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ASSOCIATIVE IMPACT OF TNFα GENE ALLERIES ON THE REPRODUCTIVE FUNCTION OF COWS OF "BAYSERKE-AGRO" LLP

Abstract. The authors of the article carried out work on the genotyping of Holstein breeding cows in Bayserke-Agro LLP on the TNFα gene locus and identified animals with the desired genotype (GG genotype) on the locus under study. SNP polymorphism in the promoter part of the TNFα gene at position 824 A → G in Holstein cows is represented by the following genetic variants: AA - 22.4%, AG - 63.8%, GG - 13.8%, the frequency of A and G alleles was 0 54 and 0.46. In the studied population, there was an excessive occurrence of the heterozygous genotype AG +21.49, according to other genotypes, there was a deficiency of homozygous variants GG and AA, respectively - 11.16 and -10.32 individuals. Reproductive rates were high in cows with the GG genotype: the interval between calving and fruitful insemination was 89 days, the insemination index was 1.63, the proportion of animals inseminated after more than 30 days was minimal (52%) in individuals of the homozygous GG genotype. It has been established that the use of the PCR-RFLP method of analysis allows genetic certification of breeding animals and predict their reproductive function.

Key words: promoter part of TNFα gene, PCR-RFLP, reproductive function of cows, DNA markers.

Studies by Japanese scientists have established the effect of polymorphism in the promoter part of the TNFα gene and SNP replacement of one nucleotide in the exon part of the named gene on the immune status and reproductive function in cows. The authors of the study population of dairy cows for the TNFα locus identified the following genetic variants: A / A, A / G, G / G and T / T, T / C, C / C in the promoter and exon parts of the gene, respectively [1].

The interval between calving and the first ovulation was short in cows with a heterozygous genotype A / G and a homozygous genotype G / G compared with animals with a homozygous genotype A / A. Polymorphism of the promoter portion of the tumor necrosis factor (TNFα) gene in cows does not affect the rate of apoptosis of polymorphonuclear leukocytes. However, the rate of transmigration was significantly higher in animals with genotypes A / A and A / G compared to animals with homozygous genotype G / G. A correlation was found between the expression level of the mRNA of the promoter part of the TNFα gene and the formation of interleukin 8 (IL-8), which performs a protective function in the body. Thus, mRNA expression of polymorphonuclear leukocytes and peripheral blood mononuclear cells was higher in cows with genotype A / A compared to genotype G / G. The results indicate that TNFα gene allele polymorphism has a significant effect on immune function and reproductive performance in cows. Thus, according to the results of Japanese scientists, the proportion of cows that showed ovulation within three weeks after calving in individuals with homozygous GG and heterozygous AG genotypes in the SNP polymorphism locus of the TNFα promoter part was the same, 59.5% and 57.1%, respectively, alleles of this gene did not affect the number of insemination [1].

Associative data and some studies suggest that inhibiting the expression of the TNFα gene contributes to liver obesity with an energy deficit in dairy cattle. Experimentally, in vitro cultivation, the ability of TRLP to inhibit TNFα signaling on primary cattle hepatocytes with recombinant TNFα has been

proven. Four Holstein breed lactating cows injected TRLP subcutaneously for 24 hours with an interval of 4 hours at a rate of 0.15 and 3.0 mg per kg body weight and intravenous recombinant TNFα at a dose of 5 μg per kg body weight of the cow. According to the results of the study, injection of recombinant TNFα and TRLP for 2 hours provides a reduction in the amount of non-esterified fatty acid in plasma (non-esterified fatty acid, NEFA), which indicates a change in the metabolic process in the body of cows. Despite the fact that TRLP inhibited signals of bovine TNFα using recombinant TNFα for 7 days did not change the metabolism in cows with a negative energy balance [2].

Thus, the development and introduction into the DNA selection practice of markers of the reproductive function of animals, milk and meat productivity, the creation of a population of animals that are resistant to diseases, the prediction of useful traits is an urgent problem of molecular and population genetics.

The goal of the study was genotyping of Holstein cows of Bayserke-Agro LLP at the TNFα gene locus by PCR-RFLP analysis, studying the effect of the alleles of the gene under study on the manifestation of reproductive function.

Material and method. The experiments were carried out on 152 Holstein cows of the Canadian breeding farm breeding; blood for the study was taken from the jugular or caudal vein into the vacuum tube with EDTA. DNA isolation was carried out by the phenol method. Amplification of the TNFα gene region was performed on an Efendorf amplifier (Germany) using primers: F 5′-GAGAAATGGGACAACCTCCA-3 ′ and R: 5′-CCAGGAACTCGCTGAAACTC - 3 ′ [3].

The length of the obtained amplification of the tumor necrosis factor (TNFα) gene was 249 bp. (figure1), for the detection of SNP polymorphism at position 824 A → G, Sac I restriction enzyme, which has the restriction site GAGCT / C, was used after restriction of the PCR product, depending on the genotype of animals, electrophorems appeared fragments: in individuals with a heterozygous genotype AG - 249 , 168 and 81 bp, for homozygous AA and GG, respectively, 168, 81 bp. and 249 bp (figure2). To visualize the results of electrophoresis, Infinity VX2 3026 gel-documenting system was used, WL / LC / 26M X-Press, Vilber Lourmat (USA), as a DNA marker plasmid pUC19 / MspI (Thermo Fisher Scientific).

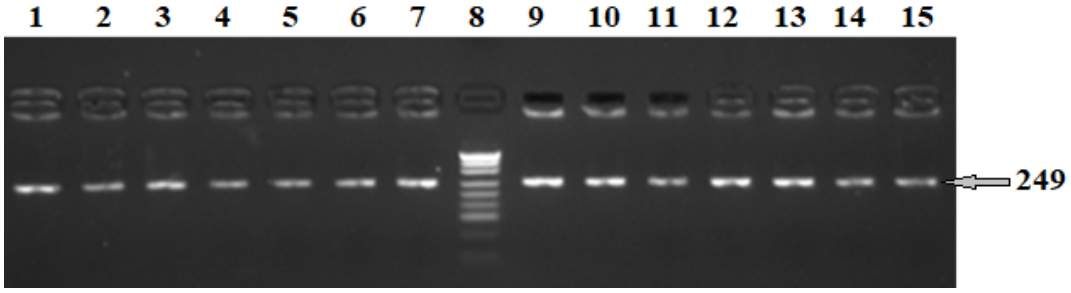

Figure 1 – Electrophoregram amplification of the TNFα gene, agarose 3%, lanes 1-7, 9-15 PCR product, lane 8 — DNA marker pUC19 / MspI

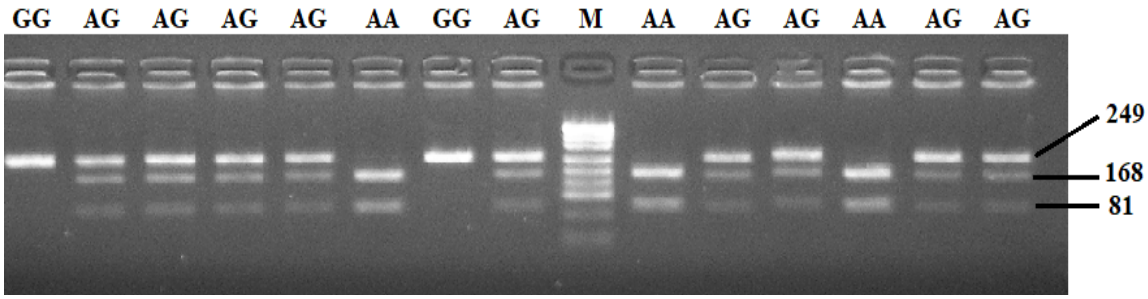

Figure 2 – Electrophoregram of the restriction enzyme Sac I PCR gene product TNFα, agarose 3%, M - DNA marker pUC19 / MspI, genetic variants GG, AG, AA

Results. The results of identification of SNP polymorphism in the promoter part of the TNFα gene at position 824 A → G in Holstein cows in the amount of 152 heads of the breeding farm "Bayerke-Agro" LLP indicate a shift in the frequency of allele A compared to G, 0.54 and 0.46, respectively. In the studied group of animals, the heterozygous AG genotype prevails and its prevalence is 63.8%, the occurrence of homozygous genotypes was: GG -13.8% and AA - 22.4%.

In cows with a homozygous genotype GG (n = 19), the interval between calving and fruitful insemination was 89 days, in individuals with a heterozygous AG genotype (n = 50), this indicator had a value of 128 days, the intermediate position (95 days) was occupied by animals with genotype AA (n = 27). There is a correlation between the interval from calving to fruitful insemination and the insemination index in animals of all three groups, the low insemination index (1.63) was in cows with the homozygous GG genotype (the interval duration was 89 days), the high insemination index (2.76) was heterozygous animals (the duration of the interval is 128 days). In homozygous individuals with genotype AA, the insemination index had a value of 1.85, the duration of the interval between calving and fruitful insemination was 95 days.

Indicators of reproductive function of cows with different genotypes at the TNFα gene locus in cows (n = 96)

Animals with genotype TNFα (n=96)	Interval between calving and fruitful insemination (days)	Cow insemination index	The number of fruitfully inseminated cows in the period more than 30 days after calving
GG (n=19)	89	1,63	9/47,36%
AG (n=50)	128	2,76	43/86,0%
AA (n=27)	95	1,85	17/62,96%

Discussion. Analysis of the literature shows that most authors study polymorphism in the promoter part of a gene, since the level of gene expression depends on the functional activity of the promoter part of the corresponding gene [4].

We found that in all three genetic variants in cows there is a discrepancy between the actual distribution of genotypes and the theoretically expected number of genotypes, an excessive occurrence of the heterozygous genotype AG +21.49 was found, on the contrary, other genotypes showed a deficit of homozygous variants GG and AA, respectively -11 16 and -10.32 individuals. Similar results were obtained by foreign authors, so the distribution of genetic variants of the promoter part of the TNFα gene in Japanese dairy cows was AA - 36 (16%), AG - 108 (48%), GG 80 (36%) heads [1]. An SNP study of the polymorphism of the promoter part of the TNFα gene at position 824 A → G in cows (n = 127) of Holstein breed shows a more even distribution of genetic variants: AA - 26.0%, AG -37.8% and GG - 36.2% [five]. As can be seen from table 1, there is an association of the homozygous GG genotype with reproductive function indicators in the studied group of animals (the minimum interval between calving and fruitful insemination, a low insemination index, the minimum number of cows (47.36%) fruitfully inseminated during more than 31 days after calving).

Conclusion. Thus, the studied locus of the promoter part of the TNFα gene in Holstein cows is polymorphic, the prevalence of genetic variants is: AA - 22.4%, AG - 63.8% and GG -13.8%. The positive effect of the GG genotype of Holstein cows on reproductive function was established, and in heterozygous animals the reproductive abilities were low.

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TNFα ГЕН АЛЛЕЛЬДЕРІНІҢ «БАЙСЕРКЕ-АГРО» ЖШС СИЫРЛАРЫНЫҢ РЕПРОДУКТИВТІ ФУНКЦИЯСЫНА ТИГІЗЕТІН АССОЦИАТИВТІ ӘСЕРІ

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АССОЦИТАТИВНОЕ ВЛИЯНИЕ АЛЛЕЛЕЙ ГЕНА TNFα НА РЕПРОДУКТИВНУЮ ФУНКЦИЮ КОРОВ ТОО «БАЙСЕРКЕ-АГРО»

Аннотация. Авторами статьи проведена работа по генотипированию племенных коров голштинской породы в ТОО «Байсерке-Агро» по локусу гена TNFα и выявлены животные с желательным генотипом (генотип GG) по изучаемому локусу. SNPполиморфизм в промоторной части гена TNFαв позиции 824A→Gy коров голштинской породы представлены следующими генетическими вариантами: AA - 22,4%, AG - 63,8%, GG -13,8%, частота аллелей A и G составила 0,54 и 0,46. У исследуемой популяции выявлена избыточная встречаемость гетерозиготного генотипа AG+21,49, по другим генотипам отмечается дефицит гомозиготных вариантов GGи AA, соответственно на -11,16 и -10,32 особей. Показатели репродуктивной функции были высокими у коров с генотипом GG: интервал между отелом и плодотворным осеменением составил 89 дней, индекс осеменения1,63, доля животных, осемененных по истечению более 30 дней была минимальной (52%) у особей гомозиготного генотипа GG. Установлено, что использование метода ПЦР-ПДРФ анализа позволяет проводить генетическую паспортизацию племенных животных и прогнозировать их воспроизводительную функцию.

Ключевые слова: промоторная часть гена TNFα, ПЦР-ПДРФ, репродуктивная функция коров, ДНК маркеры.

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