

Productivity of Cows of the Ukrainian Red-Spotted Dairy Breed with Different Genotypes According to the TLR1, SLC11A1 and CSN2 Loci

Oksana Ivashchenko^{*}, Roman Kulibaba

National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

Abstract. The use of methods of marker-associated selection opens up new perspectives in the context of maximum realization of the productive potential of animals, which makes the issue of the application of modern scientific approaches in animal husbandry particularly relevant. Considering this, the analysis of the productive qualities of cattle with different genotypes according to the set of candidate genes is one of the most urgent tasks of animal genetics and breeding. Therefore, the purpose of this study is to analyze the productive qualities of cows of the Ukrainian red-spotted dairy breed with different genotypes according to the TLR1, SLC11A1 and CSN2 loci. Genotyping of cattle individuals was performed using the PCR-RFLP method for TLR1 and SLC11A1 and AS-PCR for CSN2. The analysis of productive qualities of animals was carried out by comparing the parameters of three lactations for each group of animals according to the indicators of average milk yield for 305 days of lactation (kg), protein content in milk (%) and milk fat content (%). The study was carried out on the basis of previously obtained results of individual typing of individuals according to research loci. According to the results of studies conducted in the experimental population of cows for the toll-like receptor 1 (TLR1) locus for the first and third lactations, the dominant values of the yield index are characteristic of homozygotes with the GG genotype, while for the second lactation the highest values of yield are observed for homozygous individuals for allele A. At the same time, a probable difference between the indicators of individuals with different genotypes was not established. No significant difference between the groups of cows with the studied genotypes in the parameters of fat and protein content in milk by the TLR1 locus was also found. SLC11A1 is characterized by a characteristic difference from the previous gene, as two mutations forming haplotypes – CC-AA, CG-AA and CG-AT out of nine potentially possible variants were found in this locus. According to indicators of milk productivity, animals with the CG-AA haplotype had higher values of milk compared to other haplotypes during all three lactations (the difference is probable). The greatest differences were observed with the CG-AT haplotype. According to the indicators of the content of milk protein and fat, no probable differences between individuals with different haplotypes were established. The variability of signs for all lactations was at a low level. As for the beta-casein locus, according to the results of the conducted research, no significant difference in the indicators of milk productivity of cows with different genotypes was found. The highest confidence values are characteristic of individuals with the A1A1 genotype, however, the difference with individuals with other genotypes is unlikely. In any case, the A2 allele, according to the value of the indicators of homozygous individuals (genotype A2A2), does not have a negative effect on the parameters of milk productivity of animals

Keywords: polymorphism, allele, population, productivity, cows

INTRODUCTION

The basis of the high efficiency of selection work in animal husbandry is based on the principle of following the advanced achievements of genetics as the fundamental basis of selection. Over the past several decades, DNA-oriented technologies have become the most widely used – from the use of various types of

molecular genetic markers to genomic selection. The possibility of direct assessment of an animal's genotype by DNA, in the absence of phenotypic assessment at this stage, made it possible to carry out large-scale passporting of populations of representatives of various agricultural animal species, as well as to identify carriers of productive and counterproductive alleles

Article's History:

Received: 18.01.2022

Revised: 17.03.2022

Accepted: 26.04.2022

Suggested Citation:

Ivashchenko, O., & Kulibaba, R. (2022). Productivity of cows of the Ukrainian red-spotted dairy breed with different genotypes according to the TLR1, SLC11A1 and CSN2 loci. *Ukrainian Black Sea Region Agrarian Science*, 26(2), 35-42.

and genotypes. Today, in Ukraine, despite the difficult circumstances associated with the difficulties of financing scientific research programs in animal husbandry, work is being carried out on the use of methodical approaches of marker-associated selection (MAS), aimed at determining the characteristics of the genetic and population parameters of research groups of animals, as well as for the analysis of the productive qualities of individuals with different genotypes based on the set of potential candidate genes. Toll-like receptor 1 (TLR1), transmembrane protein SLC1 (SLC11A1) and beta-casein (CSN2) genes are among the most promising in cattle genetics. The presence of marker alleles, which are associated with economic and valuable traits of animals within experimental groups, is quite important for targeted selection in order to maximize the productive potential of cattle. Undoubtedly, traditional methods of animal selection based on phenotypic traits (selection by phenotype) are of great importance in the selection process. However, from the point of view of world practice, it is the selection by genotype (MAS) that makes it possible to significantly increase the efficiency of the work being carried out.

According to the results of numerous studies, the presence of a number of mutations in the above-mentioned loci has been established, which in some breeds of cows are associated with adaptive qualities of animals [1-3]. On the other hand, the relationship with indicators of milk productivity of animals (protein content, fat content, etc.) has practically not been investigated. Considering the above, the purpose of further research was to analyze the productive qualities of cows of the Ukrainian red-spotted dairy breed with different genotypes according to the TLR1, SLC11A1 and CSN2 loci.

Vertebrate TLR1 and SLC11A1 genes are associated with the regulation of immune responses against a wide range of pathogenic microorganisms, and their products, proteins, by their function mostly belong to receptors involved in the processes of innate immunity. Individual mutations of the TLR1 gene reduce the effectiveness of the immune response against lipopeptide and lipopolysaccharide components of the cell wall of various types of bacteria [4; 5]. In turn, the SLC11A1 gene encodes a transmembrane protein known as one of the potential targets that contribute to innate immunity against various intracellular pathogens (tuberculosis [1], bovine brucellosis [2; 3]).

An important role of the TLR1 gene in the implementation of adaptive immunity lies in its ability to influence antimicrobial activity in the skin, mucous membranes of the respiratory tract, and the gastrointestinal tract of animals [6]. With the help of phylogenetic analysis, it was established that the TLR1 gene, together with TLR6 and TLR10, is characterized by a common origin and is located in a tandem that forms a gene cluster of ~69 kb TLR6-TLR1-TLR10 on *Bos taurus* chromosome 6 (bta6) [7]. According to the results of the mapping of quantitative traits QTL (Quantitative Trait Loci) in the cattle genome, it was established that this cluster is located in a dense QTL region, which is

important for a number of traits of technical parameters of milk and the manifestation of clinical mastitis [8]. Under these circumstances, the TLR1 locus turned out to be a promising candidate for the study of animal resistance/susceptibility parameters to diseases such as mastitis [9]. Different authors found out that mutations in genes of the TLR family lead to a decrease in the efficiency of pathogen recognition and activation of the link of the innate immune system [10; 11]. In particular, Cinar and co-authors [12] revealed the association of research genotypes of cows with the risk factor for infection of animals with paratuberculosis (John's disease, pTB). Based on the results of immunoenzymatic analysis, it has been proven that East Anatolian Red and Anatolian Black breeds are more resistant to paratuberculosis compared to Holsteins, therefore, selection of animals for a certain genotype of TLR1 (+1380A/G) can potentially reduce the risk of developing this disease in cattle. Other scientists have demonstrated that some mutations of the TLR1 gene are associated with the manifestation of clinical signs of such infections as brucellosis and bovine tuberculosis [13; 14]. Instead, Russell and co-authors established an association between different SNPs in the TLR1 locus, reduced levels of clinical mastitis (CM) and increased protein and fat content in milk [15].

The SLC11A1 gene, located on chromosome 2, consists of 15 exons and 14 introns, with a total size of ~11 kb. In addition to associations of this gene with parameters of resistance/sensitivity to various infectious diseases, a reliable connection between research genotypes of the Holstein breed and the manifestation of clinical mastitis has been proven. It can be assumed that the SLC11A1 locus is a potentially valuable candidate gene, first of all, for improving resistance to mastitis, as well as for determining the parameters of productive traits in dairy cattle populations [16]. This is confirmed by the studies of other scientists, who found a correlation between NRAMP1 (alternative name SLC11A1) polymorphisms and the number of somatic cells in the milk of cows [17].

In contrast to the above, the beta-casein gene refers to objects related not to the functioning of the body's immune system, but to milk proteins. In cow's milk, beta-casein belongs to one of the most common fractions, while two variants – A1 and A2 – are the most interesting and promising for research. Variant A1 is (potentially) associated with a set of pathological effects on the human body if milk containing beta-casein of this type is consumed. In turn, the A2 option is completely devoid of these features. However, the question of potential counterproductiveness of the "desired" A2 allele (association with low milk yield compared to A1) remains open. In this regard, in addition to the issues of general typing of animals of different cattle populations according to A1 and A2 alleles, it is also advisable to investigate the productive qualities of individuals with different genotypes.

Previously, in our research, we determined the features of the genetic structure of research populations

of cattle of various breeds based on the TLR1, SLC11A1, and CSN2 loci [18]. In this work, we focus on the study of productive parameters (milk yield) of cattle (Ukrainian red-spotted dairy cow breed) with different genotypes according to the above-mentioned loci.

MATERIALS AND METHODS

The study was conducted in the laboratory of molecular genetic and physiological and biochemical research in animal husbandry of the Institute of Animal Husbandry of the National Academy of Sciences and in the laboratory of molecular genetic research of the Department of Animal Biology of the National University of Biore-sources and Nature Management of Ukraine.

As an object of research, we used a population of cows of the Ukrainian red-spotted dairy breed (DPDG "Gontarivka", Kharkiv region, Vovchanskyi district). DNA was isolated from individual samples of biological material (hair bulbs) using a commercial kit of reagents "DNA-Sorb-B" (Amplisens) according to the manufacturer's recommendations. Genotypes were determined using the PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphism) method for TLR1 (Bc1I polymorphism) and SLC11A1 (PstI polymorphism), and using AS-PCR for CSN2.

To separate the restriction/amplification fragments, electrophoresis was performed in a 1.5% agarose gel with the addition of ethidium bromide as a dye.

A UV transilluminator with a wavelength of 312 nm was used for visualization.

The value of average milk yield for 305 days of lactation (kg), indicators of milk fat content (%) and protein content in milk (%) were used to study parameters of milk productivity of animals. Performance analysis was performed by comparing the parameters of the first three lactations for each studied locus. The analysis of productive parameters of cows with different genotypes for the TLR1, SLC11A1 and CSN2 loci was carried out using one-way analysis of variance (ANOVA) and the Tukey-Kramer multiple comparisons test as a tool for post-hoc testing. Calculations were made in Microsoft Excel using the Real Statistics Resource Pack. Distribution was tested for normality using the Shapiro-Wilk test. In case of deviation from the normal probability distribution, the non-parametric Mann-Whitney U-test was used.

RESULTS AND DISCUSSION

Based on the results of the research, the milk productivity parameters of cows of the Ukrainian red-spotted breed with different genotypes according to the TLR1 locus were determined (Table 1). Taking into account the peculiarities of the genetic structure of the experimental group of cows, parameters were compared between individuals with all possible (according to this locus) genotypes – AA, AG and GG.

Table 1. Indicators of milk productivity of cows of the Ukrainian red-spotted breed with different genotypes according to the TLR1 locus

Indicator	Genotype		
	AA	AG	GG
First lactation			
Milking, 305 days, kg	5370.5±306.02 ^a	5135.9±158.20 ^a	5375.7±280.27 ^a
C _v , %	13.96	14.11	18.79
Fat, 305 days, %	3.82±0.021 ^a	3.86±0.017 ^a	3.87±0.034 ^a
C _v , %	1.32	2.05	3.13
Protein, 305 days, %	3.32±0.048 ^a	3.31±0.028 ^a	3.319±0.022 ^a
C _v , %	3.55	3.86	2.35
Second lactation			
Milking, 305 days, kg	6214±267.85 ^a	5949.3±159.42 ^a	6127.5±281.33 ^a
C _v , %	10.56	12.28	16.55
Fat, 305 days, %	3.73±0.032 ^a	3.76±0.015 ^a	3.77±0.026 ^a
C _v , %	2.11	1.81	2.48
Protein, 305 days, %	3.26±0.066 ^a	3.25±0.025 ^a	3.26±0.026 ^a
C _v , %	4.95	3.58	2.88
Third lactation			
Milking, 305 days, kg	6760.5±301.21 ^a	6576.5±129.53 ^a	6783.7±240.83 ^a
C _v , %	10.91	9.03	12.80
Fat, 305 days, %	3.67±0.030 ^a	3.69±0.013 ^a	3.70±0.022 ^a
C _v , %	2.02	1.56	2.12
Protein, 305 days, %	3.19±0.057 ^a	3.17±0.027 ^a	3.15±0.032 ^a
C _v , %	4.41	4.02	3.63

Note: different subscripts (a, b) indicate the probability of difference ($p < 0.05$) within the indicator

According to the research results, it was found that for both the first and third lactation, the maximum values of the lactation index are characteristic of homozygotes for the G allele, however, AA homozygotes have practically identical values. In turn, on the second lactation, the situation changes somewhat – the highest milk yield values are characteristic of individuals homozygous for the A allele, while for homozygotes GG, a slight difference in this indicator was noted (87 kg). Based on the obtained results, no probable difference between the values of the indicators was found (Table 1). In addition, according to the yield index for 305 days, the value of the coefficient of variation was mostly within the average level of variability of the indicator (it did not exceed the limit of 19%) for all three lactations.

It should be noted that the sample was characterized by a small number of individuals of individual genotypes, which is explained by the inclusion of only individuals of the first three lactations, which thus reduced the total number of animals in groups relative to one or two lactations.

No significant difference was found between the groups of cows with the tested genotypes for the TLR1

locus regarding the indicators of milk fat and protein content. According to the parameters of the content of milk fat and protein in relation to the value of the coefficient of variation, all groups of animals are characterized by a low level of variability (the value of Cv in each case is below 5%).

The SLC11A1 locus is significantly different from the TLR1 described above. In this locus, two mutations that form haplotypes – CC-AA, CG-AA and CG-AT out of nine possible variants were investigated. Other variants of haplotypes were not detected in the research population of cows. In more detail, the results of studies of the features of the genetic structure of cow populations of various breeds of Ukrainian selection based on the TLR1 and SLC11A1 genes are considered in our previous work [18]. Features of the distribution of haplotypes in the research population of animals (ratio of frequencies of individuals with different haplotypes) made it possible to analyze the productive indicators of cows (Table 2). It should be noted that in this case, the analysis of performance indicators of animals with different haplotypes has more practical value compared to individual genotypes. This is due to the fact that the haplotype is a single functional unit.

Table 2. Indicators of milk productivity of cows of the Ukrainian red-spotted breed with different haplotypes at the SLC11A1 locus

Indicators	Haplotype		
	CC-AA	CG-AA	CG-AT
First lactation			
Milking, 305 days, kg	5006.8±129.79 ^{ab}	5587.5±222.17 ^b	4814.6±211.28 ^{ac}
C _v , %	9.69	17.3	11.61
Fat, 305 days, %	3.88±0.026 ^a	3.84±0.02 ^a	3.89±0.031 ^a
C _v , %	2.57	2.28	2.11
Protein, 305 days, %	3.31±0.027 ^a	3.335±0.029 ^a	3.29±0.029 ^a
C _v , %	3.09	3.76	2.40
Second lactation			
Milking, 305 days, kg	5884.4±119.33 ^{ab}	6295.9±233.92 ^b	5696.3±213.31 ^{ac}
C _v , %	7.59	16.19	9.90
Fat, 305 days, %	3.776±0.019 ^a	3.74±0.019 ^a	3.78±0.021 ^a
C _v , %	1.91	2.29	1.48
Protein, 305 days, %	3.26±0.029 ^a	3.26±0.029 ^a	3.22±0.034 ^a
C _v , %	3.42	3.87	2.84
Third lactation			
Milking, 305 days, kg	6518.2±133.62 ^{ab}	6899.7±200.68 ^b	6358.1±67.03 ^{ac}
C _v , %	7.67	12.68	2.79
Fat, 305 days, %	3.72±0.017 ^a	3.68±0.016 ^a	3.7±0.019 ^a
C _v , %	1.74	1.94	1.35
Protein, 305 days, %	3.18±0.027 ^a	3.17±0.029 ^a	3.13±0.057 ^a
C _v , %	3.24	4.07	4.84

Based on the results of the research, it was found that according to the indicators of milk productivity, individuals with the CG-AA haplotype are characterized by higher yield values compared to individuals with other

haplotypes (the difference is probable, $p < 0.05$). Moreover, the biggest difference is observed with the CG-AT haplotype (772.9 kg). This tendency (predominance of index values in individuals with the CG-AT haplotype)

was noted during all three lactations. For individuals with the CG-AA haplotype, the maximum value of the coefficient of variation was also established, which indicates the average level of trait variability, but these values do not go beyond the definition of the homogeneity of the sample. In our opinion, this is due, in addition to everything else, to the need to analyze a much larger sample of animals in the case of a large number of potential variants of variability (9 possible haplotypes).

Based on the indicators of the content of milk protein and fat, no probable differences between individuals with different haplotypes were established. The variability of signs for all lactations was within the insignificant (low) level.

It should be noted that the situation may be different for the SLC11A1 locus due to the variability of the distribution of haplotypes in the group, but, in any case, the results of the research indicate the perspective of further research on other populations and breeds of cattle (subject to the mandatory condition of increasing the sample of animals, which will it is possible to analyze the productive parameters of individuals with other haplotypes).

Regarding the above two loci, it should be noted that most studies are devoted to the analysis of the relationship between TLR1, SLC11A1 gene polymorphisms and resistance/susceptibility to various infectious diseases, in contrast to our study, which covers the parameters of milk productivity of animals. In various scientific

works, it is stated that genes of the TLR family are associated with the innate immune response in various animal species [1; 12]. In the studies of Bjelka and co-authors [19], performed on breeding cows of the Czech Red breed, the association of the synonymous polymorphism 798 C>T TLR1 with reproductive and physical characteristics of cows, namely the connection with ease of calving and duration of productivity, was proven.

In the case of SLC11A1, Bagheri and other authors established [16] that the g.1139 C>G mutation in exon 11 of the indicated gene of the Holstein breed had a probable connection with the signs of clinical mastitis ($P<0.05$). A. Ateya et al., [20], using the example of Holstein and Brown Swiss dairy cattle, also found nucleotide sequence variations (SNPs) associated with resistance/sensitivity to mastitis, which, in turn, makes it possible to consider the SLC11A1 gene as a valuable gene – a candidate for solving the problems of improving resistance to mastitis and, accordingly, productive traits in dairy cattle populations.

According to the results of research on the features of the genetic structure of the experimental group of cows, the productive parameters of animals with different genotypes at the CSN2 locus were analyzed (Table 3). It should be noted that in this case, all possible variants of the genotypes – A1A1, A1A2 and A2A2 – were analyzed, but the number of individuals for each fluctuated significantly (which was reflected in the error value of the mean).

Table 3. Indicators of milk productivity of cows of the Ukrainian red-spotted breed with different genotypes according to the CSN2 locus

Indicator	Genotype		
	A ¹ A ¹	A ¹ A ²	A ² A ²
First lactation			
Milking, 305 days, kg	5502,0±410.12 ^a	5170,0±173.39 ^a	5266,4±213.42 ^a
C _v , %	14.90	13.83	17.66
Fat, 305 days, %	3.81±0.038 ^a	3.86±0.022 ^a	3.88±0.022 ^a
C _v , %	2.02	2.28	2.50
Protein, 305 days, %	3.36±0.024 ^a	3.31±0.024 ^a	3.32±0.029 ^a
C _v , %	1.40	3.02	3.86
Second lactation			
Milking, 305 days, kg	6384,00±363.55 ^a	5967,4±169.32 ^a	6047,2±214.12 ^a
C _v , %	11.39	11.70	15.43
Fat, 305 days, %	3.68±0.027 ^a	3.76±0.014 ^a	3.78±0.020 ^a
C _v , %	1.49	1.54	2.33
Protein, 305 days, %	3.22±0.040 ^a	3.24±0.019 ^a	3.27±0.033 ^a
C _v , %	2.17	2.50	4.40
Third lactation			
Milking, 305 days, kg	6754,3±269.69 ^a	6727,9±158.81 ^a	6603,4±181.90 ^a
C _v , %	7.99	9.73	12.01
Fat, 305 days, %	3.64±0.029 ^a	3.69±0.013 ^a	3.70±0.018 ^a
C _v , %	1.57	1.41	2.11
Protein, 305 days, %	3.16±0.024 ^a	3.16±0.027 ^a	3.18±0.033 ^a
C _v , %	1.52	3.54	4.56

According to the results of the research, no significant difference in milk productivity indicators of cows with different genotypes at the beta-casein locus was found. The highest values of confidence are characteristic of individuals with the A1A1 genotype, but, as already noted, the difference is unlikely. According to the value of the coefficient of variation, the lactation indicator is characterized by an average level of variability, its variability slightly decreases by the third lactation. In any case, the A2 allele, according to the value of the indicators of homozygous individuals (genotype A2A2), does not have a negative effect on the parameters of milk productivity of animals. The research results are consistent with the data obtained by other authors on various breeds of cattle [21; 22]. Thus, the differences between individuals with different genotypes at the CSN2 locus lie in the qualitative composition of milk beta-casein (beta-casein type – A1 or A2). However, this conclusion can be true only for the experimental group of cows (Ukrainian red-spotted dairy breed). In the case of another population, provided that the modifying effect of the genetic background is taken into account (the peculiarities of the influence of the genetic structure at a number of other loci), the effect may be different, which indicates the need for analytical studies in each individual situation.

Targeted studies on the study of the genetic structure of populations by gene complexes that are related to productive parameters are of great importance for selection work on improving economic and useful traits in various breeds of cattle, in particular breeds of Ukrainian selection and preserving their genetic potential. Based on the results of the conducted research, it is possible to draw a conclusion regarding the need for targeted and intensive selection work with various breeds of farm animals, taking into account the data on genotyping individuals by a number of markers and analyzing their associations with economic and useful traits of animals.

Taking into account the results of the research, only the analysis of individuals by genotype according to the set of candidate genes and the use of modern genetics methods will allow obtaining experimental groups

of cows that will be characterized by clearly defined complex genotypes (at certain loci), which in the future will allow to carry out directed selection work with the aim of maximum implementation of the productive potential of animals.

CONCLUSIONS

Based on the results of the research, it was established that for cows of the Ukrainian red-spotted dairy breed, according to the locus of toll-like receptor 1 (TLR1) according to the parameter of standard milk, the maximum values of the indicator for the first and third lactations are characterized by individuals with the homozygous genotype GG, while for the second lactation, the highest milk yield values are typical for individuals homozygous for allele A. Probable differences in milk productivity indicators between individuals with different genotypes for the TLR1 locus (1596G>A) have not been established.

Based on the results of the analysis of the productive qualities of animals with different haplotypes at the SLC11A1 locus, it was found that during all three lactations, animals with the CG-AA haplotype were characterized by higher milk yield values in 305 days compared to individuals of other haplotypes. At the same time, the biggest difference is observed with the CG-AT haplotype. According to the indicators of the content of milk fat and protein, no significant differences between animals with different haplotypes were established. The variability of signs for all lactations was at a low level.

According to the beta-casein (CSN2) locus, according to the results of research conducted for individuals with different genotypes (A1A1, A1A2 and A2A2), no probable differences were found during three lactations in terms of milk productivity (milk yield, fat content, protein content).

According to the results of the research, promising genotypes for the TLR1, SLC11A1 and CSN2 loci were identified in experimental groups of cows of the Ukrainian red-spotted dairy breed, which makes it possible to use the obtained data for further breeding work to obtain experimental populations of animals with the desired genotypes.

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