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EFFECT OF BETA-LACTOGLOBULIN GENOTYPES ON COMPOSITION AND TECHNOLOGICAL PROPERTIES OF MILK IN UKRAINIAN BLACK-AND-WHITE DAIRY CATTLE

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A total of 200 Ukrainian Black-and-White dairy cows were genotyped for the beta-lactoglobulin gene (β-LGB). The β-LGB genotypes were analyzed using PCR-RFLP method. In this breed, the frequencies of alleles were follows: A=0,630 and B=0,370. The frequencies of AA, AB and BB genotypes were 0,430, 0,400 and 0,170 respectively. Results showed that β-LGB AA genotype had higher milk yield, whole protein, total solids and milk solids non-fat (MSNF) content than AB and BB individuals. With respect to milk fat content (%), the BB genotype had higher levels than the AA and AB individuals. The results showed that milk with best cheese-making properties was obtained by cows with the genotype BB. Their milk had higher casein content and casein number and shorter coagulation time. At the same time the heat stability of this milk was less compared to milk from animals with the β-LGB AA and AB genotypes. The results presented here demonstrate that the β-LGB genotypes may be used as selection criteria in programs of Ukrainian Black-and-White dairy cattle breeding and improvement.

Key words: **milk production traits, beta-lactoglobulin gene, genotypes, milk yield, milk composition, cheese-making properties, heat stability**

ВПЛИВ ГЕНОТИПІВ ГЕНА БЕТА-ЛАКТОГЛОБУЛІНУ НА ХІМІЧНИЙ СКЛАД ТА ТЕХНОЛОГІЧНІ ВЛАСТИВОСТІ МОЛОКА УКРАЇНСЬКОЇ ЧОРНО-РЯБОЇ МОЛОЧНОЇ ХУДОБИ

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Проведено аналіз генетичної структури української чорно-рябої молочної худоби (n=200) за локусом гена бета-лактоглобуліну. Для ідентифікації генотипів було використано метод ПЛР-ПДРФ. У дослідженій мікропопуляції тварин виявлено два алелі гена бета-лактоглобуліну – A і B – з частотами 0,630 і 0,370 відповідно. Частоти генотипів AA, AB та BB становили відповідно 0,430, 0,400 та 0,170. Результати досліджень показали, що корови-носії генотипу AA мали вищі показники надою, масової частки загального білка, сухої речовини та сухого знежиреного молочного залишку, ніж носії генотипів AA і AB. Водночас гомозиготи BB мали молоко з вищим умістом жиру (%) порівняно з генотипами AA і AB. Молоко з кращими показниками сиропридатності отримували також від корів з генотипом BB. Таке молоко мало більшу масову частку казеїну, більшу частку казеїну у загальному білку і коротший час зсідання згустку під впливом сичужного ферменту. Водночас його термостабільність була нижчою порівняно з молоком тварин з генотипами AA і AB. Відтак, генотип тварин за геном бета-лактоглобуліну можна використовувати як селекційний критерій у програмах розведення і удосконалення української чорно-рябої молочної худоби.

Ключові слова: **молочна продуктивність, ген бета-лактоглобуліну, генотипи, надій, хімічний склад молока, сиропридатність, термостабільність**

ВЛИЯНИЕ ГЕНОТИПОВ ГЕНА БЕТА-ЛАКТОГЛОБУЛИНА НА ХИМИЧЕСКИЙ СОСТАВ И ТЕХНОЛОГИЧЕСКИЕ СВОЙСТВА МОЛОКА УКРАИНСКОГО ЧЕРНО-ПЕСТРОГО МОЛОЧНОГО СКОТА

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Проведен анализ генетической структуры украинского черно-пестрого молочного скота (n=200) по локусу гена бета-лактоглобулина. Для идентификации генотипов использован метод ПЦР-ПДРФ. В исследованной микропопуляции животных выявлено два аллеля гена бета-лактоглобулину – А и В – с частотой встречаемости 0,630 и 0,370 соответственно. Частоты генотипов АА, АВ и ВВ составляли соответственно 0,430, 0,400 и 0,170. Результаты исследований показали, что коровы-носители генотипа АА характеризовались более высокими показателями удоя, массовой доли общего белка, сухого вещества и сухого обезжиренного молочного остатка, чем обладатели генотипов АА и АВ. В то же время от гомозигот ВВ получали молоко с более высоким содержанием жира (%) по сравнению с генотипами АА и АВ. Молоко с лучшими показателями сыропригодности получали также от коров с генотипом ВВ. Такое молоко содержало больше казеина, имело большую долю казеина в общем белке и более короткое время сычужного свертывания. Однак его термостабильность была ниже по сравнению с молоком животных с генотипами АА и АВ. Таким образом, генотип животных по локусу гена бета-лактоглобулина можно использовать в качестве селекционного критерия в программах разведения и совершенствования украинского черно-пестрого молочного скота.

Ключевые слова: **молочная продуктивность, ген бета-лактоглобулина, генотипы, удой, химический состав молока, сыропригодность, термостабильность**

Introduction. The development of intensive technologies of high quality milk production is a priority of modern farming. Intensive technologies include the improving of cattle breeds by using of DNA-technologies. Molecular-genetic markers associated with milk production traits are used in animal breeding of numerous countries. Beta-lactoglobulin (β-LG) gene belongs to these markers. Since the discovery of alleles A and B of β-LG in cattle, genetic polymorphism in milk proteins has raised great interest in animal breeding and dairy industry.

Many studies were performed to investigate the effect of β-LG genotypes on milk production traits, milk composition and quality. They found that the AA genotype of β-LG had a favorable effect on protein yield, and the association of significantly higher fat content, protein, casein, true protein, and total solids with BB variant had been reported [2, 3, 6, 7].

Many studies have shown a beneficial effect of the β-LG B allele on technological properties of milk [8, 9]. They claimed that milk with B variant of β-LG was associated with better coagulation properties, shorter coagulation time, faster syneresis, higher curd firmness and higher yield of cheese compared to A variant.

Until now, β-LG has been a popular genetic marker for the genetic characterization of cattle populations. However, allele frequencies of the polymorphic β-LG and the influence of these alleles on milk performance traits are not consistent across the diverse range of dairy cattle.

Based on important role of β-LG gene in milk related traits and their genetic trends in dairy cattle, the **aim** of this study was to evaluate the effect of β-LG gene types in Ukrainian Black-and-White dairy cattle on the composition and technological properties of milk.

Materials and methods. A total of 200 Ukrainian Black-and-White dairy cows were genotyped for the β-LG gene. The cows were kept in the herd of «Agrocompany Kolos» (Skvira district, Kiev region). During the experiment the investigated livestock was located in the same conditions of feeding and keeping, the animals were clinically healthy.

Genomic DNA was isolated from blood samples of dairy cows using «DNA-sorbB» (Amplisence, Russia) according to recommendations of producer.

The polymorphism at beta-lactoglobulin gene was identified by conducting PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) studies [5].

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. Milk density was measured using a lactodensimeter [4].

Rennet coagulation time of milk was measured in minutes, including the time since the addition of the enzyme to the formation of a firm clot. Clot without isolation of whey and gas bubbles, and also without cracks and voids was considered firm. When turning the tube clot is not dropped or fell only a few particles.

Clot was considered soft when it contained a few gas bubbles and cracks. When turning the tube the clot deformed and less than 50 % fell out. Clot permeated gas bubbles, torn into particles was considered flaccid, flaky. Turning the tube led to a complete or partial (over 50 %) loss of the clot.

Heat stability of milk was determined using a so called «tiglova probe» [1].

Statistical analysis was carried out in program Statistica 6.0.

Results and discussion. Molecular-genetic analysis in the studied population of Ukrainian Black-and-White dairy cows showed a distribution of genotypes on β-LG gene locus: AA –43 %, AB – 40 %, BB – 17 % (table 1).

1. Allele and genotype frequencies of β-LG gene in Ukrainian Black-and-White dairy cattle

Locus	Genotype	Number of animals	Genotype frequency	Allele	Allele frequency
β-LG	AA	86	0,430	A	0,630
	AB	80	0,400	B	0,370
	BB	34	0,170		

The results subjected to analysis of variance showed β-LG genotypes to be a factor that significantly differentiated the yield of milk and the cows' milk nutrient content. The highest milk yield for 305 days of lactation (5617 kg) was observed in animals with genotype AA. Cows with the β-LG BB genotype obtained 256 and 122 kg less milk yield compared to animals with the β-LG AA ($P<0,05$) and AB ($P<0,05$). In addition, the milk produced by cows with the BB genotype had the lowest protein, total solids and milk solids non-fat (MSNF) content. Based on Table 2, the difference in milk composition between cows with the β-LG BB and AA genotype was 0,04 % for protein content, 0,05 % – for total solids content ($P<0,05$) and 0,13 % – for MSNF ($P<0,001$). At the same time cows with genotype BB had the highest fat content, the difference with AA and AB genotypes was 0,08 ($P<0,05$) and 0,07 % ($P<0,05$) respectively. The highest lactose content (4,52 %) was revealed in milk from cows with heterozygote genotype.

The results obtained in our study are confirmed by the findings of other authors [2, 3, 6, 7]. They also reported that milk composition was significantly related to β-LG genotype.

Cheese-making properties and heat stability are the most important technological traits of milk because they determine the degree of raw material use in dairy industry. In cheese manufacture the cheese yield does not only depend on the whole protein content of the milk, but on the casein content, which is measured by the casein number. Therefore it is also necessary to mention the β-LG genotypes which are determining the casein number of the milk. The casein number indicates the percentage of casein of the whole protein fraction and is the other key factor for cheese making.

Investigations showed that the β-LG genotype BB is correlated with a higher casein content and casein number than the genotypes AB and AA. We did not revealed the statistically significant differences between β-LG genotypes on casein content. At the same time casein number of milk from cows with BB genotype was 1,8 % ($P<0,01$) higher than in milk from animals with AA genotype.

2. Effect of β-LG polymorphism on milk composition and technological properties in Ukrainian Black-and-White dairy cows, $\overline{X} \pm m_x$

Traits	*Genotypes*			*Difference*	
	AA	*AB*	*BB*	*BB-AA*	*BB-AB*
n	86	80	34		
Fat content, %	3,83±0,032	3,84±0,023	3,91±0,032	***+0,08****	***+0,07****
Protein content, %	3,14±0,011	3,12±0,012	3,10±0,012	***–0,04***	***–0,02***
Casein content, %	2,38±0,011	2,40±0,073	2,41±0,034	***+0,03***	***+0,01***
Casein number, %	76,0±0,86	77,1±1,21	77,8±0,75	***+1,8*****	***+0,6***
Lactose content, %	***4,48±0,023***	***4,52±0,023***	***4,49±0,033***	***+0,01***	***–0,03***
Total solids content, %	***12,50±0,052***	***12,47±0,071***	***12,45±0,046***	***–0,05****	***–0,02***
MSNF content, %	***8,67±0,063***	***8,63±0,042***	***8,54±0,062***	***–0,13*******	***–0,09****
Density, °A	***28,1±0,13***	***27,9±0,15***	***27,5±0,24***	***–0,6*****	***–0,4****
Rennet coagulation time, min	***31,5±0,53***	***29,3±0,67***	***26,9±0,98***	***–4,6****	***–2,4***
Percentage of animals with milk curd, %:					
dense	***30***	49	78	***+48***	***+29***
soft	***54***	38	22	***–32***	***–16***
flaccid, flaky	***16***	***13***	–	***–16***	***–13***
Heat stability of milk, min	***60,2±4,56***	58,7±2,78	53,6±3,44	***–6,6****	***–5,1****

Note.* – P<0,05; ** – P<0,01; *** – P<0,001.

Table 2 shows the effect of β-LG polymorphism on the state of casein curd and also on rennet coagulation time of milk. Milk with rennet coagulation time from 15 to 40 minutes is the most desirable in the cheese-making. If the clotting lasts more than 40 minutes this results in a large loss of raw materials and a small cheese yield.

Differences (P<0,05) between the cows with different β-LG genotypes for rennet coagulation time were observed. Clotting lasted 26,9 minutes in milk from cows with genotype β-LG BB. These animals showed shorter milk coagulation time (–4,6 and –2,4 min) than AA and AB individuals.

From 51–70 % cows with allele β-LG A in genotype the soft and flaccid milk curds were obtained. The presence of B allele in the genotype significantly improved the state of casein curd. Casein curds from milk of 78 % cows with BB genotype were firm. Thus, the milk from β-LG BB individuals has higher cheese yielding capacity. It is an important attribute which has to be considered by the cheesemaker because it eventually determines the profitability of the operation.

Milk from animals with β-LG AA genotype had the highest heat stability – 60,2 min by «tiglova probe». The difference in heat stability between cows with the β-LG BB and AA genotype was 6,6 min (P<0,05). Heat stability of milk plays a significant role in the manufacture of certain high heat treated/concentrated milk products.

Conclusion. The results of this study confirmed the association of β-LG polymorphism with milk production traits, particularly with milk composition and technological properties. Significant differences have been revealed between different β-LG genotypes in Ukrainian Black-and-White dairy caws. Animals with β-LG AA genotype were characterized by higher milk yields, higher protein, total solids and MSNF content, as well as higher density and heat stability compared to genotypes AB and BB. The highest fat content and the most favorable properties for cheese making were observed in milk from cows with β-LG BB genotype. Their milk had a high casein content and good characteristic of casein curd. β-LGB genotypes may be used as selection criteria in programs of breeding and improvement of these cattle.

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