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GENETIC SPECIFICITY OF THE WHITE-HEADED UKRAINIAN BREED ACCORDING TO THE *BoLA-DRB3* GENE

Abstract: The study of the genetic specificity of local breeds is a promising direction in the context of preserving the biodiversity of cattle breeds in the world. The purpose of research was to analyze the allelic polymorphism of the *BoLA-DRB3* gene of the White-Headed Ukrainian breed. The study was carried out with blood samples from 49 animals (11 bulls and 38 cows) of this breed. The allelic spectrum of the *BoLA-DRB3* gene was detected based on two-step PCR (primers *HLO-30, HLO-31* and *HLO-32*). Restriction was performed with endonucleases *RsaI, HaeIII, BstYI*. Restriction fragments were separated by electrophoresis in 9-12 % polyacrylamide gel. According to the test results, 29 alleles were detected. Seven alleles (*03, *11, *13, *15, *22, *23 and *24) were determined with a frequency of over 5 %, that was 65.3 % of the allele pool of the breed. The most common was allele *24 with a frequency of 15.3%. In the experimental sample, 37 genotypes were identified. The predominant variant was *11/*24 (16.2%). A slight excess of heterozygotes was detected (F_{is} = – 0.035). White-Headed Ukrainian breed is characterized by a significant level of differentiation (or specificity) according to the *BoLA-DRB3* gene (Wright fixation index H_e = 0.959, Shannon-Wiener index $H`$ = 2.93), that confirms the thesis of the important role of local breeds in preserving the biodiversity of genetic resources of cattle.

Keywords: White-Headed Ukrainian breed, *BoLA-DRB3* gene, alleles, observed and expected heterozygosity, Wright fixation index, Shannon-Wiener index

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ГЕНЕТИЧЕСКАЯ СПЕЦИФИЧНОСТЬ БЕЛОГОЛОВОЙ УКРАИНСКОЙ ПОРОДЫ ПО ГЕНУ *BoLA-DRB3*

Аннотация: Изучение генетической специфичности локальных пород – одно из перспективных направлений в контексте сохранения биоразнообразия пород крупного рогатого скота в мире. Цель исследования – определение и анализ аллельного полиморфизма гена *BoLA-DRB3* белоголовой украинской породы. Изучение проведено на образцах крови 49 животных (11 быков и 38 коров) этой породы. Аллельный спектр гена *BoLA-DRB3* определяли на основе двухступенчатой ПЦР (праймеры *HLO*-30, *HLO*-31 и *HLO*-32). Рестрикцию проводили эндонуклеазами *RsaI, HaeIII, BstYI*. Фрагменты рестрикции разделяли электрофорезом в 9–12%-ном полиакриламидном геле. В результате исследований обнаружено 29 аллелей. Семь аллелей (*03, *11, *13, *15, *22, *23 и *24) определялись с частотой более 5 %, что составляло 65,3 % аллельного фонда породы. Наиболее распространенным с частотой 15,3 % выявлял-

ся аллель *24. В экспериментальной выборке идентифицировано 37 генотипов. Преобладающий вариант – *11/*24 (16,2 %). Выявлен небольшой избыток гетерозигот (F_{IS} = -0,035). Характерной особенностью белоголового украинского скота является существенная дифференциация (или специфичность) по гену *BoLA-DRB3* (индекс фиксации Райта H_e = 0,959, индекс Шеннона-Винера $H`$ = 2,93), что подтверждает тезис о важной роли местных пород в сохранении биоразнообразия генетических ресурсов крупного рогатого скота.

Ключевые слова: белоголовая украинская порода, ген *BoLA-DRB3*, аллели, наблюдаемая и ожидаемая гетерозиготность, индекс фиксации Райта, индекс Шеннона-Винера

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Introduction. The study of the genetic specificity of local breeds is one of the promising areas for studying the biodiversity of cattle breeds in Ukraine. After all, in indigenous breeds, allelic variants of various genes have been preserved, which are lost in modern highly specialized commercial breeds due to unidirectional selection. Prolonged selection for high milk productivity leads to decrease in animal health. The possibility of using the genetic potential of local breeds is worth studying for a wide range of breeding programs.

The White-Headed Ukrainian breed was created based on local Polissya cattle by the method of reproductive crossing with bulls of the Groningen herd of the Dutch breed [1]. The breed was one of the planned dairy breeds in Ukraine, 60 years ago its number reached 500-700 thousand [2]. Today, the only breeding herd of the White-Headed Ukrainian breed, consisting of 300 cows, has survived. The White-Headed Ukrainian breed belongs to the first category of domestic gene pool species being on the verge of extinction [3].

Intensification of animal husbandry requires further development of theoretical foundations and improvement of organizational forms of farm animal selection through the involvement of new methods for assessing their genotypes. Such methods include the use of different types of molecular markers.

The *BoLA-DRB3* gene is unique in all the variety of molecular genetic markers. The second exon of this gene has the highest rate of polymorphism among all studied loci of the main histocompatibility complex of cattle. The high allelic diversity of the *BoLA-DRB3* gene is associated with the formation of the body's immune response to viral and bacterial infections, which is primarily relevant for the treatment of disease resistance [4, 5]. Significant polymorphism of the gene allows to study on its basis the population-genetic structure and to assess the level of biodiversity of the population or individual herds among themselves. Due to their close localization on chromosome 23 to the locus of the prolactin gene, alleles of the *BoLA-DRB3* gene are associated with the milk productivity of cattle [6].

The polymorphism of the *BoLA-DRB3* gene, that arose evolutionarily due to the need for a variable immune response to various foreign protein antigens, has both geographical and breed variability, which is the basis for considering the features of its polymorphism both within each breed and individual herds. Recently, there has been a growing number of publications where the *BoLA-DRB3* gene polymorphism is used to assess the biodiversity of cattle populations. To date, the allelic diversity of the gene has been studied for over 30 species of *Bos taurus*, and the research continues [7].

Based on the detected allele frequencies, the population-genetic structure was studied in South African [8], Mongolian, Yakut and Kalmyk [9], Indian [10], Argentinean Creole [11], Chilean [12], Japanese [13, 14], Philippine native [15] and South American [16] cattle.

At the same time, domestic breeds of cattle, according to the *BoLA-DRB3* gene, had not been characterized as widely and systematically as foreign ones. To date, a number of studies on polymorphism of the *BoLA-DRB3* gene for Ukrainian cattle have been performed. A distribution of alleles for Ukrainian Black-and-White, Ukrainian Red-and-White Dairy and Ukrainian Gray breeds has been established [17, 18]. This work is a continuation of research on the genetic specificity of Ukrainian breeds of cattle. Its purpose is to detect the genetic features of the White-Headed Ukrainian breed according to the *BoLA-DRB3* gene.

Materials and methods. The biological material of White-Headed Ukrainian breed animals (38 cows, 11 bulls) was used for research. Blood samples were obtained from cows at Podilsky Gospodar LLC (Antoniny village, Krasyliv district, Khmelnytsky region); sperm-doses of bulls are kept in the

cryostorage of the Bank of Animal Genetic Resources of the Institute of Animal Breeding and Genetics and at the M.V. Zubets National Academy of Sciences of Ukraine and Khmelnytsky regional breeding center. Molecular genetic research was performed in the genetics laboratory of the Institute of Animal Breeding and Genetics.

Allele frequencies were determined using analysis of restriction products obtained after amplification of exon 2 of the *BoLA-DRB3* gene. DNA from blood samples was isolated using "DIAtom™ DNA Prep 200" kits ("Izogen Laboratory Ltd", Russia). The isolated DNA (yield 5-10 *mg* from 200 *ml* of whole blood) has a high molecular weight (40-50 *bp*), optical density ($OD_{260/280nm}$=1.6-2.0); it is a pure substance. Amplification of exon 2 of the *BoLA-DRB3* gene was performed by PCR [19] using "GenPak ™ PCR Core" kits ("Izogen Laboratory Ltd.", Russia). The total volume of the mixture (20 *μl*) contained 60 *mM* Tris-HCL (*pH* 8.8), 2.5 *mM* $MgCl_2$, 20 *mM* KCl, 15 *mM* $(NH_4)_2SO_4$, 10 *mM* Mercaptoethanol, 0.1% Triton X-100, 0,2 *mM* dNTP, 10 units of Klentaq DNA polymerase, 10 *pM* of each primer, template DNA. Primers were used for amplification: for the first reaction round - *HLO*-30 (5'-3 ': *TCCTCTCTCTGCAGCACATTTCC*) and *HLO*-31 (5'-3': *ATTCGCGCTCACCTCGCCGCT*); for second round - *HLO*-30 and *HLO*-32 (5'-3': *TCGCCGCTGCACAGTGAAACTCTC*). For PCR-second round 2 μl of the product of the first round was used. The first period: denaturation of DNA at 95°C during 5 min. Then 10 cycles of denaturation (94°C during 1 min.), hybridization of primers (62.5°C during 2 min.), DNA synthesis (72°C during 1 min.) and the final synthesis (72°C during 7 min). Second period: initial denaturation (95°C during 5 min.), 35 cycles of denaturation, primer hybridization and DNA synthesis (94, 68 and 72°C during 0.5 min.) and final synthesis (72°C during 7 min.).

PCR products were treated separately with three restriction endonucleases: *RsaI, HaeIII, XhoII* ("Promega", "New England BioLabs", USA; and "SibEnzim", Russia). Restriction fragments were separated by electrophoresis in 9-12% polyacrylamide gel. The molecular weight marker "GeneRuler ™ Ultra Low Range DNA Ladder" ("Fermentas", Lithuania) was used to estimate the length of the fragments.

Based on the analysis of electrophoregrams (Fig. 1), restriction patterns and corresponding allele variants (*BoLA-DRB3* genotypes) of the tested animals were identified.

Amplification of exon 2 with subsequent analysis of the length of restriction fragments and comparison of DNA samples obtained using three endonucleases allows identifying 54 nomenclature alleles [19-21]. Allele frequencies are determined taking into account the number of homozygotes and heterozygotes. The standard error of allele frequencies is determined by the formula (p_i - frequency of the corresponding allele; n - sample size):

$$SE = \sqrt{0,5p_i(1-p_i)/n}\,.$$

The observed heterozygosity was determined by directly counting the number of detected heterozygotes (N_2):

$$H_0 = N_2/n.$$

The expected heterozygosity is determined by the formula [22]:

$$H_e = 1 - \sum_{i=1}^{n} p_i^2.$$

The correspondence between the observed and expected distribution of genotypes was checked by the value of Pearson criteria (χ^2-test).

An individual Wright fixation index was used to detect deviations from panmixia [23]:

$$F_{ik} = (H_e - H_o)/H_e.$$

Quantitative assessment of alelic diversity of the breed is based on the Shannon-Wiener index [24]:

$$H' = -\sum_{i=1}^{n}(p_i \times \ln p_i).$$

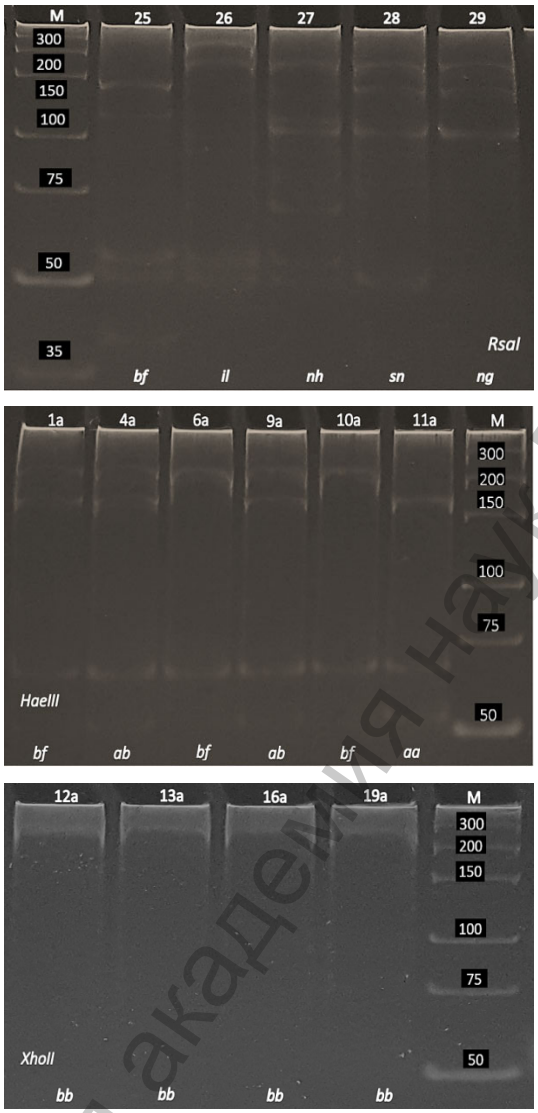

Fig. 1. Electrophoregrams of products of *BoLA-DRB3.2* gene amplification, obtained on the DNA of cow's of Ukrainian White-Headed breed using various endonucleases. Marking: at the top - sample number, below - variants of DNA-patterns, M - molecular weight marker

Statistical data processing was performed in the Microsoft Excel 2013 standard package using author's programs. Verification of the normal distribution of allelic frequencies was performed based on Shapiro-Wilk (SW) and Kolmogorov-Smirnov (KS) criteria in the standard IBM SPSS Statistics V24.0 package (https://www.ibm.com/support/knowledgecenter/ru/SSLVMB_24.0.0/spss/product_landing.html).

Results and discussion. According to the test results, 29 alleles were detected (average frequency 3.45 %) (Table 1).

Analysis of allelic diversity is to identify alleles that are properly informative and meaningful. The test results showed that in the experimental sample seven alleles were revealed with a frequency of over 5 %: *03, *11, *13, *15, *22, *23 and *24, that was a total of 65.3% of the allele pool of the breed. The most common type was allele *24 with a frequency of 15.3%. In addition, it was most pronounced in the genotype of cows (15 animals were its carriers). In further studies, it is necessary to pay attention to the *BoLA-DRB3.2**08 allele, which has a detection rate of 4.08%, closest to the limit of informative value among other options.

The predominant number of alleles in the experimental sample were rare because they were determined only 1-2 times. Also, among the uncommon, six variants (**nab, *mdb, *iab, *gbb, *fbd, *naa*) were found belonging to the so-called alleles "without established nomenclature".

T a b l e 1. **Allelic polymorphism in animal's White-Headed Ukrainian breed**

Alleles	Frequency, %	χ^2	SE	Animals with an allele	
				total	%
***02**	1.02	1.71	0.01	1	1.02
***03**	8.16	6.45*	0.028	8	8.16
***06**	1.02	1.71	0.01	1	1.02
***08**	4.08	0.12	0.02	4	4.08
***10**	1.02	1.71	0.01	1	1.02
***11**	9.18	9.54**	0.029	9	9.18
***12**	2.04	0.57	0.014	2	2.04
***13**	6.12	2.07	0.024	5	5.1
***14**	2.04	0.57	0.014	2	2.04
***15**	8.16	6.45*	0.028	8	8.16
***16**	3.06	0.04	0.017	3	3.06
***19**	1.02	1.71	0.01	1	1.02
***22**	10.2	13.2***	0.031	9	9.18
***23**	8.16	6.45*	0.028	8	8.16
***24**	15.3	40.8***	0.036	15	15.3
***26**	1.02	1.71	0.01	1	1.02
***32**	1.02	1.71	0.01	1	1.02
***33**	2.04	0.57	0.014	2	2.04
***35**	1.02	1.71	0.01	1	1.02
***36**	2.04	0.57	0.014	2	2.04
***39**	1.02	1.71	0.01	1	1.02
***45**	2.04	0.57	0.014	2	2.04
***54**	2.04	0.57	0.014	2	2.04
***nab**	1.02	1.71	0.01	1	1.02
***mdb**	1.02	1.71	0.01	1	1.02
***iab**	1.02	1.71	0.01	1	1.02
***gbb**	2.04	0.57	0.014	2	2.04
***fbd**	1.02	1.71	0.01	1	1.02
***naa**	1.02	1.71	0.01	1	1.02

* $P < 0,05$; *** $P < 0,01$; *** $P < 0,001$.

An allele as a unit of genetic information is always a potential DNA marker. It can be transformed into a real (significant) one only when a close connection (association) is established between it and a certain feature (diseases, economic and useful qualities, etc.). Alleles for which the value of the Pearson test exceeded the critical level $\chi^2 = 3.84$ (CI = 0.95; dF = 1) were considered significant [18]. In this case, the null hypothesis of a normal (uniform) distribution was rejected.

Analysis using the χ^2 test showed that six alleles fell into this category: *03, *11, *15, *22, *23, and *24. The uneven distribution was significant at dF = 28 both for the total frequency spectrum ($\Sigma\chi^2 = 111.4$; $P < 0.001$) and for the frequency of alleles detected in the genotype of cows ($\Sigma\chi^2 = 97.9$; $P < 0.001$). The obtained result was confirmed by verification according to the criteria of Kolmogorov-Smirnov and Shapiro-Wilk (Table 2).

T a b l e 2. **Checking the normality of the frequency distribution of alleles and genotypes**

Frequency distribution	Criteria					
	Kolmogorov-Smirnov*			Shapiro-Wilk		
	indicator value, KS	degree of freedom, *dF*	probability value	indicator value, SW	degree of freedom, dF	probability value
Alleles	0.192	28	0.004	0.622	28	0.0
Genotypes	0.191	28	0.003	0.612	28	0.0

* With correction of the significance of Lilliefors.

There are 37 genotypes with uniform distribution were found in 49 cows. There is only one genotype *11/*24 (16.2%) in the sample, that is significantly distinguished by the frequency of stay. With a frequency of over 5%, five more variants were identified: *13/*23 (10.8%), *03/*24, *15/**gbb*, *16/*22 and *22/*24 (5.41%, each). All other genotypes occurred only once.

When determining the alleles that characterize the sample, it is necessary to take into account that some of them were found in the homozygous genotype. In total, the study identified two variants of homozygotes for alleles *13 and *22 for one animal per corresponding genotype. The presence of homozygous genotypes somewhat reduces the informative value of alleles in comparison with heterozygous variants. The informative value of the allele as a genetic marker decreases with increasing homozygosity of the herd.

In addition to allele frequencies in a single locus, that indicate the genetic variability of the population, other most important indicators characterizing the structure of the herd are homo- and heterozygosity (Table 3).

T a b l e 3. **Genetic structure of animals of the experimental sample of the Ukrainian White-Headed breeds according to the *BoLA-DRB3* gene**

Indicator			Indicator value
Number of animals			49
Number of homozygotes	Observed		2
	Expected		3,6
Number of heterozygotes	Observed		47
	Expected		45.4
Alleles	Found total		29
	With a frequency of more than 5%	Number, %	*03(8.16), *11(9.18), *13(6.12), *15(8.16), *22(10.2), *23(8.16), *24(15,3)
		Total, %	65.3
Genotype	Total		37
	Predominant, %		*11/*24 (16.2%), *13/*23 (10.8%), *03/*24, *15/**gbb*, *16/*22 and *22/*24 (all by 5.41%)
Heterozygosity	Observed		0.959
	Expected		0.927
Wright fixation index (χ^2)			-0.035 (0.059)
Shannon-Wiener index			2.93

The frequency of heterozygotes is an important indicator because each heterozygous individual carries different alleles, which illustrates the presence of variability. A comparative analysis of the presence of homo- and heterozygotes among the tested animals showed that the White-Headed Ukrainian breed has a slight excess of heterozygotes (F_{is} = -0.035). A slight excess of heterozygotes of 3.5%, determined by the individual Wright fixation index, is not the result of inbreeding due to genetic pressure of external factors. The reliability of the determined deviation from normal distribution according to Hardy-Weinberg's law for the confidence interval CI = 0.95 is not confirmed by the χ^2-test. For the critical level of statistical significance $P < 0.05$, the value of χ^2 is less than the tabular value of $\chi^2 = 3.84$. The detected deviation of the distribution from Hardy-Weinberg's Equilibrium is not significant, although the effect of selection pressure on the established excess of homozygotes can be completely rejected only after additional studies.

The total number of identified variants of *BoLA-DRB3* gene characterizes the genetic variability of the breed. A large number of alleles indicate a variety of immune response options. The maximum nomenclature alleles are found in Mongolian (35) and Kalmyk (34) cattle [9]. There were 29 alleles in our study. We also revealed 6 alleles "without established nomenclature". Such alleles are reported in many researches. So, in the study of Jersey breed [25], as many as 11 alleles "without the established nomenclature" were found (**fab, *fbb, *fbe, *iaa, *ibb, *ibe, *laa, *iae, *kba, *nbe* and **obe*), that totaled to 12.6% of allele pool. In the autochthonous Gray Ukrainian breed 6 alleles were found (**jab, *jba, *jbb,*

nad, *nda* and **had* – a total of 7.14 %) [18], and in the Polish Holstein cattle - 4 similar variants (gba, *jba, *jbb* and **nbd* – a total of 10.5%) [26].

The genes that occur in at least 5% of cases are considered polymorphic. They are informative, and the ones for which the χ^2 test value exceeds the critical value are also significant. This threshold is quite conditional, but as a criterion of informative value, is used by most researchers [27, 28]. We found 7 informative alleles with a total frequency of 65.3%. If such alleles have a majority, then they can be considered being able to characterize the breed. For commercial breeds, the consolidation of 6-8 alleles often exceeds 70%. For example, in Holstein cows (n = 1100) from 93 dairy herds in the states of Iowa, Wisconsin, Minnesota and Illinois [29] seven alleles with a frequency of over 5% had total of 75.6%. An analogous study in the Holstein cows (n = 835) in Ontario (Canada) [30] showed that seven informative alleles occupy 88.7% of allele spectrum. The largest consolidation among the Ukrainian breeds (7 informative alleles occupy 59.4%) was found in Red-and-White dairy cattle [18]. The high level of consolidation of the alleles indicates decrease in the genetic variability of the breed. This feature is characteristic of commercial cattle. The breeding of "clean" lines with intraspecific crossing leads to reduction and even loss of individual gene options.

In order to estimate the level of genetic diversity of White-Headed cattle behind the *BoLA-DRB3* gene, similar indicators for other breeds are to be considered (Table 4).

T a b l e 4. **Population genetic indicators for different breeds according to *BoLA-DRB3* gene [31]: expected (H_e) and observed (H_o) heterozygosity, Wright fixation index (F_{IS}) and Shannon-Wiener index ($H`$)**

Cattle population		Sample size, N	Number of alleles from $P_a > 5$ %	$\Sigma P_a > 5$ %	N_a	H_o	He	$_{FI}S$	$H`$
Ukraine	Black-and-White milk	293	7	55.8	28	0.922	0.942	0.021	3.13
	Red-and-White milk	117	7	59.4	22	0.864	0.939	0.08	3.77
Holstein	USA	1100	7	88.7	26	–	0.741	–	2.44
	Canada	835	7	76.2	27	–	0.872	–	2.37
	Argentina	424	7	70.7	33	0.84	0.912	0.079	2.61
	Chile	113	7	80.1	21	0.84	0.893	0.059	2.45
	Japan	101	9	86.7	18	0.921	0.901	-0.02	2.47
Native cattle	Mongolian	71	8	52.1	35	0.775	0.953	0.187	3.25
	Kalmyk	62	4	39.5	34	0.708	0.946	0.252	3.27
	Yakut	42	5	77.3	5	0.12	0.38	0.684	1.74
	Philippine	471	4	30.5	71	0.913	0.959	0.048	3.57
	Saavedreno	125	7	70.0	22	–	0.919	–	2.7

Among the presented breeds, white-headed cattle stand out for two indicators. This breed has the highest indicator of observed heterozygosity and, accordingly, the maximum excess of heterozygotes, determined by the Wright fixation index. Such a result for the *BoLA-DRB3* gene is possible for one of the hypotheses that explain the high level of polymorphism of MHC genes has super domination. Polymorphism of the MHC molecule determines the ability of different immune reactions. It was found that particular products had variability to provide T-cells with alien protein. Selection favoring heterozygotes leads to creation of a stable polymorphic balance conditioned by antigen-binding functions of the molecules of the complex. Therefore, in populations affected by numerous pathogens, heterozygous individuals will have an advantage because they are able to bond with a large number of alien antigens [31].

The data obtained confirm that large variability in the marginal population may be associated with special conditions of existence (for example, a limited population size) that contribute to the selection of heterozygotes. This increases the adaptive potential of the population due to high genetic diversity, which is characterized by a large allele polymorphism associated with a greater fraction of rare alleles. The once numerous White-Headed Ukrainian breed today is a small herd of about 300 heads. It is the

small number of the herd that determines the advantage of heterozygotes in this population. Also, this situation can occur in case of a certain selection pressure during breeding work. Also, this situation can occur in case of a certain selection pressure during breeding operation associated with the insemination of cows by Holsteins.

A fairly high level of biodiversity of the White-Headed breed according to the *BoLA-DRB3* gene is evidenced by the Shannon-Wiener index. This index characterizes the biodiversity of the breed. In general, it can be used to assess the complexity of systems of any type, including genetic research. This indicator is proposed to assess the complexity of the structure of animal communities. The maximum value of the index corresponds to the maximum chaotic distribution of alleles. Otherwise, the system will be ordered, which in the genetic sense indicates the impoverishment of biodiversity. The value of $H`$ is in the range from 1.5 to 3.5 rarely exceeding 4.5. Index $H` = 2.93$ obtained in our study indicates a fairly high level of biodiversity of the White-Headed Ukrainian breed according to the *BoLA-DRB3* gene. Table 4 shows the data on the dimension of the index determined based on the study of the polymorphism of various breeds behind the BoLA-DRB3 gene. Among domestic breeds, the maximum value of the indicator was found for the Ukrainian Black-and-Red Dairy breed ($H` = 3.77$). As expected, commercial breeds have a lower index score, indicating a decline in the biodiversity of such livestock.

Conclusions. For the first time, an analysis of the allelic polymorphism of the *BoLA-DRB3* gene for a local Ukrainian White-Headed Dairy breed was performed. 29 alleles with an average frequency of 3.45% were detected. The breed has its own unique set of alleles of the *BoLA-DRB3* gene. 37 genotypes with uniform distribution were found in the population. The *BoLA-DRB3* gene indicates a high level of biodiversity of the breed. The results of the study significantly expand the research data on the diversity and polymorphism of the *BoLA-DRB3* gene and the genetic specificity of one of the local breeds of Ukraine. They will be useful in selection programs to improve dairy cattle breeding.

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