

**Single nucleotide polymorphism in dairy cattle populations of West Siberia**

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**ABSTRACT:** SNP data on tumor necrosis factor-alpha (TNF- $\alpha$ ) and TNF-R1 -1703T/C taken from 367 Red Steppe and Black and White cows bred in 3 Siberian farms were analyzed. The G allele frequencies in Red Steppe and Black-and-White population were found to be 0.510 and 0.526 respectively. It was estimated that milk and fat yield in first lactation per 305 lactation days of G/G Red Steppe cows was higher than in animals with A/A genotype. Milk and fat productivity were higher in G/G animals (farm 2) in comparison to heterozygotes for TNF- $\alpha$  -824 A/G SNP.

However, it was dissimilarity among cows possessed different genotypes in farm 1. Milk yield was not associated with genotypes on TNF-R1 -1703T/C SNP. In fact, only cows with complex genotypes TNF- $\alpha$  -824 A / G and TNF-R1-1703T/C AA/TT and AA/CC at first lactation had a higher milk yield than AG/TT cows.

**Keywords:** SNP; breed; Red Steppe; Black-and-White; milk yield.

### Introduction

Tumor necrosis factor (TNF- $\alpha$ ) in animals plays an important role in development of mammary gland, corpus luteum, synthesis and apoptosis cells (Sacumoto et al (2014)), involved into progression of bovine leukemia virus-infection (Konnai et . al. (2006); Rodrigues et. al. (2011)). That is associated with the level of cows' milk production (Yudin et. al. (2013); Lyukhanov et. al. (2014)).

TNF- $\alpha$  is a nonglycosylated protein with a molecular weight of 17 kDa. There are 2 immunologically distinct receptors (TNF-R1 and TNF-RII). The TNF- $\alpha$  gene is located in bovine chromosome 23 and consists of 2,773 base pairs. The type I receptor (TNF-R1) is located in chromosome 5 and found to have single nucleotide polymorphism (SNP) at -1703T/C position.

The research is aimed to study genetic structure of Red Steppe and Black and White cattle populations, to assess relationship of TNF- $\alpha$  -824 A/G и TNF-R1 -1703T/C SNP with biochemical and milk productivity parameters.

### Materials and Methods

**Heifer rearing facility.** The research was conducted at the base of three farms located in Novosibirsk region and Altai Territory. 100 animals of Red Steppe and 267 of Black and White breeds were used to take samples

for further analysis. Dairy cows were kept either in a free-stall barn or in a pasture. TNF- $\alpha$  -824 A/G SNP was determined by PCR-RFLP method using 5'-CCGAGAAATGGGACAACCT-3' and 5'-GCCATGTATCCCCAAAGAAT-3' reverse primers. It was putted EcoICRi restriction endonuclease and estimated the results in polyacrylamide gel. TNF-R1 -1703T/C SNP was identified by the method of allele-specific PCR inserting the investigated DNA into 2 tubes. Forward 5'-TCCGAGCCCCGCCTTCTGT-3', 5'-TCCGAGCCCCGCCTTCTGT-3' and reverse 5'-GGTGCCAGCATCGTGCCTGC-3' primers were employed to allow determination C and T alleles respectively. Renaturation temperature for all the primers was 60<sup>o</sup>C. The amplification products were evaluated in the gel. Ethidium bromide was used to stain the gel.

The milk productivity was assessed for 305 lactation days using fat and protein milk content in percentages. It was found the content of the following biochemical parameters in blood serum of Red Steppe cows: bilirubin, total bilirubin direct, cholesterol, alkaline and acid phosphatases activity, creatinine, generation protein, albumins,  $\alpha$ -globulins,  $\beta$ -globulins,  $\gamma$ -globulins, ALT and AST activity, urea, GGTP, LDG, glucose,  $\alpha$ -amilase activity, TG, LHD, LLD, Ca, P, Fe, Mg and etc.

**Statistical analyses.** Allele frequencies were estimated by the direct counting way. Phi and t-tests were conducted for animal groups to be compared on qualitative traits. The estimation was done for the concordance of genotype frequencies with those theoretically anticipated in case of genetic equilibrium of the population following Hardy-Weinberg's law (Weir (1996)). In that way the disequilibrium  $D_A$  and chi-square criterion were estimated. Analysis was performed by R environment.

### Results and Discussion

Single nucleotide polymorphism in the populations of Red Steppe and Black and White cattle bred in West Siberia was identified on the TNF- $\alpha$  (824A/G) gene. A and G alleles frequencies was equal to 0.490 and 0.510 respectively (table 1). The same frequency of A allele (0.521) was established in Grey Ukrainian breed by other researchers. The frequency of A allele in Yakut cattle was noticeably lower (0.227) at the same environment conditions (Yudin et al., (2013)). Our results were also corresponded with ones obtained by Japanese scientists

which studied Japanes Holstein-Friesian cattle populations for A and G alleles (Konnai et. al., (2006)). Further analysis showed no differences among the breeds in alleles and genotype frequencies.

**Table 1. The Frequency of genotypes and alleles in populations of cattle of Siberia**

Breeds	Parameter	Genotypes			Alleles	
		A/A	A/G	G/G	A	G
Red Steppe	n	26	46	28		
	frequency	0.260	0.460	0.280	0.490	0.510
Black-and White, farm 1	n	43	77	40		
	frequency	0.269	0.481	0.250	0.509	0.491
Black-and White, farm 2	n	22	46	39		
	frequency	0.206	0.430	0.364	0.421	0.579
Black-and White, farm 1+2	n					
	frequency	0.243	0.461	0.296	0.474	0.526

There were no differences or correlations among the biochemical indices in relation to genotype in Red Steppe breed. Milk yield and milking rate were significantly higher in G/G genotype in comparison with homozygous A/A cows ( $p < 0.001$ ). Heterozygous animals took the median position.

In case of comparison of fat and protein percentages within the available animals grouped by the genotypes we could not estimated any statistically significant differences. The same results were established for Black and White cattle (farm 1). At the same time, the milk yield and milk fat production in G/G homozygous animals were uncovered to be higher. In fact, A/A and G/G cows had the same milk yield (Yudin et al., (2013)). Thus, it is possible to use SNP as DNA-marker in some populations of the cattle.

The frequencies of C and T alleles (TNF-R1 gene) in Black and White cattle were 0.719 и 0.281 (Table 2). It was also noteworthy the low concentration of T/T genotype (6,3%).

**Table 2. Genotypes and alleles frequencies in Black and White cattle for TNF-R1 -1703T/C gene**

	Genotypes			Alleles	
	C/C	C/T	T/T	C	T
Number of cow	80	70	10		
Frequency	0.500	0.437	0.063	0.719	0.281

There were no differences among the milk productivity indices for the genotypes (TNF-R1 -1703T/C). The milk yield at the first lactation however was in 1.15 times higher in animals (farm 1) with complex genotypes TNF- $\alpha$  -824 A/G и TNF-R1 -1703T/C AA/TT и AA/CC than in AG/TT animals ( $p < 0.001$ ). The rest of parameters studied were not shown significant differences within the genotypes.

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