GENETIC DIVERSITY OF FOUR LITHUANIAN CATTLE BREEDS BASED ON BLOOD PLASMA PROTEIN AND ERYTHROCYTE ANTIGEN SYSTEM POLYMORPHISM

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Summary. Four blood plasma proteins and erythrocyte antigen system F were studied and used as genetic markers in four Lithuanian cattle breeds in order to characterise the populations, to investigate genetic diversity in Lithuanian local and commercial cattle breeds and to determinate genetic relations of Lithuanian cattle populations.

An average number of alleles, expected and observed heterozygosities, pairwise DA genetic distances and F statistics were calculated, exact test for population differentiation was done. About 4.7% of the total genetic variability was due to differences between breeds, indicating a moderate subdivision. A neighbour-joining tree was constructed by using the DA genetic distances. The branching pattern of the tree confirms the grouping of studied cattle breeds into two main groups and outlying the Lithuanian Red population from Lithuanian Black and White breed that belongs to the Holstein type.

Further obtaining of alleles and genotypes in Lithuanian cattle breeds suggests that all four studied Lithuanian cattle breeds represent separate gene pools, and although there may have been crossing between breeds, it has not been sufficient to make a decision that breeds are not different. Estimated breeds exposing their own features, might be selected for different purposes in husbandry and kept as genetic resource for the future.

Keywords: blood plasma proteins, transpherin, ceruloplazmin, amylase-1, amylase-2, erythrocyte antigen system, polymorphism, cattle.

Abbreviations: TF-transpherin, CP-ceruloplazmin, AMY-1-amylase-1, AMY-2-amylase-2, EAF-erythrocyte antigen system F, LWB-Lithuanian White Backed cattle breed, LBW-Lithuanian Black and White cattle breed, LR-Lithuanian Red cattle breed, LLG-Lithuanian Light Grey cattle breed.

KETURIŲ LIETUVOS GALVIJŲ VEISLIŲ GENETINĖS ĮVAIROVĖS NUSTATYMAS PAGAL KRAUJO PLAZMOS BALTYMŲ BEI KRAUJO ANTIGENINIŲ FAKTORIŲ SISTEMOS TYRIMUS

Santrauka. Šio darbo tikslas yra ištirti keturių Lietuvoje auginamų galvijų veislių, tokių kaip Lietuvos juodmargiai, Lietuvos žalieji, Lietuvos šėmi bei Lietuvos baltnugariai, bioįvairovę keturių kraujo plazmos baltymų bei vienos kraujo antigeninių faktorių sistemos – F, kaip genetinių žymeklių pagalba.

Galvijų tyrimas kraujo plazmos baltymų bei kraujo antigeninių faktorių polimorfizmo atžvilgiu leidžia genetiškai įvertinti tiriamas galvijų veislės, nustatyti jų biologinę įvairovė bei panašumas, įvertinti genetinius atstumus bei ryšius tarp dviejų Lietuvos vietinių ir dviejų komercinių galvijų veislių.

Tiriamų veislių vidutinis nustatytų skirtingų alelių skaičius, heterozigotiskumo laipsnis, F statistikos pagrindiniai rodikliai, genetiniai atstumai DA bei kitos statistinės vertės buvo apskaiciuotos kompiuterinių statistinių programų, tokių kaip DISPAN (Ota 1993), GENEPOP bei FSTAT (versija 2.9.3.2, 2002) pagalba. Vietinės galvijų veisles, lyginant jas su kultūrinėmis, neparodė mažesnio viduveislinio kintamumo. Nustatyta, kad vidutiniskai 4,7% bendros tiriamų veislių įvairovės yra nulemta tarpopuliacinio skirtingumo, o likusi dalis priklauso nuo kiekvienos veislės įvairoves viduje.

Kaimyninės jungties medžio pagalba tiriamos veislės buvo sugrupuotos i dvi pagrindines grupes: Lietuvos vietinių galvijų grupė, kuriai priskiriamos: Lietuvos šėmų ir Lietuvos baltnugarių veislės; kultūrinių arba pagerintų galvijų veislių grupė, kuriai priskiriamos: Lietuvos juodmargių ir Lietuvos žalųjų veislės. Lietuvos žalieji galvijai, savo ruožtu, sudarė atskirą pogrupį, genetiškai atitolusį nuo Holšteinų – Fryzų tipo galvijų.

Raktažodžiai: galvijai, polimorfizmas, įvairovė, kraujo plazmos baltymai, kraujo antigeniniai faktoriai.

Introduction. Worldwide the livestock cattle breeds are evaluated as important part of world biodiversity (FAO 2000). Many historically important European cattle breeds have been isolated and such isolation promoted the genetic separation of populations. At the same time, breeds, being at risk of loss, may have unique, not found in other breeds flexibility to local environment, adaptation to local climate, nutrition and diseases, which would be difficult to retrieve or even to recreate. The local breeds have been also selected for different intents and purposes that were important to the local human community. Genetic drift, as well as selection, has contributed to the differentiation of breeds, and individual breeds may possess unique combinations of genes as a result of the action of these different evolutionary forces (Blott et al., 1998). Many native cattle breeds became to be extinct because of farmer's concentration on the new highly productive cattle breeds. Around 16% of native cattle breeds become to be extinct and 15 % are known as rare (FAO, 2000). Many breeds become rare either because their characteristics do not suit present-day requirements or because their qualities have not been well known; simultaneously specialized breeds of great productivity cattle were spreading rapidly because of intensification of cattle breeding. Predominance of a few breeds in species and predominance of a small number of sires in a breed generate a possibility for decrease in cattle genetic diversity. Long – term selection oriented only to milk yield rise and mixing animals with only a few wide – spread breeds may determine a lack of heterogenity and increase homogenity of cattle populations; simultaneously the loss of the biodiversity is conditioned by the declining of diverse phenotypes and growing of the number and frequency of the same alleles in different populations (Vagonis et al., 1975).

In Lithuania there are two main commercial cattle breeds - Lithuanian Black and White (LBW) and Lithuanian Red (LR). These breeds form correspondingly, 62% and 32% of country's cattle population. Also, there are two native Lithuanian cattle breeds - Lithuanian Light Grey (LLG) and Lithuanian White Backed (LWB), which were almost replaced, mainly by black and white or other highly productive cattle breeds.

Lithuanian Black and White cattle breed was developed from Lithuanian local black and white cattle by crossing them with highly productive imported black and white cattle. Lithuanian Red cattle breed was formed from local red cattle by classified mating, selection and crossing with imported improved breeds (Jukna 1998). The hybrids of those breeds were selected for milk production and nowadays both breeds belong to clearly defined dairy type (FAO, 2000).

It is known that Lithuanian Light Grey and Lithuanian White Backed cattle were bred in Lithuania from the olden times and are typical to the country. At the present time, Lithuanian White Backed and Lithuanian Grey cattle comprise very small populations and still are an endangered. In the second half of the 20th century, as a result of intensification of agriculture and high expansion in a distribution of a few highly productive breeds, the Lithuanian native cattle decreased in a number remarkably. Furthermore, for a long time native cattle were kept only by private owners and bred without any straight breeding system. Nevertheless, Lithuanian local breeds still have characteristics familiar to them: typical coat colour, body formation, qualities common to dairy cattle, high adaptation and resistance against local diseases (Kuosa 1997). Local Lithuanian Light Grey and Lithuanian White Backed cattle breeds were recognized as unique and are included into Catalog of Diversity of World Agricultural Animals (Word Watch List).

Research and analysis of genetic biodiversity of Lithuanian cattle breeds in different levels are the part of the system, which comprises investigation and maintaining of genetic biodiversity of agricultural animals worldwide. Information about a genetic structure of local cattle breeds is essential for determination its importance and place among other breeds in the world.

Many studies on cattle genetic diversity are based on data determinated from typing of bovine erythrocyte antigens and plasma protein polymorphism (Kantanen et al., 2000). Blood serum is used to study blood protein and enzymes, which are secreted by other tissues and carried in blood. The variations among blood serum proteins and red cell antigens of different individuals are normal with respect to their biological function and differ from each other in a few amino acids or other components. Amino acid substitutions occurring in proteins as a result of mutation alter the charge of the protein. Most common studied proteins include albumin, amylase, ceruloplasmin, esterases, haemoglobin, transpherin and alkaline phosphatase. Erythrocyte antigens systems are the first genetic markers used for characterization of Lithuanian cattle breeds and were used widely for parentage verification and control in cattle. In general 11 erythrocyte antigen systems are known in cattle (Brænd et al., 1962).

In this paper we present data on the subject of four blood proteins: transpherin (TF), ceruloplazmin (CP), amylase-1 (AMY-1), amylase-2 (AMY-2) and erythrocyte antigen system F (EAF) polymorphism in four Lithuanian cattle breeds. The selected systems were used to investigate current genetic diversity in Lithuanian local and commercial cattle breeds, to estimate biodiversity among and within studied breeds and to determine genetic relations of Lithuanian cattle.

Material and methods.

Data. The blood samples for analysis were collected from animals which are kept by private farmers, agricultural companies and relict – gene pool herds. Pedigree information was available for two commercial breeds completely and studied animals are unrelated mostly to third generation. For two native cattle breeds about one third of samples pedigree information was not available. In respect that the studied animals of native cattle breeds are kept mostly by private holders who are located far away from each other, we can make the conclusion that animals with unknown pedigree information might be not related. A total of 113 animals were included in the analysis. The number of animals ranged from 23 (Lithuanian Light Grey) to 30 (Lithuanian White Backed, Lithuanian Black and White and Lithuanian Red).

Detection of blood plasma protein and erythrocyte antigens variations. The genetic polymorphism of four blood plasma proteins was estimated: transpherin (TF), ceruloplasmin (CP), amylase-1 (AMY-1) and amylase-2 (AMY-2) and one erythrocyte antigen system F (EAF).

Blood was taken in citrated vacuum tubes directly from the jugular vein. The samples were centrifuged at 1500 rpm for 10 minutes to separate erythrocytes and serum fractions.

The electrophoretic detection of blood plasma proteins was done in a laboratory, which has taken part in the Cattle ISAG Comparison Tests (International code is EST/L), at Estonian Agricultural University, Institute of Animal Science (Tartu, Estonia).

The variations of the blood serum proteins were detected by using a few different electrophoretic determination methods with technique modified to local conditions. For phenotyping of samples for ceruloplazmin (CP), amylase-1 (AMY-1) and amylase-2 (AMY-2) proteins variation was used horizontal 10-13% concentration starch gel in a discontinuous buffer system described by Ebertus (1967) and Smithies (1955). Phenotyping of samples for transpherin (TF) protein variation has been done using horizontal polyacrilamide gel (Gahne et al., 1977). Different antigens within F red cell antigen system (EAF) were detected by using internationally accepted reagents for standard haemolysis reaction (Grosclaude et al., 1979).

Analysis of statistical data. Within-breed genetic variation of blood plasma protein and erythrocyte antigens were evaluated by direct calculating the number of different alleles within each locus and breed, frequency of different alleles and phenotypes, percentage of polymorphic loci, observed and expected heterozygosities. Loci were defined as polymorphic when the frequency of the most common allele was less than 0.95 (95%). Estimates of within-breed variation were derived using the GENEPOP 3.1 version (Raymond and Rousset, 1995) computer program.

An exact test was used to determine possible deviations from Hardy-Weinberg proportions and existence of non-random associations of genotypes across polymorphic codominant locus was executed using computer program GENEPOP 3.1 version (Raymond and Rousset, 1995).

Population subdivision was examined with Wright's fixation index using based method of Weir and Cockerham (1984). Using computer program FSTAT (Goudet, 1995) F – statistics were computed. Standard deviations of the F statistics parameters were estimated for each locus across breeds by the jackknife procedure (Weir 1990).

Within-breed genetic variation is based on frequency of codominantly inherited alleles (Cornuet and Luikart 1996). Expected heterozygosities (H_{exp}) estimated assuming Hardy-Weinberg equilibrium were compared with the theoretical values calculated on the basis of the number of sampled alleles. This statistical test is known as sign test for heterozygosity excess. The theoretical heterozigosity at each locus under the supposition of mutation-drift equilibrium was obtained by 1000 iterations according to the infinite allele model (IAM) and alleles were clasified into 10 frequency classes using the programme BOTTLENECK (Piry et al., 1999).

The D_A genetic distances were computed with DISPAN computer program (© T. OTA, Pennsylvania State University, Pa, USA).

Results. Genetic diversity within each breed and between breeds was investigated according to estimated present diversity of blood plasma proteins and erythrocyte antigen F system phenogroups.

Mean frequency of detected blood plasma diverse alleles in four Lithuanian cattle breeds is shown in Table 1. A total of 10 different alleles were detected at four blood plasma protein loci. The number of different alleles varied from 4 (TF) to 2 (CP, AMY-1 and AMY-2). The average number of detected alleles over all protein and erythrocyte antigen F system loci and their standard errors are shown in Table 4. The average number of alleles was at the same level of 2.4 in each of studied breeds.

Table 1. **Mean frequency of different alleles for each studied blood plasma protein locus detected in four Lithuanian cattle breeds**

Locus	Allele	Breed			
		Lithuanian Red (LR)	Lithuanian Black and White (LBW)	Lithuanian White Backed (LWB)	Lithuanian Light Grey (LLG)
TF	A	0.267	0.533	0.317	0.543
	D1	0.283	0.217	0.250	0.217
	D2	0.267	0.183	0.333	0.217
	E	0.183	0.067	0.100	0.038
CP	A	0.700	0.600	0.433	0.326
	C	0.300	0.400	0.567	0.674
AMY-1	B	0.667	0.467	0.417	0.457
	C	0.333	0.533	0.583	0.543
AMY-2	A	0.167	0.033	0.050	0.043
	B	0.833	0.967	0.950	0.957

One of the alleles was found as a predominant in all studied populations (Table 1). B allele at AMY-2 locus with almost the same high frequency, which varied from 0.833 (LR) to 0.967 (LBW), was found as predominant in all four studied breeds. The highest frequency across all four studied plasma proteins was showed by B allele at AMY-2 locus. The lowest frequency in all studied cattle populations showed E allele at TF locus and varied from 0.038 (LLG) to 0.183 (LR); and A allele at AMY-2 locus, ranging from 0.033 (LBW) to 0.167 (LR).

Blood plasma protein transpherin as genetic marker is used while estimating differences between or inside breeds for a long time. Allele A of TF locus was detected as most frequent in LBW and LLG breed and varied from 0.533 to 0.543 respectively. Alleles D1 and D2 did not show any large differences or high variation across all studied breeds. Allele A at CP locus was detected as most frequent in LR and LBW populations and varied from 0.700 to 0.600 respectively, while C allele, ranging from 0.567 to 0.674 respectively, was found as most common in LWB and LLG populations. Alleles B and C at AMY-1 locus showed relatively high frequency in all four studied cattle breeds. Only in LR population allele C was found with lower frequency (Table 1).

Frequency of detected different phenotypes is presented in Table 2. In general, the frequency of different phenotypes ranged from 0.033 to 0.933. The highest variation in phenotypes TF locus showed. The highest frequency over all estimated breeds phenotype AD2 showed. Only in two cattle populations (LR and LWB) phenotypes D2D2 and D2E were found. Phenotype D1E was not found in LLG cattle breed. In general, 3 different phenotypes (D2D2, D1E and D2E) were not found in LLG breed at transpherin locus.

Table 2. **Mean frequency of phenotypes of blood plasma proteins detected in four Lithuanian cattle breeds**

Protein	Phenotype	Breed			
		Lithuanian Red (LR)	Lithuanian Black and White (LBW)	Lithuanian White Backed (LWB)	Lithuanian Light Grey (LLG)
TF	AA	0.100	0.267	0.100	0.304
	AD1	0.067	0.200	0.133	0.087
	AD2	0.167	0.267	0.233	0.348
	AE	0.100	0.067	0.067	0.043
	D1D1	0.100	0.033	0.033	0.130
	D1D2	0.167	0.100	0.267	0.087
	D2D2	0.067		0.033	
	D1E	0.133	0.067	0.033	
	D2E	0.067		0.100	
CP	AA	0.500	0.433	0.167	0.130
	AC	0.400	0.333	0.533	0.392
	CC	0.100	0.233	0.300	0.478
AMY-1	BB	0.433	0.233	0.100	0.218
	BC	0.467	0.467	0.633	0.478
	CC	0.100	0.300	0.267	0.304
AMY-2	AA	0.033		0.033	
	AB	0.267	0.067	0.033	0.087
	BB	0.700	0.933	0.933	0.913

According to estimated frequencies, phenotype AA at CP locus is more characteristic for commercial breeds, phenotype AC is highly frequent in all four studied populations; and phenotype CC was found as more specific to LLG breed. Phenotype BC at AMY-1 locus was found with relatively high frequency in all studied populations. Phenotype BB at the same locus was found as more common to LR breed. Phenotype AA at AMY-2 locus was not found in LBW and LGG breeds, while in other two breeds it was detected with the same very low frequency of 0.033.

Depending on the investigations of the erythrocyte antigen F system and blood plasma protein, studied loci were 100 % polymorphic in LWB and LR breeds, and 80 % polymorphic in LLG and LBW cattle populations (Table 4). In general, all blood plasma protein loci and erythrocyte antigen F system were found as polymorphic in all studied breeds.

The results of F statistics among four studied breeds are showed in Table 3 and were not significantly different from zero. The main F statistic indexes, such as F_{IT} (a measure of the individuals relatedness to the total population), F_{ST} (a measure of the effect of population subdivision) and F_{IS} (a measure of the reduction in heterozigosity in an individual within its subpopulation) (Weir and Cocherham, 1984) were calculated for estimation of differences among Lithuanian cattle breeds by codominantly inherited markers. In general, the estimated indexes show that all studied populations are more likely heterogenetic than homogenetic and distinguish themselves as polymorphic by phenogroups of blood plasma proteins and erythrocyte antigen F system.

Lack of heterozygosity was not found in any of investigated loci among populations. The lowest coefficient of inbreeding was found at AMY-1 locus (-0.068); the highest was found at locus CP (0.134). In general about 4.7% of the total genetic variability was due to differences between breeds, indicating a moderate subdivision.

The average degree of observed heterozigosity is showed in Table 4 and varies from 0.37 in LBW breed

Table 3. **Results of F statistics for each studied blood plasma protein locus and erythrocyte antigen F system across the breeds**

Locus	F_{IT}	F_{ST}	F_{IS}
TF	0.002 (0.067)	0.026 (0.008)	-0.025 (0.062)
CP	0.205 (0.098)	0.082 (0.061)	0.134 (0.115)
AMY-1	-0.031 (0.087)	0.035 (0.048)	-0.068 ()0.081
AMY-2	0.185 (0.109)	0.038 (0.053)	0.153 (0.147)
F	0,124 (0,072)	0,058 (0,037)	0,069 (0,059)
Average	0.071 (0.056)	0.047 (0.014)	0.025 (0.046)

Table 4. **Mean observed (H_{obs}) and expected (H_{exp}) heterozygosities, mean number of alleles (A) and percentage of polymorphic protein loci ($P_{0.95}$) in four Lithuanian cattle breeds**
Standadr errors are presented in parenthesis.

Breed	H_{obs}	H_{exp}	A
Lithuanian Red (LR)	0.40 (0.09)	0.42 (0.10)	2.4 (0.4)
Lithuanian Black and White (LBW)	0.37 (0.11)	0.41 (0.09)	2.4 (0.4)
Lithuanian Light Grey (LLG)	0.38 (0.08)	0.42 (0.09)	2.4 (0.4)
Lithuanian White-Backed (LWB)	0.44 (0.14)	0.40 (0.12)	2.4 (0.4)

to 0.44 in LWB breed. High differences between observed and expected heterozygosities were not detected. The average of observed heterozygosity is lower than expected one in LLG, LWB and LR respectively, 0.04, 0.04 and 0.02. The larger heterozygosity than expected one was found only in LWB breed. According to the results LWB breed is more genetic polymorphic breed comparing with the other ones.

According to Infinite Allele Model (IAM) statistically observed heterozygosity excess was found not significant in LLG, LWB, LBW and LR cattle breeds (Table 5). In LLG, LBW and LR breeds 4 loci with excess of heterozygosity were found and in LWB breed - 3 loci. That explains the lower level of variation in LWB breed.

Table 5. **Number of loci showing heterozigosity excess or deficiency, and probabilities obtained from the sign test assuming that factors evolve according to the model of infinite mutations (IAM)**

Breed	Heterozigosity excess		Heterozigosity deficiency	$P_{0.95}$
	H_{exp}	H_{obs}	H_{obs}	
Lithuanian Red (LR)	2.29	4	1	0.136
Lithuanian Black and White (LBW)	2.25	4	1	0.128
Lithuanian Light Grey (LLG)	2.28	4	1	0.134
Lithuanian White-Backed (LWB)	2.26	3	2	0.410

The permutation test showed that all breeds are different from each other. The value of D_A distances varied from 0.0243 (between Lithuanian White Backed and Lithuanian Light Grey) to 0.0677 (between Lithuanian Black and White and Lithuanian White Backed), (Table 6).

Table 6. **Estimation of DA genetic distances of four Lithuanian cattle breeds**

	Lithuanian Red (LR)	Lithuanian Black and White (LBW)	Lithuanian Light Grey (LLG)
Lithuanian Black and White (LBW)	0.0521		
Lithuanian Light Grey (LLG)	0.0533	0.0474	
Lithuanian White-Backed (LWB)	0.0671	0.0677	0.0243

Allele frequencies were used to generate the D_A genetic distances and construct the neighbour-joining tree (Figure 1). The branching pattern of the tree suggested a grouping of studied cattle populations into two groups: native (Lithuanian Light Grey and Lithuanian White Backed) and commercial (Lithuanian Red and Lithuanian Black and White). Lithuanian Red cattle formed an outgroup with a deep branch. According to the length of branches we could see that most effectively the population size decreased in LR breed. LLG and LWB cattle breeds distinguish themselves in bigger genetic diversity than cultural Lithuanian breeds of cattle –LBW and LR.

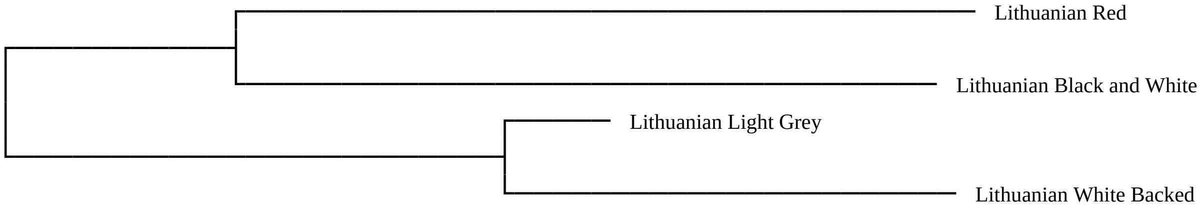

Figure 1. **Neighbour-joining tree constructed from the D_A distances.**

The normal distribution of alleles formative “L” constructed according to detected distribution of alleles frequency into 10 allele frequency classes was found only in LWB cattle breed (Figure 2.). In general, the highest frequency of alleles was shown at the lowest class of frequency from 0.201 to 0.300 in LLG and LR; and from 0.601 to 0.700 in LBW population.

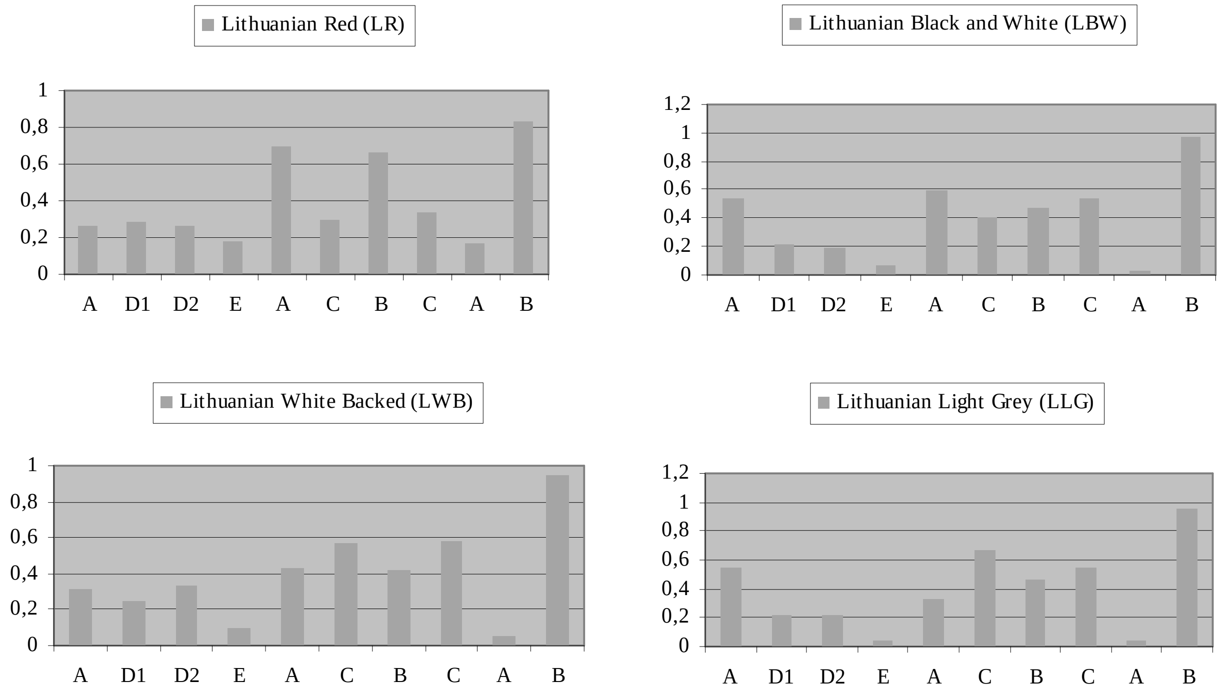

Figure 2. **Distribution of alleles frequency in four Lithuanian cattle breeds.**

Discussion and conclusions. The novelty and practical meaning of this work is that for the first time investigation and comparison of biological characteristics of Lithuanian native LLG and LWB cattle breeds was carried out. For the first time genetic variation of LLG, LWB, LBW and LR cattle breeds was studied by using biochemical markers. These studies are important for decisions concerning breed conservation (Kantanen et al., 2000).

Selected population data of breeds, investigated biological and genetic features of studied breeds allowed to draw LLG and LWB cattle breeds in the world’s biodiversity data basis – Hanover, FAO and DAD-IS.

The results show that all studied Lithuanian cattle breeds display not low gene diversity and the genetic divergence can be described as moderate. It is known that there are positive relationships between the degree of heterozigosity and the level of polymorphism, because as higher the amount of polimorphic phenotypes in a population is the more heterozygous genotypes can be. Detected average heterozigosity in Lithuanian cattle breeds was more close to the high value and it shows high intra-breed genetic variation.

The allele frequency did not show that breeds have unique alleles, which may be native and adaptive. It is known that allele frequency among the subpopulations is influenced by the size of the population and may be different because of random genetic drift as well as by natural selection, or because of gene migration among the subpopulations, located at the same area. In spite of the fact that native Lithuanian White Backed and Lithuanian Light Grey cattle have relatively small population size, in general, the frequency of phenogroups is at the same level as in the studied breeds which contain the largest part of the whole Lithuanian cattle population.

Weir and Cockerham F statistics is based on the analysis of variance of gene frequencies. According to Wright's suggested qualitative guidelines for the interpretation of FST measure, the estimated results indicate a moderate genetic differentiation among the subpopulations.

The overall pattern of breed relationship in the neighbour-joining tree presented in this paper confirms the grouping of studied cattle breeds into two main groups and outlying of the Lithuanian Red population. The outlying of the Lithuanian Red cattle population could be explained if we assume that this breed was not influenced by gene flow from other studied breeds, or breeds that belong to the Holstein type. Previously studied genetic relationships between 14 Balkan and crossbred Balkan-Alpine (Medjugorac et al., 1994) and 20 North European (Kantanen et al., 2000) cattle breeds estimating genetic distances according the typing of erythrocyte antigens and blood plasma proteins showed similar distribution of indigenous and commercial cattle populations in Europe.

Local LLG and LWB cattle breeds are unique as historical – cultural heritage. Specific colours and features for local cattle survived until nowadays, although cattle were improved intensively with bulls of Black and White and Red breed in Lithuania in latest decades. This fact shows that some of economic valuable and useful features are typical for them and stable. Most of the owners of local cattle have a positive opinion about their good adaptation, resistance against diseases, quiet temper and other useful traits. Considering that, it is necessary to create selection programmes for LLG and LWB breeds and to increase a number of cattle in relict – gene pool herds by using pure breeding. In order to preserve genetic and economical valuable features specific for local cattle breeds, it is necessary to use the sperm of only the same breed bulls for LLG and LWB cows' artificial insemination.

In summary, it has been shown that all four studied Lithuanian cattle breeds represent separate gene pools, and that although there may have been crossing between breeds it has not been sufficient to make the breeds become not differentiated and breeds reveal their own features.

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