POLYMORPHISM OF GENES OF THE PROTEIN AND LIPID EXCHANGES IN MODERN UKRAINIAN BREEDS OF CATTLE BRED FOR DAIRY PRODUCTIVITY

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Genetic structure of animals of several Ukrainian modern breeds analysed by genes of protein and lipid exchange CSN3 (kappa-casein), BLG (betalactoglobulin), LEP (leptin), Pit-1 (pituitary transcription factor), TG-5 (thyroglobulin) were investigated in cows of three domestic breeds (Ukrainian Red Dairy, Ukrainian Black-speckled Dairy, Ukrainian Red-speckled Dairy). Polymerase chain reaction, followed by restriction fragment length polymorphism was used for the molecular-genetic analysis.

Key words: structural genes, homozygosity, genetic equilibrium, milk productivity.

INTRODUCTION

Development of methods using genetic diagnostics in the practice of cattle breeding is an urgent task of fundamental and applied genetics. A necessary precondition for carrying out DNA-diagnostics is genetic polymorphism, which is the basis of hereditary change in an organism.

DNA markers provide exact tools to investigate genetics of varieties. Use of the polymorphic area of DNA as marker systems allows testing the genetic unsteadiness at the molecular level of organisation in organisms at the level of genes, that is genotype. Using DNA markers in selection of pedigree cattle gives the opportunity of determining their real genetic potential. Further, genotyping of organisms allows to conduct selection by identifying valuable alleles of different genes within farm populations. The DNA markers give a possibility of identification of any DNA fragment, which is necessary for marker assisted selection (MAS) (Sulimova *et al.*, 2002).

These diagnostic tools are useful for determining potential improvement of cattle breeds, and have become required

procedures in Europe, and are expanding in dairy cattle breeding in Ukraine.

Due to the market demand for a higher quality of milk, especially for the quantity of protein, its qualitative composition, and for cheese-making characteristics of milk, breeding requires use of genetic markers associated with qualitative and quantitative signs of milk production. Thus, allelic variants of genes associated with protein and milk fat production are important markers in dairy cattle (Kopylov, 2011).

The search for new molecular-genetic markers of genes associated with key traits and their mapping on chromosomes in farm animals is aimed to identify the genetic potential of animals, irrespective of age, sex, and physiological state and allows breeding at the level of DNA (Kim *et al.*, 2001). Research on breeds of cattle to determine the main loci of quantitative traits associated with milk production has focused on the main genes affecting qualitative and technological parameters of milk (Kopylov, 2011).

The aim of this work was to study polymorphism in genes of protein and lipid exchanges — CSN3 (kappa-casein),

BLG (beta-lactoglobulin), *LEP* (leptin), and *Pit-1* (pituitary transcription factor), *TG-5* (thyroglobulin) in cows of three native breeds within a pedigree farm and to determine their genetic structure associated with milk productivity loci. The study was carried out on three Ukrainian breeds in a farm with the same feeding and diet conditions, located in the southern region of the country. The examined loci directly affect the qualitative and quantitative indicators of milk productivity.

MATERIALS AND METHODS

Milk breeds of cattle Ukrainian Red Dairy (URD, $n = 32$ heads), Ukrainian Black-speckled Dairy (UBSD, $n = 32$ heads), and Ukrainian Red-speckled Dairy (URSD, $n = 28$ heads) in the herd of the pedigree farm PSP "Kolos-2011" in Ochakov district, Mykolaiv region, were tested.

Genomic DNA was isolated from peripheral blood of animals as previously described (Maniatis *et al.* 1984; Zinovieva 2002), using the standard commercial set "DNA sorb B" produced by "Amplisens" (Russia), according to the recommendation of producer. The concentration of DNA was determined by electrophoresis in 2% agarose gel. Polymorphism of the structural genes in extracted DNA was determined by electrophoretic distribution of proteins in the starched horizontal gel and in the vertical polyacrylamide gel with further histochemical painting.

The characteristics of polymorphism of the tested genes were determined by RFLP (Restriction Fragment Length Polymorphism) (Alexander *et al.* 1988; Grodziker *et al.*, 1974; Sulimova *et al.*, 2002). The PCR reactive mixture (10 μ l) contained 4.3 μ l dH₂O, 2.0 μ l PCR buffer (15 mM Mg-1.0 mol); 0.8 μ l of 2 mM dNTP mixture, 0.8 μ l of two primers (70 ng each), 0.1 μ l Taq-polymerase (1 ml / 1000 U) and 2.0 μ l DNA (50–100 ng). The restriction products were separated by electrophoresis in 2% agarose gel with further staining by bromide ethidium (Oblap *et al.*, 2010). Visual evaluation was made with a transilluminator in ultraviolet light and electrophoregrams taken by a digital camera was applied. Differentiation of amplicons by size was carried out with the help of markers of molecular weight GeneRuler™ 50 bpDNA ladder, SM0378 ("Fermentas", Lithuania; Kopylov 2011).

The temperature conditions and number of cycles of PCR-amplification required for every gene were determined separately. Restrictases selected for every locus were used for analysis of polymorphism of the structural loci κ -Cn, β LG, TG, PIT-1 and LEP (Table 1) after PCR-analysis. The composition of the restriction mixture was: H₂O – 3.0 μ l, buffer (10 mM MgCl₂, 100 mM KCl, 0.1 mg/ml BSA) — 1.5 μ l, restrictase — 0.5 μ l, and PCR-product — 10 μ l.

Amplification of fragments of tested genes was carried out with using the specific primers, given in Table 1.

The following parameters of genetic structure were determined: frequency of alleles and genotypes, the level of real (H_o) and expected heterozygosity (H_e), and index of polymorphism (PIC). Estimation of compliance of genotype frequency to the equilibrium Hardy-Weinberg equation was carried out by the Pearson (χ^2) criterion. The genetic differentiation of the tested population was estimated by the fixation index (F_{IS}), which quantitatively reflects declination from panmixis and is determined by relation of average real heterozygosity to the average expected. Population genetic and biometrical analysis of the obtained results was conducted using the χ^2 , Student's T, and Fisher's exact tests.

Statistical processing of the data was conducted using the software Microsoft Excel 2013 and integration of the GenAIEx 6.5 add-on (<http://biology-assets.anu.edu.au/GenAIEx/Download.html>). Analysis of other indices was conducted using IBM SPSS Statistics V24.0 package (https://www.ibm.com/support/knowledgecenter/en/SSLVMB_24.0.0/spss/product_landmg.html).

RESULTS

Distribution of the allelic variants of the structural genes of the protein and lipid exchange in the above-mentioned breeds are given in Table 2.

Two alleles of gene kappa-casein A and B and three genotypes were revealed: AA with fragment length 273 n.p., AB with restriction fragments of length — 273, 133, 91 and 49 n.p. and genotype BB with restriction fragments of length 133, 91, and 49 n.p. (Fig. 1).

Table 1. Nucleic succession of primers and restrictases

Gene	Succession of primers 5'→3'	Restrictase	Reference
κ -Cn	5'-GAAATCCCTACCATCAATACC-3' ta 5'-CCATCTAC CTAGTTTAGATG-3'	HinfI/HindIII	Eggen, 1998, Pider, 1991
β LG	5'-GTG CTGGACACCGACTACAAAAAG-3' ta 5'-GCTCCCGGTATATGACCACCCTCT-3'	Hae III	Medrano, 1990
TG-5	5'-GGGGATGACTACGAGTATGACTG-3' Ta 5'-GTGAAAATCTGTGGAGGCTGT-3'	PstI	Alison, 2007
LEP	5'-GTCACCAGGATCAATGACAT-3' ta 5'-AGCCCAGGAATGAAGTCCAA-3'	Sau3AI	Pomp, 1997
Pit-1	5'-CAAT GAGAAAGTTGGTGC-3' ta 5'-TCTGCATTTCGAGATGCTC-3'	HinfI	Moody, 1996

Table 2. Distribution of allelic variants by loci *CSN3*, *BLG*, *LEP*, *Pit-1* and *TG-5*

Locus, allele	Breed		
	Ukrainian Red Dairy	Ukrainian Black-speckled Dairy	Ukrainian Red-speckled Dairy
<i>CSN3</i> (n)	16	15	15
A	0.81	0.80	0.73
B	0.19	0.20	0.27
<i>BLG</i> (n)	16	15	15
A	0.59	0.27	0.27
B	0.41	0.73	0.73
<i>Pit-1</i> (n)	16	15	15
A	0.31	0.37	0.50
B	0.69	0.63	0.50
<i>TG-5</i> (n)	16	15	15
C	0.91	0.97	0.93
T	0.09	0.03	0.07
<i>LEP</i> (n)	16	15	15
C	0.56	0.37	0.80
T	0.44	0.63	0.20

Among populations of the three cattle breeds (Table 3), the frequency of allele A in the group of cows of Ukrainian red dairy breed was 0.813. More than half of cows of this breed were homozygotic for allele AA and 37.5–40.0 per cent of the tested animals of the breeds were carriers of the heterozygotic genotype AB *CSN3*, and only representatives of the Ukrainian red-speckled dairy breed were characterised by the presense of female cows with homozygotic genotypes BB (7.7 per cent), which may be explained by their origin.

The distribution of the locus *CSN3* is uneven among the cattle breeds with greater frequency of heterozygotes (62.5 percent) among the cows of Ukrainian Red Dairy breed. The frequency of the desired allele *CSN3* B is low (0.188–0.267) because of wide use of the Holstein breed in breeding for greater yield in cows. In Ukrainian Red Dairy and Ukrainian Black-speckled dairy cows, the homozygote BB is not found. Comparison of frequency of the allele showed that in the tested breeding stock of this breed in the pedigree farm PSP “Kolos-2011” the allele *CSN3* B had 2.7–4.3 times higher frequency than in other farms and, opposite, the lowest frequency in cows of the Red-speckled colour breed. There was a loss of genetic equilibrium in locus *CSN3* in favour of homozygotes in cows of breeds of Ukrainian Red Dairy and Ukrainian Black-speckled Dairy,

Table 3. Polymorphism of the gene *CSN3* in cows of Ukrainian breeds

Breed	<i>n</i>	Frequency of genotype						Frequency of allele A/B	Heterozygosity		χ^2	<i>PIC</i>	<i>F_{IS}</i>
		AA		AB		BB			<i>H_o</i>	<i>H_e</i>			
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%						
URD	16	10	62.5	6	37.5	0	–	0.813/0.187	0.375	0.315	0.852	0.305	-0.192
URSD	15	8	53.3	6	40.0	1	6,7	0.733/0.267	0.400	0.405	0.008	0.391	0.011
UBSD	15	9	60.0	6	40.0	0	–	0.800/0.200	0.400	0.331	0.938	0.320	-0.208

Fig. 1. Electrophoregramme of the products of restriction of amplified fragments of kappa-casein gene of locus *CSN3*

(1 – product of amplification of gene; 2 – genotype AA; 3,4,6,7,10 – genotype AB; 5, 8, 11, 12 – genotype BB; 13 – marker of molecular mass)

Fig. 2. Electrophoregramme of the restriction products of amplified fragments of beta-lactoglobulin gene by locus *BLG*

(1, 2, 3, 4, 8, 9 – genotype AB; 5, 6, 7, 10 – genotype BB; PCR-product of amplification; M – molecular mass marker)

which is not typical for cows Ukrainian Red-speckled Dairy of the same age, shown by the indices H_o and H_e . This was confirmed by the fixation index (1.1%) obtained for Ukrainian Red-speckled Dairy animals, compared with to 19.2 and 20.8% for the others breeds.

Figure 2 shows the results of restriction of products of amplification of beta-lactoglobulin gene.

Three restrictive fragments with length 148, 99, and 74 n.p. belong to genotype AB, and two fragments with length 99 and 74 n.p. to genotype BB. The protein contains sulphur (7–12% of the total protein content). The produced β -globulin contained in the serum of blood is transmuted in milk is globulin of milk. Globulin is also present in milk in the

Table 4. Polymorphism by genome *BLG* of cows of Ukrainian breeds

Breed	<i>n</i>	Frequency of genotype						Frequency of allele A/B	Heterozygosity		χ^2	<i>PIC</i>	<i>F_{IS}</i>
		AA		AB		BB			<i>H_o</i>	<i>H_e</i>			
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%						
URD	16	7	43.8	5	31.2	4	25.0	0.59/0.41	0.312	0.498	1.98 5	0.482	0.372
URSD	15	3	20.0	2	13.3	10	66.7	0.27/0.73	0.133	0.405	6.516	0.391	0.670
UBSD	15	1	6.7	6	40.0	8	53.3	0.27/0.73	0.400	0.405	0.008	0.391	0.011

form of membranes of fat balls, and with increasing fat content, the proportion of globulin is increased (Godovac-Zimmermann *et al.*, 1990).

The Ukrainian Red Dairy breed estimated in our research (Table 4) has almost even distribution of the allelic variants: frequency 0.59 for *BLG* AB and 0.41 for *BLG* B. However, the frequency of homozygotes of genotype AA was 0.438, BB — 0.250. The cows of the Ukrainian Red-speckled Dairy breed of the same age had a greater quantity of homozygotes BB (frequency 0.667), and the frequency of B was 0.73, that is, 66.7 per cent of animals were homozygotic for allele B. The Ukrainian Black-speckled Dairy breed also had greater frequency of allele B (0.73); the frequency of animals homozygotic for allele B was 53%.

The largest frequency of homozygotes (43.8% genotype AA) for genome *BLG* was observed for the cows of the Ukrainian Red Dairy breed, compared with 66.7% genotype BB in the Ukrainian Red-speckled Dairy breed and 53.3% genotype BB in Ukrainian Black-speckled Dairy cows. Deviation of real heterozygosity for locus *BLG* was expressed clearly in animals of the Ukrainian Red-speckled Dairy breed (0.133–0.405), indicating displacement of the genetic equilibrium. The fixation index indicated a considerable advantage of homozygotes over heterozygotes in cows of the Ukrainian Red Dairy (37.2 per cent) and Ukrainian Red-speckled Dairy (67.0 per cent) breeds, but this was not seen in this locus for animals of the Ukrainian Black-speckled Dairy breed, which were supposed to be genetically free from gene complexes of control of the red colour.

The results of restriction analysis of the products in amplification of the leptin gene is shown in Figure 3.

The Ukrainian Red-speckled Dairy breed had greater frequency of allele C (0.800) and homozygote CC (0.667), and the frequency of heterozygote CT was 0.267 (Table 5). For the Ukrainian Black-speckled Dairy breed there was a higher frequency of T (0.63), and the frequency of heterozy-

Fig. 3. Electrophoregram of the restriction products of amplified fragments of leptin gene of locus *LEP*

(1, 3, 6, 8, 9, 10 – animals with genotype (AA 730 n.p., 690 n.p., 400 n.p.); 4, 7 – animals with genotype AB (730 n.p., 690 n.p., 400 n.p., 310 n.p. and 90 n.p.); 5 – animals with genotype AC (730 n.p., 690 n.p., 470 n.p., 400 n.p. and 220 n.p.); 2 – animals with genotype BC (730 n.p., 690 n.p., 470 n.p., 310 n.p. and 90 n.p., 220 n.p.); 11 – PCR-product of amplification, 1820 n.p.; 12 – marker of molecular mass DNA Ladder)

gote CT and homozygote TT was almost identical — 46.7 and 40.0%, respectively.

Cows of the same age of the red dairy breed had a slightly higher frequency (by 1.27 times) of allele C over T and there has a significant proportion of cows (62.5%) that were heterozygotic for locus *LEP*.

Analysis of the real and expected heterozygosity does not reveal significant divergence in cattle of the three tested breeds, but higher real heterozygosity was found in cows of the Ukrainian Red Dairy breed, and higher expected heterozygosity for the other breeds. Considerable predominance of homozygotic over heterozygotic animals was shown by the fixation index for locus *LEP* (F_{IS} = 2.9... 19.4 %), which was specific to breeds with red-speckled colour.

The pituitary transcription factor (*Pit-1*) is important in assessment of milk productivity. PCR of *Pit-1* was conducted by DNA amplification of fragment length 1355 n.p. (Anderson *et al.*, 1994). Genotype AA was characterised by fragments — 660, 425, 270 n.p.; for BB — 660, 385 and 270

Table 5. Polymorphism by gene *LEP* of cows of Ukrainian breeds

Breed	<i>n</i>	Frequency of genotype						Frequency of allele C/T	Heterozygosity		χ^2	<i>PIC</i>	<i>F_{IS}</i>
		CC		CT		TT			<i>H_o</i>	<i>H_e</i>			
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%						
URD	16	4	25.0	10	62.5	2	12.5	0.56/0.44	0.625	0.508	1.165	0.492	-0.230
URSD	15	10	66.7	4	26.7	1	6.6	0.80/0.20	0.266	0.311	0.417	0.320	0.194
UBSD	15	2	13.3	7	46.7	6	40.0	0.37/0.63	0.466	0.480	0.000	0.464	0.029

Fig. 4. Electrophoregramme of the products of restriction of amplified fragments of gene *Pit-1*

(8, 9, 11 – genotype AB; 2, 3, 4, 5, 6, 7, 10 – genotype BB; PCR-product of amplification; M – marker of molecular mass)

n.p.; and for AB — 660, 425, 385 and 270 n.p. (Fig. 4). In the Ukrainian Red Dairy breed, the frequency of allele B was 2.22 times higher than for A. The frequency of homozygotes of allele A was 0.31, though in fact the genotypes AA was not observed (Table 6).

Ukrainian Red-speckled Dairy cattle had identical frequency of the alleles A and B, with 60% of cows heterozygotic for locus *Pit-1*, and 20% homozygotic (BB or AA).

For Ukrainian Black-speckled Dairy cows, the frequency of allele A (0.37) was lower than for B (0.63) by 1.7 times and heterozygotes AB in the breed dominated (60%). In all of the studied of dairy cattle breeds in the locus *Pit-1* expected heterozygosity in fact was corresponding to estimated. There was a non-compliance with genetic equilibrium in the locus of hypophysis factor of transcription in favour of heterozygotes in cows of the three breeds (URD, URSD and UBSD), indicated by the fixation index with 40.9, 16.0 and 24.9%, elimination of homozygotes.

The amplified gene fragment of thyroglobulin had 548 n.p. The lengths of restricted fragments were: CC – three fragments 295, 178 and 75 n.p.; CT – four fragments of 473, 295, 178 and 75 n.p.; TT – two fragments 473 and 75 n.p. (Fig. 5).

Fig. 5. Electrophoregramme of the restriction products of amplified fragments of gene thyroglobulin of locus *TG-5*

(1– amplificate; 2, 4, 6, 7, 8, 11, 13 – genotype CT; 3, 8 – genotype TT; 5, 7, 9, 10, 12, 14, 15, 16 – genotype CC; M – marker of molecular mass)

For locus *TG-5*, in all Ukrainian contemporary breeds of dairy cattle, a high frequency of allele C (0.91–0.97) was observed, and the frequency of homozygotes of this genotype was 0.812–0.933 (Table 7). For all estimated populations, expected and real heterozygosity of locus *TG-5* differed and there was a displacement of genetic equilibrium in favour of heterozygotes in cows of breeds URD and URSD, indicated by the fixation index — 6.9 and 3.6%, respectively.

DISCUSSION

Polymorphic protein systems are the products of allelic genes of separate loci that in the process of selection are supported by selection (natural or artificial). Thus, every allele has a selective value, which may be shown in indices of productivity.

kappa-casein is a basic gene with quantitative features, the polymorphism of which affects qualitative and quantitative indices of milk productivity. The product of gene amplification of *CNS3* with primers includes the fourth exon and the

Table 6. Polymorphism by gene *Pit-1* of cows of Ukrainian breeds

Breed	n	Frequency of genotype						Frequency of allele A/B	Heterozygosity		χ^2	PIC	F_{IS}
		AA		AB		BB			H_o	H_e			
		n	%	n	%	n	%						
URD	16	0	-	10	62.5	6	37.5	0.31/0.69	0.625	0.444	3.306	0.430	-0.409
URSD	15	3	20.0	9	60.0	3	20.0	0.50/0.50	0.600	0.517	0.600	0.500	-0.160
UBSD	15	1	6.7	9	60.0	5	33.3	0.37/0.63	0.600	0.480	1.278	0.464	-0.249

Table 7. Polymorphism in gene *TG-5* of cows of Ukrainian breeds

Breed	<i>n</i>	Frequency of genotype						Frequency of allele A/B	Heterozygosity		χ^2	<i>PIC</i>	<i>F_{IS}</i>
		CC		CT		TT			<i>H_o</i>	<i>H_e</i>			
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%						
URD	16	13	81.2	3	18.8	0	–	0.91/0.09	0.187	0.175	0.171	0.170	–0.069
URSD	15	13	86.7	2	13.3	0	–	0.93/0.07	0.133	0.129	0.077	0.124	–0.036
UBSD	15	14	93.3	1	6.7	0	–	0.97/0.03	0.066	0.067	0.018	0.064	0.000

fourth intron of the gene. After restriction of this fragment by Hind III restrictase, two allelic variants A and B are produced. Variant B of gene *CSN3* is characterised by two spot mutations in state 136 and 148, which arrest amino acid substitutions of Tyr for Iso and Ala for Asp (Kaminski *et al.*, 1993). Gene *CSN3* is associated with protein of milk and its coagulative characteristics. Allele B, one of the most valuable, is associated with a higher content of proteins and fat, as well as with optimum properties for production of cheese. Allele A influences the yield of milk, fat and protein (Tyulkin *et al.*, 2005; Kalashnikova *et al.*, 2006). Among different breeds for production, there is a preference for allele A over allele B (Tyulkin *et al.*, 2005; Kalashnikova *et al.*, 2006; Khaizaran *et al.*, 2014; Pavlova *et al.*, 2016), but some studies suggest the opposite (Kemenes *et al.*, 1999; Lazebnaya *et al.*, 2016).

There is a large amount of information on the relationships between genotypes (AA, AB and BB) and yield and mass of fat and protein, cheese-suitability of milk and other features in different breeds of cattle (Nekrasov *et al.*, 2017; Shendakova *et al.*, 2014). It is known that the presence of allelic variant B in locus *CSN3* is associated with increased content of casein proteins and higher biological value and milk with considerable amounts of all amino-acids (valine – 7 %, leucine – 12 %, lysine – 7 %). Irreducible amino-acids of casein are synthesised from free amino-acids of blood. Synthesis of milk proteins depends on nitrogen-containing substances of blood — amino-acids and polypeptides. A higher level of amino acids in blood plasma is associated with a higher protein content in milk. At the expense of increasing protein content in milk, the amounts of common proteins and amino-nitrogen in serum of blood become lower, which is explained by intensive protein metabolism for synthesis of milk protein.

Presence of allele B in locus κ -casein is associated with a trend of milk productivity. It is also economically important for production of cheese, as it improves the quality of hard cheese and accelerates the speed of curdling of milk. The allele is associated with increased content of casein proteins and milk fat, dry matter of milk, and also concentration of ionic Ca, which is 13 times higher than in blood (Zhuravel *et al.*, 1997). Animals which are homozygotic in allele A produce milk that has a low protein index, and retention in selection of this allele results in production of milk that is called “drinkable”, but has poor (bad) technological features with low dry matter content (Epishko *et al.*, 2010).

The next most important gene with effect on quantitative and qualitative indices of milk is beta-lactoglobulin, the size of which is 4662 n.p.; it is formed from seven exons and six introns (Karimi *et al.*, 2009). Allele *BLG* A has one restriction site for restrictase Hae III, which leads to formation of two restriction fragments — 148 and 99 n.p., and further restriction of the 148 n.p. fragment or forms two fragments with length 74 n.p. The gene of beta-lactoglobulin is polymorphic, which has allelic variants, the most common of which are variants A and B (Eigel *et al.*, 1984; Tsiaras *et al.*, 2005).

Beta-lactoglobulin gene is another genetic marker with significance for milk productivity and technological features of milk: allele *BLG* B is associated with a higher content of casein proteins, high percent fat and better casein coagulation parameters. Presence of the *BLG* genotype in an animal is associated with a high content of serum proteins and higher common yield (Sheiko *et al.*, 2009; Novikova *et al.*, 2010). Serum proteins enter milk directly from blood, but in considerably less quantity. In this case, blood globulin transmutes into globulin of milk. Expression of variant B is linked with a high content of casein protein in milk, higher percent fat and better casein coagulation parameters. Variant A results in a high content of both serum proteins and total content of proteins of milk. In milk of cows with genotype AB, both allelic forms of *BLG* are present with preference of form A (Kim *et al.*, 2001).

The leptin gene affects assimilation of nutrient matter, its metabolism and regulates the synthesis of fatty acids and their distribution in the organism. The content of triglycerides, which is the main component of milk fat, is reduced by increasing the yield. Some studies showed that allele T of the leptin gene is more desirable than allele C, since allele T is associated with increased fat and protein content in milk, but some data shows that allele C is associated with increasing yields (Gonyon *et al.*, 1987).

Glycerides of milk include in their composition greater quantities of low molecular weight fatty acids: saturated — palmitic, stearin, and myristic; unsaturated — butyric, capronic, caprylic, and lauric.

At the starting stages of embryogenesis, the locus *Pit-1* is well-known to direct differentiation of hypophysis cells, determine development of the zones associated with synthesis of somatotropin, prolactin, and thyrotrop hormone, as well as regulate the activity of hormones that influence the level of yield and filtration and synthesis of components of milk in the udder. The allele A variant is associated with increased yield, and expression of the allele B is connected with increased content of fat in milk (de Mattos *et al.*, 2004).

Another tested gene is *TG-5* which is responsible for underskin ball and for common per cent of fat in tissues, including forming of milk, since iodotyronines influence on differentiation of adipocytes (Kopylova *et al.*, 2011). Thyreoglobulin is a glucoprotein hormone that is synthesised in follicular cells of the thyroid gland and is the precursor of tricodteranin and tetraiodteranin. It affects the percent fat content in tissues and participates in formation of triglycerids (Aihaund, 1992). The sources of light fatty acids of milk fat are acetic, propionic and butyric acids, but the glycerine part of milk fat is synthesised from carbohydrates. Therefore, fatty-milky cows have a low content of sugar in blood (Bogatova *et al.*, 2004). It is also known that allele T is associated with greater content of fat in milk (Larionova, 2006).

CONCLUSIONS

The study of polymorphisms of structural genes in native breeds (Ukrainian Red Dairy, Ukrainian Black-speckled Dairy, and Ukrainian Red-speckled Dairy) was based on the analysis of the distribution of allelic variants of genes *CSN3*, *BLG*, *LEP*, *Pit-1* and *TG-5* and analysis of breeds genotypes in connection with their unique phenotypic features. Our findings can be useful for cow selection by DNA markers in the following directions:

- increased yields of milk in the breeds Ukrainian Red Dairy (*CSN3^{AA}*, *TG-5^{SS}*), Ukrainian Black-speckled Dairy (*CSN3^{AA}*, *TG-5^{SS}*) and Ukrainian Red-speckled Dairy (*TG-5^{SS}*, *LEP^{SS}*);

- increased content of fat in milk — in breeds Ukrainian Red Dairy (*Pit-1^{BB}*), Ukrainian Black-speckled Dairy (*BLG^{BB}*; *LEP^{TT}*) and Ukrainian Red-speckled Dairy (*BLG^{BB}*);

- better technological qualities of processing of milk for hard cheese — in the Ukrainian Red-speckled Dairy (*CSN3^{BB}*);

- increased content of common protein in milk — in breeds Ukrainian Red Dairy (*BLG^{AA}*), Ukrainian Black-speckled Dairy (*LEP^{TT}*) and Ukrainian Red-speckled Dairy (*CSN3^{BB}*);

- relatively greater content of cheese-serum protein of milk — in the breed of Ukrainian Red Dairy (*BLG^{AA}*). This breed may be used to form industrial groups or farms of definite assignment for obtaining milk raw material.

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PROTEĪNU UN LIPĪDU VIELMAIŅAS GĒNU POLIMORFISMS MODERNAJĀS UKRAINAS PIENA VIRZIENA LIELLOPU ŠĶIRNĒS

Ar RFLP (*Restriction Fragment Length Polymorphism*) metodi noteikts polimorfisms proteīnu un lipīdu vielmaiņas gēnos *CSN3* (*kappa-casein*), *BLG* (*betalactoglobulin*), *LEP* (*leptin*), *Pit-1* (*pituitary transcription factor*), *TG-5* (*thyroglobulin*) vairākām Ukrainas piena virziena liellopu šķirnēm.