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Genetic Structure's Investigation of the Ukrainian Population of the Polish Red Breed Cattle by Quantitative Trait Loci

M. L Dobrianska[1], T. S. Yaschuk[2], P. P. Dzhus[1], K. V. Kopylova[1],
O. M. Zhukorskyi[3], K. V. Kopylov[1]

[1]*Institute of Animal Breeding and Genetics NAAS*
1, Pogrebnyaka Str., Chubynske Villiage, Boryspil District, Kyiv Region, Ukraine, 08321
[2] *Ternopil Institute of Agricultural Production NAAS*
12, Troleibusna Str., Ternopil, Ukraine, 46027
[3] *National Academy of Agrarian Sciences of Ukraine,*
37, Vasylkivska Str., Kyiv, Ukraine, 03022

e-mail:cvic_ua@mail.ru

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Aim. To investigate the genetic structure of the Polish Red breed cattle population that is being reproduced in Ukraine by 5 genes participating in the formation of qualitative and quantitative performance indicators of cattle. **Methods**. Definition of individual genotypes of the animals was performed using polymerase chain reaction (PCR) followed by determination of restriction fragment length polymorphism (RFLP analysis). **Results.** The high frequency of desirable alleles in the genes of kappa-casein and beta-lactoglobulin has been reported for the investigated population. **Conclusions**. The significant genetic potential of the Polish Red breed cattle by milk production was stated.

Key words: Polish Red breed cattle, quantitative trait loci, lactation, milk yield, milk fat, protein.

INTRODUCTION

Global trend in rapid development of the molecular biology provide further intensive application of methods in all branches of agriculture, in particular, cattle breeding. Based on individual genetic approaches to determination of the usefulness of the body by genome labeling, DNA technologies allow to optimize performance evaluation of hereditary traits of cattle and establish patterns of transmission and consolidation of generations. Alongside with typing the allele variants of genes determining economically useful traits, the preconditions for the determination of the degree of expression displayed in the characteristics of the phenotype manifestations of reproductive and productive traits of animals are also created. Thus, the results of complex genetic studies are effectively used for improving the selection and breeding through a targeted staffing stud carriers of the desired alleles and genotypes for the genes of quantitative traits and biodiversity conservation programs and control organization of endangered and native species, which is a key issue in the modern livestock [1].

The Polish Red breed cattle is one of the important sites for long-term genetic and population monitoring in Ukraine. The QTL-loci research of local populations' genetic structure specific formation is of the excellent theoretical and practical significance, because the animals of this breed are often used as a starting material in the derivation of other breeds of cattle milk and meat productivity trends. Therefore, to study the species specificity at the level of genetic polymorphisms within the Ukrainian population of the Polish Red breed cattle molecular analysis of the distribution of allele variants of genes involved in the formation of qualitative and quantitative performance indicators animals has been performed.

MATERIALS AND METHODS

The genetic structure of the Ukrainian population of the Polish Red breed cattle has been studied by 5 loci of quantitative traits: kappa-casein (κ-Cn), beta-lactoglobulin (βLG), leptin (LEP), growth hormone (GH) and pituitary-specific transcription factor (PIT 1). 150 animals were kept at the farms within the Ternopil region of Ukraine were surveyed.

Genome DNA was extracted from whole blood of animals by the sorbent method. The studied genes identification was performed by the polymerase chain technique followed with the definition of restriction fragment length polymorphism (PCR-RFLP). The standard reaction mixture for PCR-RFLP analysis in the volume

of 10 µl contained: 4.3 µl of deionized water, 2.0 µl 5 × × PCR buffer (15 mmol Mg −1.0 ml), 0.8 µl 10 × dNTP mixture (2 mmol concentration each), 0.8 µl primers (70 ng each), 0.1 µl Taq-polymerase (1 ml/1000 U), 2.0 µl DNA (50−100 ng).

To amplify the gene fragment of κ-Cn, the following primers were used: 5′-GAAATCCCTACCATCAATACC-3′; 5′-CCATCTACCTAGTTTAGATG-3′. The PCR amplification temperature range for the kappa-casein gene: initial denaturation − 94 ºC, 4 min; 34 cycles: denaturation − 94 ºC, 15 s, primers annealing − 58 ºC, 15 s, synthesis − 72 ºC, 15 s; terminal elongation – 72 ºC, 5 min. The HinfI restriction enzyme (Fermentas, Lithuania) [2] was used for the analysis of gene polymorphism.

For the amplification of βLG gene fragments the following primers were used: 5′-TGTGCTGGACACCGACTACAAAAAG-3′ and 5′-CTCCCGGTATATGA-CCACCCTCT-3′. The PCR amplification temperature range for the beta-lactoglobulin gene: initial denaturation – 94 °C, 4 min; 34 cycles: denaturation – 94 °C, 15 s; primers annealing – 63 °C, 15 s; synthesis – 72 °C, 15 s; terminal elongation – 72 °C, 5 min. The Bsu RI (HaeIII) restriction enzyme [3] was used for the analysis of gene polymorphism.

For amplification of the GH gene fragments, the following primers were used: 5′-GCTGCTCCTGAGGGCCCTTC-3′; 5′-GCGGCGGCACTTCATGACCC-3′. The PCR amplification temperature range for the growth hormone gene: initial denaturation – 94 °C, 4 min; 34 cycles: denaturation – 94 °C, 15 s; primers annealing – 65 °C, 15 s; synthesis – 72 °C, 15 s; terminal elongation – 72 °C, 5 min. The Alu I restriction enzyme [4] was used for the analysis of gene polymorphism.

For amplification of the LEP gene fragments the following primers were used: 5′-GTCACCAGGATCAATGACAT-3′; 5′-AGCCCAGGAATGAAGTCCAA-3′ The PCR amplification temperature range for the leptin gene: initial denaturation – 94 °C 4 s; 40 cycles: denaturation – 94 °C, 15 s; primers annealing – 58 °C, 15 s; synthesis – 72 °C, 15 s; terminal elongation – 72 °C, 5 min. The Sau3a restriction enzyme [5, 6] was used for the analysis of gene polymorphism.

To amplify the PIT-1 gene fragment the following primers were used: 5′-CAATGAGAAAGTTGGTGC-3′ 5′-TCTGCATTCGAGATGCTC-3′. The PCR amplification temperature range for the PIT-1 gene: initial denaturation – 94 °C, 4 min; 34 cycles: denaturation – 94 °C, 15 s; primers annealing – 58 °C, 15 s; synthesis – 72 °C, 15 s; terminal elongation – 72 °C, 10 min. The HinfI restriction enzyme [7] was used for the analysis of gene polymorphism.

Products of restriction were separated by electrophoresis in 2 per cent agar gel followed by staining them with a solution of ethidium bromide. Differentiation of the amplicon by size was carried out using the molecular weight marker GeneRuler TM 100 bp DNA Ladder (Fermentas).

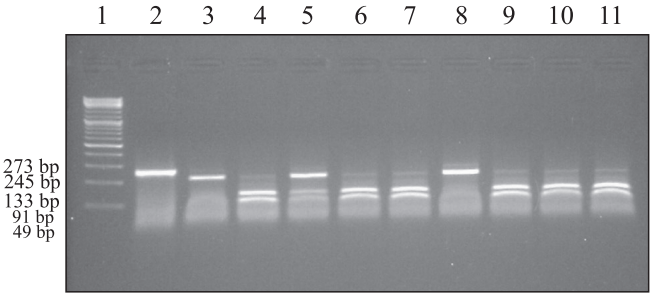

Fig. 1. Electrophoregramm of the separation products for the kappa-casein gene restriction (enzyme Hinf1) for the Polish Red breed cattle. Tracks: 1 – molecular weight marker DNA Ladder, GeneRuler™ 100 bp; 2 – PCR product without restriction (273 bp); 3, 8 – homozygous animal with genotype BB (224, 49 bp); 4, 6, 7, 9–11 – homozygous animals with genotype AA (133, 91, 49 bp.); 5, – heterozygous animals with genotype AB (224, 133, 91, 49 bp)

The data were processed statistically by means of the Microsoft Excel standard software package.

RESULTS AND DISCUSSION

Polymorphism of the genes associated with milk protein and fat content defines general characteristics of cattle milk technological properties. Kappa-casein and beta-lactoglobulin A genes' alleles are responsible for the value of milk yield per lactation. In addition, allele variants of βLG A locus are also associated with containing of the milk whey protein. Aκ-Cn B gene's allele is involved in the milk proteins synthesis, beta-lactoglobulin B gene's allele is associated with the content of casein protein and milk fat [8, 9].

According to Table 1, three genotypes of kappa-casein and beta-lactoglobulin genes were fixed within studied population of the Polish Red breed cattle.

Table 1. The Distribution of Genotypes and Allele Frequencies for the Genes Associated with Economically Useful Traits within the Studied Ukrainian Population of the Polish Red Breed Cattle

Genes	Genotype			Alleles	
	AA	BB	AB	A	B
κ-Cn	0.405	0.167	0.428	0.697	0.303
βLG	0.155	0.502	0.343	0.204	0.796
PIT 1	0.112	0.843	0.045	0.269	0.731
	Genotype			Alleles	
	LL	LV	VV	L	V
G	0.526	0.210	0.264	0.754	0.246
	Genotype			Alleles	
	AA	AB	AC	A	B	C
LEP	0.552	0.161	0.287	0.798	0.085	0.113

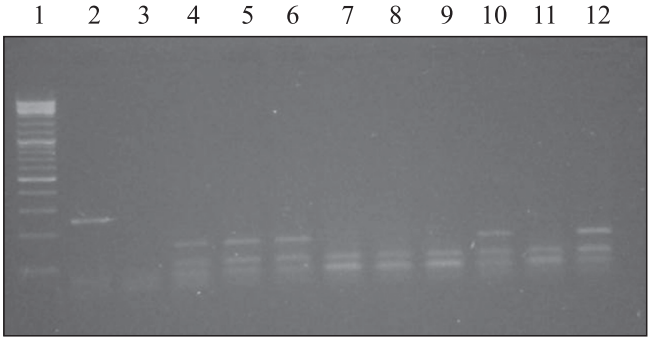

Fig. 2. Electrophoregramm of the separation products for the beta-lactoglobulin gene restriction (enzyme BsuRI) of the Polish Red breed cattle. Tracks: 1 – molecular weight marker DNA Ladder, GeneRuler™ 100 bp; 2 – PCR product without restriction (247 bp); 3 – control, 4–6, 10, 12 – heterozygous animals with genotype AB (148, 99, 74 bp); 7–9, 11 – homozygous animal with genotype BB (99, 74 bp)

For κ-Cn locus, frequency of allele A dominates over allele B by a larger carrier's concentration of the homozygous genotype AA, which frequency was 0.405. For gene βLG the opposite trend was observed. The frequency of allele B was significantly higher than frequency of allele A 0.796 and 0.204 respectively.

Concerning the distribution of genotypes for genome pituitary-specific transcription factor, it should be noted that the surveyed populations showed low concentrations of allele variant A (0.269) associated with high milk yield at low milk fat content [10]. As for the growth hormone gene, the homozygous genotype LL (0.526) and L allele variant (0.754) respectively that is responsible for the protein and fat content in the milk dominate within the studied population.

Table 2. The Distribution of Genotypes and Allele Frequencies by Genes within Cows Groups Based on the Average Level of Milk Yield per Lactation

Genes	Group of animals by milk yield			
	Milk yield			
	up to 3000 kg (fat content of 3.8–3.99 %)		more than 3000 kg (fat content of 3.71–3.8 %)	
	The frequency of genotype	The frequency of alleles	The frequency of genotype	The frequency of alleles
κ-Cn	AA – 0.077 AB – 0.923	A – 0.576 B – 0.424	AA – 0.333 AB – 0.666	A – 0.667 B – 0.333
βLG	AA – 0.052 AB – 0.422 BB – 0.526	A – 0.256 B – 0.744	AA – 0.166 AB – 0.334 BB – 0.500	A – 0.333 B – 0.667
GH	LL – 0.588 LV – 0.235 VV – 0.177	L – 0.706 V – 0.294	LL – 0.545 LV – 0.273 VV – 0.182	L – 0.682 V – 0.318
PIT-1	AA – 0.125 AB – 0.375 BB – 0.500	A – 0.312 B – 0.688	AA – 0.000 AB – 0.364 BB – 0.636	A – 0.182 B – 0.818
LEP	AA – 0.667 AB – 0.333	A – 0.833 B – 0.167	AA – 0.333 AB – 0.333 AC – 0.333	A – 0.666 B – 0.167 C – 0.167

According to the results of the locus polymorphism analysis as for the leptin within the Ukrainian population of the Polish Red breed cattle, rare allele variant C has been revealed. Its frequency is equal to 0.113, what is higher than the B allele frequency, which was the lowest – 0.085.

For the purpose of establishing the characteristics of the distribution allele variants and genotypes for κ-Cn, βLG, GH, PIT-1 and LEP loci, depending on the level of milk yield per lactation, the comparative analysis was performed. The Polish Red breed cattle cow herd was divided into two groups. The first group includes the animals with up to 3000 kg of average milk yield per lactation. The second group consists of the cows with an average milk yield higher than 3000 kg per lactation. The obtained findings are represented in Table 2.

For κ-Cn gene, lower frequency of allele A (0.576) and higher one of allele B (0.424) were observed in cows with lower milk yield indices compared to the similar values in the group of animals with higher milk yield (0.667 and 0.333 respectively). The frequency of the BLG gene A and B allele variants distributed similarly for both of the studied groups. The allele B frequency (0.744 compared to 0.667) is dominated by the first group, while allele A frequency (0.333 compared to 0.256) in the second. For PIT-1 gene, the allele A frequency was slightly higher in cows with lower milk yield (0.312). the allele B frequency (0.818 compared to 0.688) was fairly higher for cows with higher rates during lactation milk yield.

For LEP locus, no genotypes of rare C allele variant were observed in the first group of animals. As for allele B, the two experimental groups demonstrated the same frequency – 0.167. In general, the distribution of genotypes and allele frequencies of the studied genes never allowed to establish statistically significant intergroup differences. The findings on the genetic structure of the Polish Red breed cattle obtained at 5 loci of quantitative traits determine its breed specifying characteristics and features due to breeding areas of the species. For the alleles and genotypes frequencies distribution of kappa-casein gene, the current results are consistent with the results formerly got by other scholars. For example, the Polish researchers distributed the allele frequencies within 65 animal, as follows: A – 0.685, B – 0.315 [11].

Allele B frequency prevalence in the PIT-1 gene and L allele in the growth hormone gene serves as an ad-

ditional criterion for technological evaluation of milk that indicates a high fat content.

According to a comprehensive molecular genetic analysis, the animals of the Ukrainian population of the Polish Red breed cattle are marked with the considerable genetic potential for milk production as for some peculiar indicators.

CONCLUSIONS

The surveyed population of the Polish Red breed cattle is characterized by polymorphism loci associated with qualitative and quantitative indicators of milk production.

For kappa-casein gene, the allele A is a predominant marker for the level of yield, while for beta-lactoglobulin gene – allele B, which provides satisfactory performance of fat milk.

The molecular genetic analysis results of the Polish Red breed cattle reflect the specificity of genetic population structure by individual loci of quantitative traits that trace the features of breeding and its place in the breeding process.

Дослідження генетичної структури української популяції червоної польської породи великої рогатої худоби за локусами кількісних ознак

М. Л. Добрянська[1] , Т. С. Ящук[2],
П. П. Джус[1], К. В. Копилова[1],
О. М. Жукорський[3], К. В. Копилов[1]

e-mail:cvic_ua@mail.ru

[1]Інститут розведення і генетики тварин НААН України
Вул. Погребняка, 1, с. Чубинське, Бориспільський р-н, Київська обл., Україна, 08321
[2] Тернопільський інститут агропромислового виробництва НААН України
Вул. Тролейбусна, 12, Тернопіль, Україна, 46027
[3] Національна академія аграрних наук
Вул. Васильківська, 37, Київ, Україна, 03022

Мета. Дослідження генетичної структури популяції чер-воної польської породи великої рогатої худоби, що відтворюється в Україні, за п'ятьма генами, які беруть участь у формуванні якісних і кількісних показників продуктивності ВРХ. **Методи**. Індивідуальні генотипи тварин ідентифікували за допомогою полімеразно-ланцюгової реакції з подальшим визначенням поліморфізму довжин рестриктних фрагментів (ПДРФ-аналіз). **Результати**. У дослідженій популяції відмічено високу частоту обраних алелей за генами капа-казеїну і бета-лактоглобуліну. **Висновки**. Встановлено значний генетичний потенціал корів червоної польської породи за молочною продуктивністю.

Ключові слова: червона польська порода, локуси кількісних ознак, лактація, надій, молочний жир, білок.

Исследование генетической структуры украинской популяции красной польской породы крупного рогатого скота по локусам количественных признаков

М. Л. Добрянская[1] , Т. С. Ящук[2],
П. П. Джус[1], К. В. Копилова[1],
О. М. Жукорский[3], К. В. Копилов[1]

e-mail:cvic_ua@mail.ru

[1]Институт разведения и генетики животных НААН Украины
Ул. Погребняка, 1, с. Чубинское, Бориспольський р-н, Киевская обл., Украина, 08321
[2] Тернопольский институт агропромышленного производства НААН Украины
Ул. Троллейбусная, 12, Тернополь, Украина, 46027
[3] Национальная академия аграрных наук Украины
Ул. Васильковская, 37, Киев, Украина, 03022

Цель. Исследование генетической структуры популяции красной польской породы крупного рогатого скота, воспроизводящейся в Украине по пяти генам, участвующим в формировании качественных и количественных показателей продуктивности КРС. **Методы**. Индивидуальные генотипы животных идентифицировали с помощью полимеразно-цепной реакции с последующим определением полиморфизма длин рестрикционных фрагментов (ПДРФ-анализ). **Результаты**. В исследованной популяции отмечена высокая частота выбранных аллелей по генам каппа-казеина и бета-лактоглобулина. **Выводы**. Установлен значительный генетический потенциал коров красной польской породы по молочной продуктивности.

Ключевые слова: красная польская порода, локусы количественных признаков, лактация, удой, молочный жир, белок.

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