 2015). Based on the researches of Zinnatova F.F. et al. (2012), the highest milk yield had individuals with the LGBAA genotype of the Kholmogory breed of the Republic of Tatarstan.

The results of the analysis of cow milk production of various breeds depending on the genotype at the PRL locus are generalized in table 4.

Table 4. Milk production of cows depending on prolactin gene genotype

Genotype	By first lactation			Third lactation		
	yield of milk, kg	fat content of milk, %	milk fat, kg	yield of milk, kg	fat content of milk, %	milk fat, kg
Simmental breed of Austrian breeding, brought from Austria						
PRLAA (n=11)	1405 ±103	3,7 ±0,07	52,76±4,27	3461,09±243,84	3,95 ±0,08	137,67 ±11,74
PRLAB (n=6)	1194 ±101	3,71 ±0,1	44,52 ±4,2	4128,00±512,44	3,86 ±0,07	158,65 ±19,36
Simmental breed of Austrian breeding, born in Yakutia						
PRLAA (n=23)	2859 ±111	3,7 ±0,02	106,3±4,12	3639,1 ±177,5	3,8 ±0,02	139,5 ±6,93
PRLAB (n=9)	3069 ±218	3,7 ±0,03	114,0±8,39	3588,6 ±333,9	3,9 ±0.04	138,5 ±12,91
Simmental breed of local breeding SHPK «Najahy»						
PRLAA (n=12)	1663 ±101	3,8±0,03	63,5±3,98	1498 ±386	3,9 ±0,06	57,8 ±14,7
PRLAB (n=5)	1404 ±85,2	3,8±0,02	53,3±3,14	1394	3,9	54,4
Simmental breed of local breeding SPK «Lena»						
PRLAA (n=29)	1812 ±39,7	4,0±0,04	71,4±1,47	2047,0 ±62,18	4,0 ±0,03	81,1 ±2,43
PRLAB (n=9)	1747 ±56,5	4,2±0,16	72,7±3,66	2028,7 ±85,66	4,0 ±0,07	80,7 ±3,56
Kholmogory OOO «Kladovaja Olekmy»						
PRLAA (n=12)	2267 ±87,1	3,4±0,02	77,98±2,97	2818 ±489	3,75 ±0,4	121± 26,5
PRLAB (n=7)	2515 ±179	3,4±0,02	86,18±6,00	2828 ±464	3,4 ±0,88	93± 12

The results of the analysis of cow milk production of various breeds depending on the genotype at the PRL locus showed that the cows carrying PRLA gene allele in their genotype both in heterozygous, and homozygous forms have higher milk yield. In the researches of Kholmogorsky breed of the Tatarstan species, Minnakhmetov A.H. with coauthors (2013) established that cows with the genotype of PRLAA surpassed heterozygotes of PRLAB in milk yielding (Minnahmetov et al., 2015). Authors Zakirova G.M., Sultanov R.R., Zinnatova F.F. (2011) also note milk yield prevalence of cows with the PRLAA genotype in their researches. However, in Gareyeva I.T. researches (2012) it is noticed that cows of black-and-white breed with the PRLBB genotype had the highest milk yield.

4. Conclusion

Low rates of milk production of Simmental breed cows of the Austrian selection from Austria is explained by acclimatization stress. Also, their next generation had low milk yield rates in comparison with average milk production of this breed (6707 kg), though having higher rates of milk yield than in other studied selections.

The possibilities of an animal organism to adapt to the pressure caused by change of conditions of keeping are limited to quite narrow bounds. Within conservation of optimum dynamic constancy of the internal environment of an organism, the process of adaptation is accompanied by a serious pressure, which, undoubtedly, have a major impact on efficiency, and over time leads to disorder of physiological functions, and in many cases — to breakdown. Therefore, realization of genetic potential of the brought-in cattle in Yakutia will require time. The cause of low milk production of the cattle breeds adapted to local conditions is low food supply (annual food supply makes 62–65% of norm of feeding) and the shortened lactational period (8 months on average).

Thus, in case of the Simmental breed raising and development for rising level and quality of the milk received from them to breeding livestock and commercial stock, it is appropriate to consider the received results as an additional criterion in the course of selective stock breeding using of DNA markers under an animal selection. However, it is necessary to notice that the investigation of their flexibility for large animal selections is necessary for wide use of the determined patterns in practice.

Conflict of Interest

None declared.

Конфликт интересов

Не указан.

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