BF-1587
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RESEARCH ARTICLE Indian Journal of Animal Research

Genetic Variability in Lithuanian Black and White Cattle with Different Proportions of Holstein Bloodline

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10.18805/IJAR.BF-1587

ABSTRACT

Background: Maintaining genetic variability in livestock is the important step towards developing appropriate management strategies. When considering crossing native Lithuanian Black and White cattle (BW) with Holsteins (HL), comprehensive knowledge of breed characteristics, including genetic diversity, is required. The aim of this study was to determine the genetic variability between Lithuanian Black and White and Holstein cattle breeds in relation to groups with different proportions of the Holstein bloodline.

Methods: A total of 126 cattle samples were studied at 13 microsatellite loci to estimate genetic variability.

Result: The largest number of alleles was detected in the BW cattle group with at least 50% Holstein blood. Private alleles were found in all cattle groups except the BW cattle group with a 83-90% Holstein bloodline. Principal coordinate analyses (PCoA) showed the separation of the HL group and other BW groups, as well as the individual that was pure-blooded Lithuanian Black and White. The results revealed further actions breeders should take to retain the required genotype of the native Lithuanian Black and White cattle breed.

Key words: Cattle, Genetic variability, Microsatellites.

INTRODUCTION

Effective management of animal genetic diversity has become an important issue in cattle mating programs (Kukučková *et al*., 2017). Modern livestock programs can be implemented to control inbreeding in the next generation and minimize genetic relationships between selected individuals (Weigel, 2001). Furthermore, correct information about cattle pedigree is fundamental for the appropriate development of breed and selection programs to improve productivity in farming.

The Holstein cattle breed is characterized by a shorter reproduction time and produces a high amount of milk (Coffey *et al*., 2016; Pishchan *et al*., 2021). However, despite the obvious advantages, the Holstein cattle breed represents a significant threat to biological diversity worldwide due to its extensive crossbreeding with other usually local breeds (Lee and Shin, 2018). It has been established that interbreeding of Holstein cattle with other dairy cows can increase their economic efficiency due to gene variety expansion and a decrease in the cows' stress and depression levels (Begley *et al*., 2010).

The Lithuanian Black and White is an old native dairy cattle breed that makes up 70% of all cattle in Lithuania (Prusevičius, 2014). This cattle breed is part of Lithuania's cultural heritage and is exclusive to Lithuania. Like many other breeds, Lithuanian Black and White cattle have a close genetic relationship with the Holstein breed. Since 1973, Lithuanian Black and White cattle have been intensively improved using American and Canadian Holsteins, as well as Danish and German Black and White and British and Holstein Friesians (Baltrėnaitė *et al*., 2003).

In Lithuania, few studies have investigated genetic diversity and genetic relationships in cattle breeds (Peciulaitiene *et al*., 2007; Šveistienė and Jatkauskienė, 2008; Lapickis *et al*., 2021). Characterization of breeds is increasingly important in the context of assessments of genetic diversity and conservation of genetic resources (FAO, 2011). Thus, the objective of the present study was to determine the genetic variability of Lithuanian Black and White and Holstein cattle breeds by the percentage presence of bloodlines.

MATERIALS AND METHODS

The present study was conducted during 2019-2020 at the Biology department of Vytautas Magnus university. Semen or ear tissue samples (n = 126) were taken from native cattle breeds in Lithuania (Marijampolė district): Lithuanian Black and White (BW, n = 121) plus Holsteins (HL, n = 5) included

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How to cite this article: Paulauskas, A., Griciuvienė, L., Lapickis, R., Lipatova, I., Aleksandravičienė, A., Ražanskė, I. and Jurgelevičius, V. (2023). Genetic Variability in Lithuanian Black and White Cattle with Different Proportions of Holstein Bloodline. Indian Journal of Animal Research. doi: 10.18805/IJAR.BF-1587.

Submitted: 19-09-2022 **Accepted:** 09-02-2023 **Online:** 02-01-2023

in the study for reference purposes. All the samples were collected from several agricultural companies and data about the level of Holstein bloodline were provided by the Lithuanian Black and White Cattle Improvers Association. Lithuanian BW cattle were divided into five groups by their level of Holstein bloodline: individuals with more than 93.75%, individuals with 83-90%, individuals with 71-78%, individuals with 62-70% and individuals with less than 50%. Plus, five pure-blooded HL individuals (names “BREWMASTER”, “DICKEY”, “EPIC”, “FLAX”, “KESSLER-ET”) and one pure-blooded Lithuanian BW cow (name “IKRAS”).

Genomic DNA from tissue samples was extracted using the Thermo Scientific Genomic DNA Purification Kit (Thermo Scientific, Lithuania), following the manufacturer’s instructions. Sixteen microsatellites were selected from the panel recommended by the Food and Agriculture Organization and the International Society for Animal Genetics for genetic characterization of cattle breeds (Table 1) (FAO, 2011). Microsatellites were amplified in three multiplex PCRs (3 to 4 co-amplified loci). Each PCR was performed in a 15 μL reaction mixture containing 1 μL of each primer pair, 7.5 μL 2xPCRMix (Thermo Scientific, Lithuania) and 2 μL each of DNA and H_2O. Two separate reactions with different temperature requirements were used-PCR program I: denaturation at 95°C for 5 min, 35 passes of 95°C for 45 s, 50-60°C for 45 s, 72°C for 30 s and an extension 72°C for 10 min; PCR program II: 95°C for 15 min, 3 passes of 84°C for 2 min, 54°C for 20 s, 72°C for 10 s, then 35 passes of 94 °C for 15 s, 54°C for 20 s, 72°C for 10 s, followed by a final extension at 72°C for 30 min. Amplified DNA fragments were mixed with formamide and GeneScan™ 500 LIZ size standard (Applied Biosystems, USA) and then loaded in the ABI 3100 Genetic Analyzer (Applied Biosystems, USA) for capillary

Table 1: Characteristics of cattle microsatellite markers analyzed in the present study.

Marker name	Primer sequence Forward/Reverse	5’ fluorescent label	Size (bp)
AGLA232	5’-CCTTTGCAAATACCTCCTGACCAG-3’/ 5’-AATGGTTCTACATTTGCTAGGTGTC-3’	6FAM	140-160
BM723	5'-ACCCTTGGTTTTCTGCTGG-3'/ 5'-CATCCTGTGTGAGTGTTGTGG-3'	6FAM	150
BM2830	5'-ATGGGCGTATAAACACAGATG-3'/ 5'-TGAGTCCTGTCACCATCAGC-3'	CY3	143-163
BM1824	5’-GAGCAAGGTGTTTTTCCAATC-3’/ 5’-CATTCTCCAACTGCTTCCTTG-3’	6FAM	178-180
BM1225	5'-TTTCTCAACAGAGGTGTCCAC-3'/ 5'-ACCCCTATCACCATGCTCTG-3'	6FAM	254-260
BM1818	5'-AGCTGGGAATATAACCAAAGG-3'/ 5'-AAGTGCTTTCAAGGTCCATGC-3'	CY3	260-264
BM6438	5'-TTGAGCACAGACACAGACTGG-3'/ 5'-ACTGAATGCCTCCTTTGTGC-3'	6FAM	264-274
BOVIRBP	5'-GTATGATCACCTTCTATGCTTC-3'/ 5'-GCTTTAGGTAATCATCAGATAGC-3'	6FAM	144-146
BOVFSH	5'-CTTCTTGGGATATAGACTTAGTGGC-3'/ 5'-GCTTAGATTAGCCTGACTATGA-3'	6FAM	300-350
BTJAB1	5'-CATTAAGGGCTGGGATTCCT-3'/ 5'-AGATTTCTGGAGGAGGCTCACAGCA-3'	6FAM	206-228
Eth121	5'-CCAACTCCTTACAGCAAATGTC-3'/ 5'-ATTTAGAGCTGGCTGGTAAGTG-3'	6FAM	182-210
ETH10	5'-GTTCAGGACTGGCCCTGCTAACA-3'/ 5'-CCTCCAGCCCACTTTCTCTTCTC-3'	6FAM	211-215
RT9	5'-TGAAGTTTAATTTCCACTCT-3'/ 5'-CAGTCACTTTCATCCCACAT-3'	6FAM	135-141
RT29	5’-GCCTTCTTTCATCCAACAAA-3’/ 5’-CCCATCTTCCCATCCTCTT-3’	6FAM	250-300
TGLA122	5'-CCCTCCTCCAGGTAAATCAGC-3'/ 5'-AATCACATGGCAAATAAGTACATAC-3'	6FAM	141-163
TGLA53	5'-GCTTTCAGAAATAGTTTGCATTCA-3'/ 5'-ATCTTCACATGATATTACAGCAGA-3'	CY3	146-148

electrophoresis. Allele size was assigned using GeneMapper v4.0 software (Applied Biosystems, USA).

GeneAlEx 6.501 was used to estimate allele frequencies, the total number of alleles, the number of different private and dominant alleles. The principal coordinate analysis (PCoA) was performed to identify structure in the distribution of genetic variation. Nei's genetic distances and FST values were used to identify genetic differentiation between different cattle breeds (Peakall and Smouse, 2012; Nei, 1973).

RESULTS AND DISCUSSION

A total of 126 individuals' samples were successfully genotyped at 13 microsatellite loci and altogether 251 alleles were detected (Table 2). Three loci (AGLA232, BM1824, RT29), either failed to amplify or the amplified alleles were difficult to score. The number of different alleles ranged from 8 (BOVIRP) to 29 (BM2830 and TGLA122). The BW cattle group with <50 % Holstein bloodline had the largest number of alleles with 49 (Table 2). The presence of dominant alleles was also abundant, with 36 different dominant alleles appearing in 13 microsatellite loci (Table 2). The number of different dominant alleles per locus ranged from 1 (BOVIRP) to 5 (BM2830). The BW cattle group with <50% Holstein bloodline had the highest number of dominant alleles (Table 2). No private alleles were identified in two loci (BOVFSH and TGLA53) in the HL group.

Several of the loci used in this work have been analyzed in previous studies with different breeds of European cattle breeds bred in Lithuania: Danish Red, Swedish Red, German Black and White and German Red (Makštutienė *et al*., 2013). In Australia, HL cattle were investigated and found that they had an impact on other breeds over the years (Zenger *et al*., 2007). The study states that the level of individual breeding is increasing but does not yet have a significant effect on genetic differentiation. However, the level of estimated inbreeding is increasing and becoming a serious problem. In addition, it may change due to environmental impacts, local selection, genetic drifting and isolation. Similar accounts about the genetic variability of different cattle breeds have been reported in Senegal, Pakistan and Korea (Suh *et al*., 2014; Ndiaye *et al*., 2015; Hussain *et al*., 2017).

Private alleles were found in all the cattle groups except for the BW cattle group with 83-90% Holstein bloodline (Table 3). The HL had the highest number of private alleles at 10, followed by BW with >93.75% Holstein bloodline at 8. Special consideration should be given to 140 bp (RT9) and 150 bp (BM723) alleles, which only occurred in one BW cattle group (<50% Holstein bloodline) with a high frequency of 1.00 and 0.50 respectively, while the other private alleles occurred with a low frequency of less than 0.10-0.25. These results highlight the relevance of these markers for characterizing cattle breeds and potentially improving efficiencies in breeding programs.

Principal coordinates analysis (PCoA) revealed that the HL group was most distinct from the other groups as well as the individual of pure-blooded Lithuanian BW (Fig 1). As expected, the PCoA in the present study revealed that the HL cattle breed is genetically distant from the other cattle breed groups. There was a significant difference, which was clearly visually detectable. This is in complete agreement with the factorial correspondence analysis of individual cattle microsatellite genotypes calculated using GENETIX in the study by Suh *et al*. (2014) where the HL cattle breed is undoubtedly also separate.

The greatest Nei's genetic distance (0.359) was observed between pure-blooded HL group and BW cattle

Table 2: Distribution of different and dominant alleles among different groups of cattle.

Loci	Groups with different proportions (%) of Holstein bloodline												Number of different alleles	Number of alleles dominant
	100		>93.75		83-90		71-78		62-70		<50			
	df	do	df	do	df	do	df	do	df	do	df	do		
BOVIRBP	1	1	2	1	1	1	1	1	1	1	2	1	8	1
BTJAB1	2	1	3	1	3	3	5	1	4	1	5	2	22	3
BM6438	1	1	2	1	4	1	2	1	2	1	4	2	15	2
BM2830	4	4	5	2	4	1	6	1	5	1	5	3	29	5
TGLA122	7	1	5	1	4	2	5	2	3	1	5	3	29	3
ETH10	4	1	7	1	4	2	5	1	4	1	4	3	28	4
BM1225	3	1	2	1	3	1	3	1	2	1	4	1	17	2
BM1818	3	1	2	1	3	1	5	1	5	1	5	2	23	2
RT9	1	1	2	1	1	1	1	1	1	1	3	2	9	2
BM723	3	1	2	1	1	1	1	1	1	1	4	2	12	2
ETH121	6	1	3	1	3	1	5	1	5	1	2	2	24	4
BOVFSH	0	0	3	1	1	1	3	1	4	1	3	1	14	3
TGLA53	0	0	4	1	3	1	5	1	6	1	3	2	21	3
Total	35	14	42	14	35	17	47	14	43	13	49	26	251	36

df- Number of different alleles; do- Number of dominant alleles.

group with less than 93.75% Holstein blood, nevertheless the lowest (0.132) was between BW groups with less than 50% Holstein blood and 71-78% Holstein blood (Table 4). The fixation index (FST) values ranged from 0.002 to 0.257 among different groups of cattle, indicating mixed levels (from low to high) of genetic differentiation (Table 4). The highest value was between the pure HL group and the BW cattle group with less than a 93.75% Holstein bloodline.

Many of the loci used in this study have been used for different breeds, such as native Korean breeds, Senegal's Gobra zebu, Pakistan's zebu, Indian cattle and native China breeds where investigations used the HL breed as

Table 3: Summary of private alleles (allele size and frequencies) by different groups of cattle.

Loci	Groups with different proportions (%) of Holstein bloodline						Number of private alleles
	100	>93.75	83-90	71-78	62-70	<50	
BOVIRBP		143 (0.1)					1
BTJAB1	213 (0.5)			218 (0.1)			2
BM6438							0
BM2830	160 (0.3)	172 (0.1)		178 (0.1)			3
TGLA122	154 (0.1), 156 (0.1)	160 (0.1)		152 (0.2)			4
ETH10	206 (0.1), 212 (0.1)						2
BM1225							0
BM1818				260 (0.1)	272 (0.1)	262 (0.2)	3
RT9						140 (1)	1
BM723	154 (0.1), 160 (0.1) 162 (0.1)					158 (0.2), 150 (0.5)	5
ETH121	178 (0.1), 180 (0.1), 182 (0.1), 192 (0.2)	202 (0.2)			186 (0.1)		6
BOVFSH		318 (0.2)		312 (0.1)	314 (0.1)		3
TGLA53					154 (0.1), 166 (0.1), 176 (0.1)		3
Total	10	8		5	6	2	33

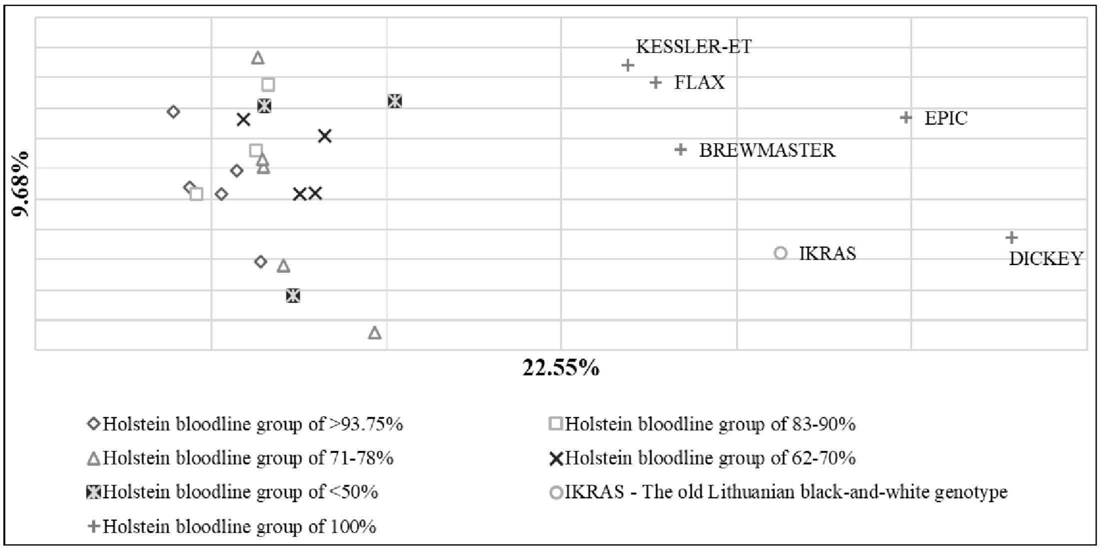

Fig 1: Principal coordinates analysis (PCoA) based on genetic distances between cattle samples.

Table 4: Pairwise FST (above diagonal) and Nei's genetic distance (below diagonal) between the studied different groups of cattle.

Groups with different proportions (%) of Holstein bloodline	100	>93.75	83-90	71-78	62-70	<50
100		0.257	0.191	0.185	0.191	0.085
>93.75	0.359		0.000	0.060	0.102	0.080
83-90	0.275	0.151		0.010	0.047	0.010
71-78	0.277	0.198	0.172		0.000	0.000
62-70	0.246	0.281	0.252	0.137		0.002
<50	0.29	0.264	0.228	0.132	0.188	

comparative genetic material (Suh *et al*., 2014; Ndiaye *et al*., 2015; Gupta *et al*., 2016; Hussain *et al*., 2017; Ni *et al*., 2018). The present findings would seem to show that Nei's statistic ranged from 0.132 to 0.359, which is very similar to that of Suh *et al*. (2014) who evaluated a range of 0.129 to 0.316. Further analysis showed FST values from 0 to 0.257 that correlate favorably with Hussain *et al*. (2017) (0.02445-0.22009) and Svishcheva *et al*. (2020) (0.0608-0.0955). Interestingly, the observation originated from the data comparison in many cattle breed microsatellites, repeating the tendency of high allelic richness and high accuracy of dominant and rare alleles (Makštutienė *et al*., 2013; Suh *et al*., 2014; Ndiaye *et al*., 2015; Hussain *et al*., 2017; Svishcheva *et al*., 2020). This may occur due to the high polymorphism level per locus (Radko, 2008). Generally, rare alleles in large populations occur because of random mutations and are distributed in the individuals in a heterogenic form. In small populations, rare alleles are distributed by possible mating between related individuals or crossbreeding (Hale *et al*., 2012).

Another reason for the appearance of random alleles is the decrease in genetic diversity that over time leads to the loss of specific genes that define the breed or are found in a very low frequency. This phenomenon is noticeable when the effective population size falls significantly. In addition, scientists have observed that the offspring kept in one herd are phenotypically similar, but genetically different (Lithuanian Black and White Cattle Improvers, 2011). The high number of rare alleles detected could include genetic drift or migration from population to population. The finding of the unique alleles in the individuals allows the assumption that these alleles survive as adaptive genes that could be significant in future usage in animal husbandry. DNA sequencing polymorphism studies provide tools to help decide which livestock populations should be targeted and which should be used to identify the DNA regions of unusual productivity and signs of wellness. This information will be used directly in future genetic improvement programs for cattle.

CONCLUSION

The results of this study showed that the genetic distance between Lithuania's Black and White cattle individuals and the pure-blooded Holstein individuals was high. The Lithuanian Black and White cattle breed is part of Lithuania's cultural heritage and is exclusive to Lithuania. Maintaining genetic diversity should be one of the major objectives of breeding programs, especially in native cattle breeds. Genetic marker information is an excellent alternative to pedigree data and could be used to reduce the chance of inbreeding and conserve genetic diversity in populations.

ACKNOWLEDGEMENT

The authors would like to thank the Lithuanian Black and White Cattle Improvers Association for providing semen and ear tissue samples. This research was funded by the National Paying Agency under the Ministry of Agriculture of the Republic of Lithuania via the Genetic Analysis of Lithuanian Black and White, Holsteins, Lithuanian Red and Lithuanian Red and White cattle breeds project, grant number MT-19-16.

Conflict of interest: None.

REFERENCES

Baltrėnaitė, L., Jeninas, E., Juškienė, V., Kriauzienė, J., Kučinskas, A., Macijauskienė, V., Miceikienė, I., Muzikevičius, A., Razmaitė, V., Svitojus, A., Šveistienė, R., Zapasnikienė, B. (2003). Lithuanian domestic animal genetic resources-Nowadays and future perspectives. Country report to the FAO. Lithuanian Veterinary Academy: Kaunas, Lithuania. pp. 1-40.

Begley, N., Buckley, F., Pierce, M.K., Evans, R.D. (2010). Breed difference and heterosis estimates for milk production and udder health among Holstein, Friesian and Norwegian Red dairy cattle. Advances in Animal Biosciences. 1: 341-341.

Coffey, E.L., Horan, B., Evans, R.D., Berry, D.P. (2016). Milk production and fertility performance of Holstein, Friesian and Jersey purebred cows and their respective crosses in seasonal-calving commercial farms. Journal of Dairy Science. 99: 5681-5689.

Food and Agriculture Organization (FAO). (2011). Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health guidelines; FAO: Rome, Italy, pp. 1-100.

Gupta, J.P., Bhushan, B., Panigrahi, M., Ranjan, S., Asaf, V.N.M., Kumar, A., Sulabh, S., Kumar, A., Kumar, P., Sharma, D. (2016). Study on genetic variation of Short Tandem Repeats (STR) markers and their association with Somatic Cell Scores (SCS) in crossbred cows. Indian Journal of Animal Research. 50: 450-454.

Hale, M.L., Burg, T.M., Steeves, T.E. (2012). Sampling for microsatellite -based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. PLoS One. 7: e45170.

Hussain, T., Ellahi Babar, M., Ali, A., Nadeem, A., Rehman, ZU., Musthafa, M.M., Marikar, F.M. (2017). Microsatellite based genetic variation among the buffalo breed populations in Pakistan. Journal of Veterinary Research. 61: 535-542.

Kukučková, V., Moravčíková, N., Ferenčaković, M., Simčič, M., Mészáros, G., Sölkner, J., Trakovická, A., Kadlečík, O., Curik, I., Kasarda, R. (2017). Genomic characterization of Pinzgau cattle: genetic conservation and breeding perspectives. Conservation Genetics. 18: 893-910.

Lapickis, R., Griciuvienė, L., Aleksandravičienė, A., Lipatova, I., Paulauskas, A. (2021). Genetic variability of dairy cattle breeds in Lithuania. Biologija. 67: 188-195.

Lee, Y.S., Shin, D. (2018). Estimation of the genetic substitution rate of Hanwoo and Holstein cattle using whole genome sequencing data. Genomics and Informatics. 16: 14-20.

Lithuanian Black and White Cattle Improvers. (2011). Development of the Lithuanian black and white cattle breed, people and their works at the turn of the century; Lithuanian Black and White Cattle Improvers: Marijampole, Lithuania. pp. 1-80.

Makštutienė, N., Liucvaikienė, K., Baltrėnaitė, L., Morkūnienė, K., Morkūnas, P., Ramanauskienė, A., Miceikienė, I. (2013). Investigation of microsatellites polymorphism in milk cattle breeds bred in Lithuania. Zootechnics. 20: 57-63.

Ndiaye, N.P., Sow, A., Dayo, G.K., Ndiaye, S., Sawadogo, G.J., Sembène, M. (2015). Genetic diversity and phylogenetic relationships in local cattle breeds of Senegal based on autosomal microsatellite markers. Veterinary World. 8: 994-1005.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences of the United States of America. 70: 3321-3323.

Ni, W., Jiang, A., Zhang, J., Guangxin, E., Huang, Y. (2018). Microsatellite marker-based estimation of the genetic diversity of cattle in Chongqing. Indian Journal of Animal Research. 52: 1543-1547.

Peakall, R., Smouse, P.E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics. 28: 2537-2539.

Pečiulaitienė, N., Miseikienė, R., Baltrėnaitė, L., Miceikienė, I. (2007). Genetic differences among native and modern cattle breeds in Lithuania based on milk protein loci polymorphism. Polish Journal of Veterinary Sciences. 10: 35-41.

Pishchan, I.S., Pishchan, S.G., Lytvyschenko, L.O., Honchar, A.O., Horchanok, A.V., Mylostyvyi, R.V., Kapshuk, N.O., Kuzmenko, O.A. (2021). Assessment of the adaptive stability of the Holstein cows in the conditions of the ecological plasticity in northern steppe of Ukraine. Indian Journal of Animal Research. 55: 1111-1115.

Prusevičius, V. (2014). Farms under modernization in the dairy sector. Revival of Lithuanian countryside. Vilnius, Lithuania. pp. 30-32.

Radko, A. (2008). Microsatellite DNA polymorphism and its usefulness for pedigree verification of cattle raised in Poland. Annals of Animal Science. 8: 311-321.

Suh, S., Kim, Y.S., Cho, C.Y., Byun, M.J., Choi, S.B., Ko, Y.G., Lee, C.W., Jung, K.S., Bae, K.H., Kim, J.H. (2014). Assessment of genetic diversity, relationships and structure among Korean native cattle breeds using microsatellite markers. Asian-Australasian journal of animal sciences. 27: 1548-1553.

Svishcheva, G., Babayan, O., Lkhasaranov, B., Tsendsuren, A., Abdurasulov, A., Stolpovsky, Y. (2020). Microsatellite diversity and phylogenetic relationships among East Eurasian *Bos taurus* breeds with an emphasis on rare and ancient local cattle. Animals. 10: 1493.

Šveistienė, R., Jatkauskienė, V. (2008). Analyses of the genetic diversity within Lithuanian white-backed cattle. Veterinarija ir Zootechnika. 44: 67-72.

Weigel, K.A. (2001). Controlling inbreeding in modern breeding programs. Journal of Dairy Science. 84: E177-E184.

Zenger, K.R., Khatkar, M.S., Cavanagh, J.A., Hawken, R.J., Raadsma, H.W. (2007). Genome-wide genetic diversity of Holstein Friesian cattle reveals new insights into Australian and global population variability, including impact of selection. Animal Genetics. 38: 7-14.