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WAYS OF REDUCTION OF DAIRY ALLERGY IN YAKUTIA BY GENOTYPING OF BETA-LACTOGLOBULIN GENE

ABSTRACT

In order to reduce the incidence of milk allergy among residents of Yakutia, Russia we studied an allelic and genotype variation of beta-lactoglobulin gene of cattle breed in the Republic of Sakha (Yakutia). Allele distribution analysis of beta-lactoglobulin gene among the studied breeds demonstrated that the frequency of allele B prevails in Kholmogor cattle, local selection of Simmental cattle and especially in Kalmyk cattle. The genotype associated with the low content of milk serum proteins, and therefore hypoallergenic milk is BB genotype. This genotype was observed in 67% of cows of Kalmyk breed and in 43% of cows of local Simmental breed.

The use of marker-assisted selection in dairy cattle will facilitate the increase in number of individuals with BB genotype of beta-lactoglobulin gene which is critical for the reduction of incidence of atopic allergies in the Republic of Sakha (Yakutia).

Keywords: Beta-lactoglobulin, genotyping, allergy, dairy cattle.

INTRODUCTION

Recently the world medical statistics indicates the growth of many allergic diseases. This is primarily connected to a number of factors promoting global allergization of the population: ecological deterioration, increased interaction of the population with chemicals, malnutrition, and consumption of food products with additives, the growing urbanization, changing in lifestyle, stress and increased consumption of medicines [7].

According to the Republic's Skin and Venereal Diseases Dispensary for the last decade atopic dermatitis is the most common type of dermatitis among children of age of 0-14 years. Allergic reaction to food products is a very common issue, especially milk allergy is widely represented among adults and children. The intolerance to cow's milk and other dairy products can be caused by many different abnormalities in carbohydrate, fat and protein catabolism. However, milk allergy is primarily caused by protein components. The major allergens of cow's milk are such components of milk serum as beta-lactoglobulin, albumin, alpha-lactalbumin, lactoferrin and immunoglobulins [5]. To reduce the allergic effect, milk is often exposed to heat inactivation, however harsh and prolonged heating decrease the nutritional value of the product. Thus, other methods of lowering the allergic effect of milk should be considered. One such method is based on production of milk with low content of beta-lactoglobulin facilitated by marker-assisted selection.

All milk proteins are characterized by the presence of genetically determined polymorphic variants with the difference in one or several amino acids, caused by nucleotide change in different alleles of a single gene. The most common variants of beta-lactoglobulin gene are *LGB A* и *LGB B,* which differ in two amino acid substitutions Asp 64 (LGB A) – Gly 64 (LGB B) и Val 118 (LGB A) – Ala 118 (LGB B) and are encoded in different alleles of the gene. The allele B of beta-lactoglobulin gene is associated with high content in casein proteins and fat, while A variant is characterized by high content of serum proteins [4].

Restriction Fragment Length Polymorphism Analysis of PCR-Amplified Fragments (PCR-RFLP) is a powerful method for the analysis of allele A of beta-lactoglobulin gene and it can be used in selection practice as a marker to produce milk with low content of beta-lactoglobulin on every stage of development of the animal [6].

Considering the connection of genes to milk allergy we propose to investigate genotype and allele variability of beta-lactoglobulin gene in cattle being breed in the Republic of Sakha (Yakutia). It is also necessary to study the polymorphism of beta-lactoglobulin gene in imported Simmental cattle of Austrian selection, Kalmyk cattle as well as in Simmental and Kholmogor cattle of local selection.

MATERIALS AND METHODS

In this study we have used the cohort of DNA samples from different breeds of cattle breed in the Republic of Sakha (Yakutia). The cohort included cows of Simmental breed ("Nayahy" and "Ust-Aldan" farms of Ust-Aldan region), Kholmogor breed (Kladovaya Olekmy, LLC, Olekminsk region and "Daiyna" farm of Namsky region), Simmental breed of Austrian selection and Kalmyk breed ("Nemygy agricultural company", Hangalassky region). In total samples from 156 animals of 4 breeds were studied including Simmental breed of local selection (n=86), Kholmogor breed of local selection (n=38), Simmental breed of Austrian selection (n=20) and Kalmyk breed (n=12).

All experimental work was performed in Genetics and Selection Laboratory of Yakut State Agricultural Academy. The blood samples for DNA isolation in the volume of 6 ml were harvested from the Jugular vein into sterile EDTA coated blood collection tubes. Genomic DNA was isolated by standard phenol-chloroform extraction method [3].

The method of Restriction Fragment Length Polymorphism Analysis of PCR-Amplified Fragments (PCR-RFLP) is based on sequence differences between allelic variants of the gene that lead to the absence or presence of specific restriction site, which can be detected and cleaved by certain restriction enzyme. For the analysis of herd structure according to beta-lactoglobulin gene we used the method described elsewhere [8].

For PCR analysis specific primers have been used, structure of which along with the location of restriction sites is presented in **Table 1.**

Table 1

Primers used for the determination of variants of beta-lactoglobulin gene

Gene	Sequence (5'-3')	Endonuclease	Restriction site	Reference
LGB	Forward: GTCCTTGTGCTGGACACCGACTACA Reverse: CAGGACACCGG CTCCCGGTATATGA	HaeIII	GG/CC	Medrano J.F. et al., 1990 [8]

According to the method of herd genotyping developed by Medrano J.F. and colleagues [6] part of beta-lactoglobulin gene is amplified resulting in PCR product with the size of 262 bp. PCR reactions were carried our using Tertsik thermal cycler, Russia. The total volume of the reaction mix was 25 µl, containing 10×Taq Polymerase Reaction buffer -2.5 µl, dNTP mix - 2.5 µl, Taq Polymerase – 0.5 µl , 0.5 µl of each primer, 18 µl of deionized water and 1 µl of sample DNA. PCR temperature conditions are shown in **Table 2**.

Table 2

PCR conditions

Gene	Amplicon size	Length of restriction fragments	PCR conditions
LGB	262	AA: 153, 109 AB: 153, 109, 79+74 BB: 109, 79+74	1. 94°C – 4 min 2. (94°C-1 min; 55°C-1 min; 72°C-1 min)*30 3. 72°C – 5 min

To determine the polymorphism of beta-lactoglobulin gene, PCR probes (20 µl) were treated with 5 units of HaeIII restriction enzyme in specific buffer at 37°C overnight. For product visualization horizontal agorose gel electrophoresis was performed. The gel contained 2% agarose and 0.5 µg/ml of ethidum bromide and was run under 15 V/cm for 50 min in 1×TBE buffer. Following genotypes were distinguished after the electrophoresis: AA with fragment

sizes of 153 and 109 bp, AB with fragment sizes of 153, 109, 79+74 bp and BB genotype with fragment sizes of 109, 79+74 bp (**Fig. 1**).

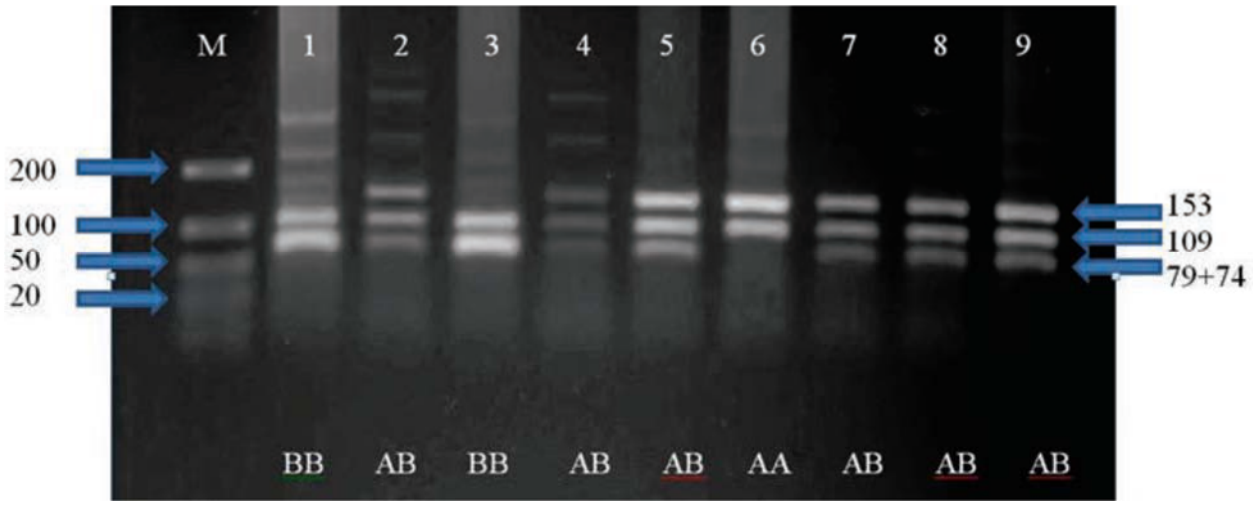

Figure 1. The data of PCR-RFLP analysis. M – Ultra Low Range DNA Ladder (Thermo Scientific); 1, 3 – BB genotype; 2,4,5,7,8,9 – AB genotype; 6 – AA genotype.

RESULTS AND DISCUSSION

With the use of PCR-RFLP analysis of DNA of different breeds of cattle we were able to reveal two alleles of beta-lactoglobulin gene (A and B) (**Table 3**).

Table 3

Frequency of alleles and genotypes of beta-lactoglobulin gene

Breed	Number of animals	Distribution *	AA		AB		BB		Allele frequency		X^2
				%	n	%	n	%	A	B	
Kholomogor breed	38			18	16	42	15	40	0.40	0.60	0.52
			.1	16	18.2	47.9	13.7	36.1			
Simmental breed of local selection	86		1	13	38	44	37	43	0.35	0.65	0.06
			0.5	12.2	39.1	45.5	36.4	42.3			
Simmental breed of Austrian selection	20			30	11	55	3	15	0.58	0.42	0.34
			.7	33.5	9.7	48.5	30.6	18.0			
Kalmyk breed	12			-	4	33	8	67	0.17	0.83	0.40
			,35	,9	,4	8,3	,75	8,8			

*A – actual genotype distribution, E – expected genotype distribution.

The calculations of correspondence of actual genotype distribution to theoretically expected distribution of the locus of beta-lactoglobulin gene revealed that all studied breeds maintain genetic equilibrium.

The frequency of B allele prevails in Kholmogor cows (0.60), local Simmental breed (0.65) and especially in Kalmyk cows (0.83) with the accuracy of $p<0.001$.

Some milk serum proteins cause allergic reaction in human body. It is known that beta-lactoglobulin is the major allergen among all milk serum proteins because it is not produced in human body during lactation.

The genotype associated with the low content of milk serum proteins, and therefore hypoallergenic milk is BB genotype [6]. This genotype was observed in 67% of cows of Kalmyk breed and in 43% of cows of local Simmental breed. Simmental cows of Austrian selection were predominantly represented by AB genotype (55%) and AA genotype (30%) of beta-lactoglobulin gene. AA genotype is not present in Kalmyk cows. AB genotype prevails in Simmental cows of Austrian selection, while local Simmentals were primarily represented by BB genotype of beta-lactoglobulin gene.This could be an indicative of directed selection. Since the majority of farms in the Republic are focused primarily on production of dairy products they prefer breeds of dairy cattle. Kalmyk cattle belongs to beef cattle, thus the selection of the breed was based on improving meat quality. Simmentals of Austrian selection belong to dairy cattle, which selection is based on high milk productivity.

CONCLUSION

The incidence of allele *LGBA* was lower in cattle following breeds - holmogorskoj (40 %) , Kalmyk (17 %) and local Simmental breeding (35 %) . Cows Austrian Simmental breeding , this figure was higher and amounted to 58 % .

The genotype associated with the hypoallergenic milk was observed in 67% of cows of Kalmyk breed and in 43% of cows of local Simmental breed. Simmental cows of Austrian selection were predominantly represented by AB genotype (55%) and AA genotype (30%) of beta-lactoglobulin gene.

The use of marker-assisted selection in dairy cattle will facilitate the increase in number of individuals with BB genotype of beta-lactoglobulin gene which is critical for the reduction of incidence of atopic allergies in the Republic of Sakha (Yakutia).

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