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EFFECT OF PROLACTIN GENE POLYMORPHISM ON MILK PRODUCTION TRAITS IN UKRAINIAN BLACK-AND-WHITE DAIRY CATTLE

Метою дослідження було з’ясувати вплив поліморфізму гена пролактину (PRL) на показники молочної продуктивності, зокрема надій, склад та технологічні властивості молока, в української чорно-рябої молочної худоби (n=200). Для визначення генотипів тварин за локусом гена пролактину було використано метод ПЛР-ПДРФ. У дослідженому стаді частота алелів A і G становила відповідно 0,120 і 0,880, частота генотипів AA, AG і GG – відповідно 0,017, 0,211 і 0,772.

Встановлено, що тварини з генотипом PRL AA мали вищі показники надою та білковомолочності (P<0,05) порівняно з тваринами альтернативних генотипів. Стосовно технологічних властивостей (тривалість сичужного зсідання, термостійкість), вищі значення показників спостерігали у носіїв генотипу AA порівняно з генотипами AG та GG. Отримані дані підтверджують, що ген пролактину може використовуватись як кандидатний ген у маркер-асоційованій селекції і є важливим джерелом інформації для виробників молочної продукції.

Ключові слова: молочна продуктивність, ген пролактину, ПЛР-ПДРФ, генотипи, надій, склад молока, сиропридатність, термостійкість.

Formulation of the problem, analysis of recent research and publications. One of the methods of improving the milk production traits in cattle (yield, content and milk technological properties) is the use of information on polymorphism of genes engaged in shaping the importance of these properties. Having this information, it has been possible to quantify genetic variability and identify the superior genetic merits of animal through mathematical models, through more and more refined means.

Among genes involved in milk production there is prolactin gene (PRL). PRL disruption experiments proved its essential roles in reproduction, mammary gland development, initiation of milk secretion, and the maintenance of lactation in mammals. In addition to being a classical pituitary hormone, PRL is also primarily responsible for the synthesis of many milk components, including milk proteins, lactose, and lipids [7]. Therefore, the bovine PRL seems to be an excellent candidate for linkage analysis of quantitative trait loci that affect milk performance traits in dairy cattle.

Many authors [1, 3, 5, 6, 8, 9] reported a polymorphism in the prolactin gene that is present in a number of breeds of cattle, including dairy cattle. The polymorphism was found to influence milk yield, percent protein, and fat in the milk. However, these authors did not evaluate the polymorphism for any effects on technological properties of milk, particularly cheese-making properties and heat stability. This study was conducted to follow up on the researches of different authors, by investigating whether the polymorphism in the prolactin gene can be used as a DNA-marker for selecting dairy cows with favorable milk cheese-making properties. Also, this research was conducted to confirm that the prolactin gene polymorphism affects milk yield and quality.

The **objectives** of this work were to study gene frequencies at the prolactin locus, and association of genetic variants of the PRL gene with milk production traits in Ukrainian Black-and-White dairy cattle

Materials and research methods. The research was conducted in the Skvira district, Kiev region on 200 Ukrainian Black-and-White dairy cows. During the experiment the investigated livestock was located in the same conditions of feeding and keeping, the animals were clinically healthy.

The blood for the tests was collected from the external jugular vein into test tubes with EDTA anticoagulant. Genomic DNA was isolated from blood samples using «DNA-sorbB» (Amplisence, Russia) according to recommendations of producer.

Prolactin genotypes were determined using method PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) [4]. Restriction fragments were separated electroforetically in a 4 % agarose gel with ethidium bromide in the presence of 1x TBE buffer and visualized in the transilluminator.

Milk yield was calculated based on the results of monthly control milking. Fat and protein content in milk was measured by means of device "Ekomilk KAM-98.2". Total solids content was determined by method of drying to constant weight, milk solids non-fat (MSNF) content and lactose content – by using milk analyzer AM-2, casein content – by formol method. The casein number was calculated as the ratio of casein to whole protein. Milk density was measured using a lactodensimeter [2].

The rennetability of milk was ascertained by the trial of rennetability. 20 cm^3 of milk was heated up to 35 ºC in water bath, 1 cm^3 of rennet Hannilase powder with the strength of 1:400 was added, milk was stirred. The time till the creation of first curd flakes was measured.

Heat stability (alcohol number) was determined by the titration with 96 % ethanol. Alcohol number expresses an ethanol consumption of given concentration for fixed bulk of milk (2 cm^3) till protein coagulation under the terms of the method [10].

Statistical analysis was carried out in program Statistica 6.0.

Results and discussion. The electrophoretic separation of the PRL/RsaI restriction fragments of the bovine prolactin gene is presented in Figure 1. In the examined herd of dairy cows, two alleles of the PRL gene (PRL A and PRL G) and three genotypes (AA, AG and GG) were identified. The conducted research showed (Table 1) that 77 % of the population had the GG genotype, 21 % had the AG genotype and 2 % had the AA genotype. This has been confirmed by the results obtained by Novak et al. [3] for Ukrainian Black-and-White dairy cattle.

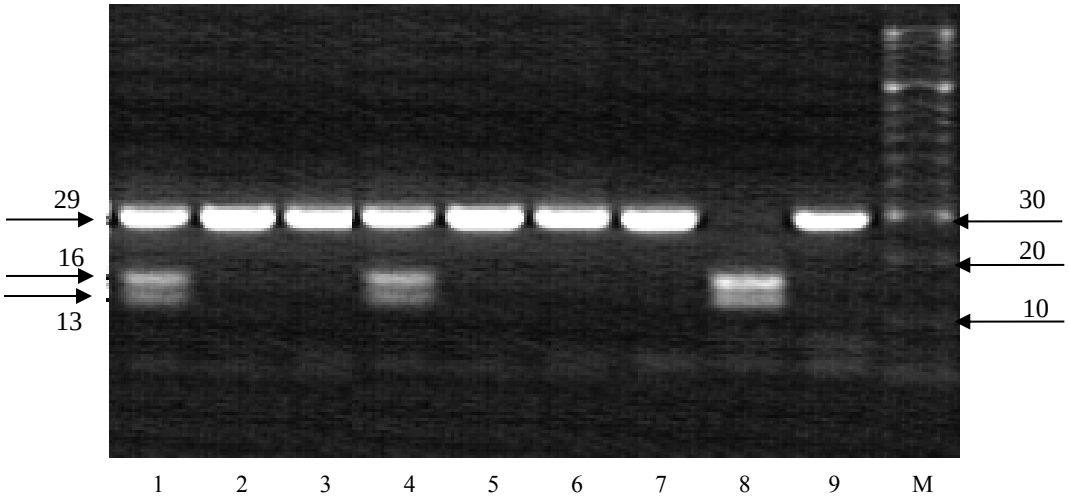

Fig. 1. **Electroforetic restriction fragments PRL/ *Rsa*1 of bovine prolactin gene**: lanes 1, 4 – genotype AG; lanes 2, 3, 4–7, 9 – genotype GG; lane 8 – genotype AA; M – DNA size marker GeneRuler 100 bp.

Table 1 – **Allele and genotype frequencies of PRL gene in cattle**

Locus	Genotype	Number of animals	Genotype frequency	Allele	Allele frequency
PRL	AA	3	0,017	A	0,120
	AG	42	0,211	G	0,880
	GG	155	0,772		

Table 2 shows the effect of the PRL polymorphism on milk production traits in cows studied. The highest milk yield (5703 kg) was characteristic of cows with the AA genotype, then those with AG and GG genotypes. Results of present study are exactly in agreement with data obtained by Brym et al. [6] on Jersey cows, Alipanah et al. [5] on Russian Pied cows and Gareeva [1] on Russian Black-and-White cows showed that PRL/RsaI had a significant effect on milk yield.

Significant differences between the cows of different PRL genotypes were not found for the content of main substances of milk. At the same time we have observed the difference ($P<0,05$) between animals with genotypes GG and AG in milk fat yield – 6,8 kg. The associations of PRL/RsaI mutation with milk fat yield and fat percent were reported by Kathami et al. [9], Dybus et al. [8], Brym et al. [6] and Alipanah et al. [5], but our result for fat percent was not similar to their outputs. Animals with genotype PRL GG had slightly higher milk fat content (3,90 %) in comparison with genotypes AA (3,82 %) and AG (3,85 %). The differences were not significant ($P>0,05$).

Table 2 – **Effect of PRL polymorphism on milk yield, composition and technological properties in Ukrainian Black-and-White dairy cows**, $\overline{X} \pm m_x$

Traits	Genotypes			Difference	
	AA	AG	GG	GG-AA	GG-AB
n	3	42	155		
Milk yield, kg	5703±109,2	5424±77,4	5347±34,3	–356	–77
Fat content, %	3,82±0,024	3,85±0,037	3,90±0,029	+0,08	+0,05
Fat yield, kg	216,5±35,16	208,5±55,32	215,3±23,45	–1,2	+6,8*
Protein content, %	3,15±0,001	3,11±0,043	3,10±0,007	–0,05	–0,01
Protein yield, kg	183,5±25,38	167,6±28,64	166,7±11,56	–16,8*	–0,9
Casein content, %	2,39±0,005	2,40±0,011	2,39±0,004	0	–0,01
Casein number, %	75,9±1,29	77,1±1,16	77,0±1,34	+1,1	–0,01
Lactose content, %	4,50±0,007	4,50±0,012	4,49±0,043	–0,01	–0,01
Total solids content, %	12,45±0,056	12,50±0,045	12,47±0,034	+0,02	–0,03
MSNF content, %	8,63±0,063	8,65±0,042	8,57±0,062	–0,06	–0,08
Density, °A	28,0±0,11	28,0±0,25	27,5±0,14	–0,5	–0,5
Rennetability, s	181,8±23,46	158,3±16,34	158,8±18,92	–23*	+0,5
Alcohol number, ml	2,32±0,126	2,18±0,144	2,18±0,144	–0,14*	0

* – P<0,05

In the case of milk protein yield in the present study it was found the PRL AA homozygotes were characterized by the highest yield (183,5 kg), whereas the lowest one was found in the GG homozygotes (166,7 kg). The difference between cows with genotypes GG and AA was significant (P<0,05). The significant association of the PRL polymorphism with milk protein yield was not previously reported for populations of Black-and-White cattle.

We determined the highest casein number (75,9 %) in the milk of cows with PRL AA genotype. It was also found a significant association of rennetability and heat stability with the genotype PRL AA. An insignificant positive effect of the G allele on the shortening of renneting time was observed both in homozygous and heterozygous genotypes. The expression of G allele had a negative effect on heat stability of milk. These results can be applied when milk is used for cheese production.

Conclusions. Our results showed that animals with genotype PRL AA had a highest (P<0,05) milk yield and milk protein yield in comparison with alternative genotypes. The positive association between allele A and rennetability and heat stability of milk were confirmed. Unfortunately, the frequency of this allele in herd studied is low (0,120). Data obtained confirmed that PRL may be a strong candidate gene for application in marker assisted selection and could be used as an important source of information for producers as well as for breeders.

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Влияние полиморфизма гена пролактина на показатели молочной продуктивносты украинского черно-пестрого молочного скота

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Целью исследования было определить влияние полиморфизма гена пролактина (PRL) на показатели молочной продуктивности, в частности удой, состав и технологические свойства молока, в украинского черно-пестрого молочного скота (n =200). Для определения генотипов животных по локусу гена пролактина был использован метод ПЦР-ПДРФ. В исследованном стаде частота встречаемости аллелей А и G составляла соответственно 0,120 и 0,880, частота встречаемости генотипов АА, AG и GG – соответственно 0,017, 0,211 и 0,772.

Установлено, что животные с генотипом PRL АА характеризовались более высокими показателями удоя и белковомолочности (P<0,05) по сравнению с животными альтернативных генотипов. Относительно технологических свойств (длительность сычужного свертывания, термоустойчивость), более высокие показатели наблюдали у носителей генотипа АА по сравнению с генотипами AG и GG. Полученные данные подтверждают, что ген пролактина может использоваться в качестве кандидатного гена в маркер-ассоциированной селекции и является важным источником информации для производителей молочной продукции.

Ключевые слова: молочная продуктивность, ген пролактина, ПЦР-ПДРФ, генотипы, удой, состав молока, сыропригодность, термоустойчивость.

Effect of prolactin gene polymorphism on milk production traits in Ukrainian black-and-white dairy cattle

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The objective of this study was to elucidate the effect of prolactin (PRL) gene polymorphism on milk production traits involving total milk yield, milk composition and technological properties in Ukrainian Black-and-White dairy cattle (n=200). PCR-RFLP method was used to PRL genotypes detecting. In this herd, the frequencies of alleles were follows: A=0,120 and G=0,880. The frequencies of AA, AG and GG genotypes were 0,017, 0,211 and 0,772 respectively.

Results showed that PRL AA genotype had a statistically significant (P<0,05) higher milk yield and milk protein yield in comparison with alternative genotypes. With respect to technological properties (milk rennetability and heat stability), the AA genotype had higher levels than the AG and GG individuals. The obtained data confirmed that PRL may be a strong candidate gene for application in marker assisted selection and could be used as an important source of information for producers as well as for breeders.

Key words: milk production traits, prolactin gene, PCR-RFLP, genotypes, milk yield, milk composition, rennetability, heat stability.

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