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RESEARCH ARTICLE

Polymorphism of the Prolactin Gene in Dairy Cows

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ABSTRACT

Modern genetic research is actively focused on studying nucleotide sequence polymorphisms, which is important not only for fundamental science but also for practical breeding of farm animals. For dairy farming, the prolactin (PRL) gene plays a crucial role. Given that prolactin directly regulates the initiation and maintenance of lactation in mammals, analyzing its genetic variants can serve as a reliable marker for the milk productivity of cattle.The aim is to establish the polymorphism of the prolactin gene and its relationship with the dairy productivity of Holstein and Ayrshire cows.

INTRODUCTION

At the present stage of development of the agro-industrial complex of the Russian Federation, considerable attention is paid to the intensification of dairy cattle breeding. The key objectives of the industry include increasing the total population of existing breeds and developing new ones that ensure high productivity performance in animals [1, p. 4].

Classical breeding methods based on pedigree and phenotypic data are limited by the fact that productive traits manifest only with age. The use of DNA markers enables early identification of genes determining desirable phenotypic traits in animals [2, p. 43; 13, p. 335].

Milk productivity in cattle is closely associated with gene polymorphism, since the application of genetic diagnostics in dairy cows allows the identification of marker allelic variants associated with superior productivity, followed by the use of marker-assisted selection (MAS) technologies in breeding programs [3, p. 190].

The PRL gene encodes one of the universal pituitary hormones belonging to the protein hormone family. Its key functions are associated with the initiation and maintenance of lactation.

Prolactin acts on the alveoli of the mammary glands and is responsible for the synthesis of the main milk components. It is involved in every stage of milk protein gene expression, including transcription, mRNA stabilization, translation, and post-translational modification of proteins. Prolactin also regulates immune functions and participates in cell differentiation and growth [4, p. 40]. The PRL gene in cattle has a structural organization consisting of five exons and four introns.

It should be noted that the PRL^{B} allele is associated with higher milk protein content, improved coagulation properties of milk, and increased cheese yield [5, p. 38]. However, a number of authors report a positive association of the PRL^{AA} and PRL^{AB} genotypes with milk yield and milk protein content in Holstein–Friesian and Black-and-White cattle populations [6, p. 4; 7, p. 107; 8, p. 7338; 9, p. 233; 10, p. 108; 11, p. 203].

The above considerations determined the need to study the polymorphism of the prolactin gene in dairy cattle breeds raised in the Rostov region.

MATERIALS AND METHODS

The genetic structure of the Holstein cattle population was analyzed in animals from the agricultural production cooperative Kolos, while the genetic structure of the Ayrshire population was studied in animals from JSC "Imeni Lenina" in the Rostov region.

All animals were maintained under optimal conditions meeting zoohygienic and zootechnical standards. The study was based on primary breeding and zootechnical records as well as the authors' own research data, including evaluation of parental productivity traits, sampling, and laboratory analysis of biomaterial. Random mating conditions were maintained, excluding the effect of directed parental selection. The age difference among young animals in the experimental group did not exceed 4 days. Full siblings were excluded from the experimental group.

During the study, milk productivity traits were recorded monthly throughout lactation. Milk yield was measured by control milking, while milk fat and protein content were determined monthly. Milk sampling was performed in accordance with GOST 26809-86 "Milk and dairy products". Milk fat content was determined using the Gerber acid method (GOST 5867-90), and protein content by the formal titration method (GOST 25179-2014).

Based on control milking data, milk yield was calculated for each month and for the standard 305-day lactation period. Milk fat and protein yields were determined by calculation.

The biomaterial consisted of DNA isolated from blood samples of Holstein and Ayrshire cows. Peripheral blood was collected by jugular venipuncture into 9.0 mL Vacuette tubes containing ethylenediaminetetraacetic acid potassium salt ($EDTA\text{-}K_3$) as an anticoagulant at a final concentration of 4.0 mg/mL.

DNA extraction from blood samples was performed using the commercial kit "Izogen" (Russia) according to the manufacturer's standard protocol.

PCR amplification of the PRL gene fragment (156 bp) was carried out using reagents produced by Sintol LLC (Russia) and the following primers:
PRL-F: 5′-CGA-GTC-CTT-ATG-AGC-TTG-ATT-CTT-3′
PRL-R: 5′-GCC-TTC-CAG-AGG-TCG-TTT-GTT-TTC-3′.

Amplification was performed using an ANK-32 thermal cycler (Sintol LLC, Russia) under the following conditions: initial denaturation at 95°C for 30 s; followed by 35 cycles of denaturation at 60°C for 30 s, annealing at 72°C for 45 s, and extension at 72°C for 30 s; with a final extension at 72°C for 10 min.

PCR products were identified by electrophoresis in a 2% agarose gel stained with ethidium bromide (EtBr) at 85 V for 60 min using a commercial kit (DNA-Technology LLC, Russia). Visualization was performed using a GenoSens 2150 gel documentation system.

At the next stage, PCR-RFLP analysis was conducted using restriction enzyme RsaI in 1× buffer (SibEnzyme LLC, Russia) at 37°C for 16 h. Fragment analysis was performed by horizontal agarose gel electrophoresis (1% agarose with ethidium bromide). Electrophoresis was carried out for 40 min at 120 V using a 50 bp DNA ladder (Evrogen, Russia). Visualization was performed using a Kvant-S transilluminator (Helicon, Russia). Fragment sizes of 156 bp corresponded to genotype AA; 156, 82, and 74 bp to genotype AB; and 82 and 74 bp to genotype BB.

Genetic parameters were calculated using PopGen 1.32 and Arlequin 3.5.2.2 software, and statistical analysis (Johnson and Bhattacharyya, 2019) was performed using Statistica 10.0 (StatSoft Inc., USA).

Post hoc comparisons and assessment of intergroup differences within analysis of variance were performed using Tukey's test. Statistical significance was accepted at $P < 0.05$: *** $P < 0.001$; ** $P < 0.01$.

Data were visualized using Microsoft Office 2010.

RESULTS AND DISCUSSION

The electropherogram is shown in Figure 1.

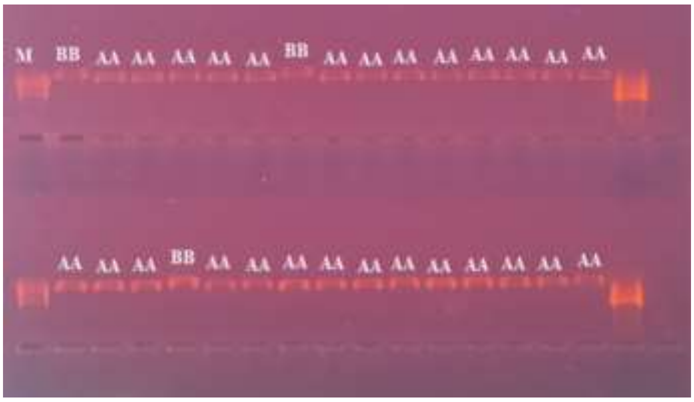

Figure 1: Electropherogram of PCR-RFLP analysis of the PRL gene

The results of DNA testing for the presence of A and B allelic variants of the PRL gene and corresponding genotypes using the PCR-RFLP method in Holstein and Ayrshire cows are presented in Table 1.

Table 1: Allele frequencies of the PRL gene in dairy cattle breeds

Breed	Allele frequencies		Genotype frequencies		
	A	B	AA	AB	BB
Holstein	0,10	0,90	0,90	0,00	0,10
Ayrshire	0,90	0,10	0,90	0,00	0,10

According to the results of molecular genetic analysis, the PRL gene polymorphism was represented by two alleles, PRL^{A} and PRL^{B}, with different frequencies of occurrence, 0.90 and 0.10, respectively. In Holstein cattle, the homozygous genotype PRL^{AA} predominated.

Table 2 presents milk productivity in cows depending on PRL genotypes.

Table 2: Milk productivity of cows with different PRL genotypes

Indicators	Genotypes			
	Holstein breed		Ayrshire breed	
	PRL^{AA}	*PRL^{BB}*	*PRL^{AA}*	*PRL^{BB}*
Milk yield for 305 days, kg	9845,5±92,3	10109,1±90,9*	6878,5±381,31	6869,63±412,52
Milk fat content, %	3,69±0,01	3,72±0,02	4,05±0,11	4,07±0,12
Milk fat yield, kg	363,3±3,3	376,1±4,2*	278,62±20,32	278,40±10,98
Milk protein content, %	3,40±0,01	3,37±0,02	3,47±±0,07	3,47±0,07
Milk protein yield, kg	334,7±3,2	340,7±3,8	238,83±13,45	233,25±2,46

The results showed that the highest milk productivity was observed in animals with the PRL^{BB} genotype (10109.1 kg), whereas individuals with the PRL^{AA} genotype had a milk yield of 9845.5 kg, which is 263.6 kg lower than that of the PRL^{BB} group (2.61%, $P > 0.95$).

Analysis of milk fat and protein content in Holstein cows with different PRL genotypes showed that milk fat percentage ranged from 3.69% in PRL^{AA} individuals to 3.72% in PRL^{BB} animals, indicating an advantage of the latter by 0.03% ($P < 0.95$). In terms of milk protein content, animals with the PRL^{AA} genotype had higher values (3.40%), whereas individuals with the PRL^{BB} genotype had a protein content of 3.37%.

Holstein cows with the PRL^{BB} genotype showed higher milk productivity than PRL^{AA} cows.

The average milk yield per lactation in Ayrshire cows was 6878.5 kg for the PRL^{AA} genotype and 6869.63 kg for the PRL^{BB} genotype. Ayrshire cows with the PRL^{BB} genotype yielded 8.87 kg less milk compared to their counterparts with the PRL^{AA} genotype.

Milk fat content ranged from 4.05% in PRL^{AA} animals to 4.07% in PRL^{BB} animals; thus, in Ayrshire cattle, individuals with the PRL^{BB} genotype showed a slight advantage of 0.02% in milk fat content. A higher milk protein yield over the lactation period was characteristic of animals with the PRL^{AA} genotype, exceeding the PRL^{BB} genotype by 5.58 kg.

The obtained data on the frequency distribution of PRL gene genotypes and alleles are consistent with findings reported by other authors. A high frequency of the PRL^{A} allele has been observed in many dairy cattle breeds, ranging from 0.524 to 1.00. Argentine researchers studying six local cattle breeds reported that some breeds were monomorphic for this allele, with an overall frequency ranging from 0.816 to 1.00 [5].

Russian studies have shown that such rare breeds as Kostroma and Yaroslavl cattle have lower frequencies of the PRL^{A} allele, 0.690 and 0.650, respectively [6]. The lowest frequency of the PRL^{A} allele (0.524) was reported in a population of Anatolian Black cattle (Turkey) [7].

CONCLUSION

1. Data on the presence of prolactin gene polymorphism in the studied populations of Holstein and Ayrshire cows were obtained.
2. The highest milk productivity was observed in Holstein cows with the PRL^{BB} genotype (10109.1 kg), whereas individuals with the PRL^{AA} genotype had a milk yield of 9845.5 kg, which is 263.6 kg lower than that of the PRL^{BB} group (2.61%, $P > 0.95$).
3. In terms of the prolactin gene, Ayrshire cows were characterized by a high frequency of the PRL^{A} allele; 90% of the population had the PRL^{AA} genotype, and the allele frequency of PRL^{A} was 0.9. No animals with the heterozygous genotype PRL^{AB} were identified. In the analyzed Ayrshire population, the PRL^{A} allele is associated with high and stable milk productivity.
4. The results of this study expand the range of applied breeding tasks, including the identification of genetic markers associated with milk productivity. Further research will enable the selection of animals with high genetic potential, taking into account both breed characteristics and population-specific features.

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