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Lebedyn cattle breed genetic pool genesis and new types and breeds developed on its base

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Abstract

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The selection and economic characteristics of layer of brown and black and white dairy cattle are studied in Sumy region of Ukraine. Based on the analysis of micro populations of Ukrainian breeds, it was found that there is a low level of genetic diversity in the presence of differences between them. The results revealed that there is low rates of genetic polymorph ability and heterozygosity, especially in animals of Sumy interbreed type of Ukrainian black and white dairy cattle, Ukrainian black and white dairy cattle breed and Lebedyn cattle, this threatens to loss of genetic diversity, loss in unique alleles and increase in inbreeding of next generations. Relying on the study results, the measures to preserve the Lebedyn breed in Sumy region are proposed.

Keywords: dairy cattle; genetic diversity; gene pool; Schwyz breed; Lebedyn breed

Introduction

Preservation of genetic resources in animal husbandry is a primary need and it is connected with productive and adaptive abilities of a some breed, so livestock, determined by their genetic pool, formed under the definite climatic and geographical conditions of a region where they were developed (FAO, 2013).

Every breed is a combined product of artificial and natural selection, that, with the time, determines the formation of adaptive genetic complexes, having agreatroleasa source of good genetic material for the processes of breed formation, improvement of existing breeds and interbreed structures (Belew et al., 2016).

Securing modern and future generations of people with food products is a question of primary importance for FAO and every country in measures of food supply security projects (FAO, 2015; Boettcher and Akin, 2010). In a context of solving these problems, the first place is given to preservation of animal genetic resources diversity, special declarations and strategic plans were specially adopted by international community, and every country has special national programs determining the form and methods of preservation, providing special systems of rational genetic resources management (Vishnevsky, 2017).

In Ukraine only several breeds are high productive, or so called "commercial" cattle breeds, which are dominant in a world, these are Holstein and partially brown breeds (Guzeev, 2014). Such breeds as Ukrainian grey cattle (928 cows, 12 bulls), Ukrainian white headed (303 cows), Ukrainian Carpathian brown (133 cows), Lebedyn cattle (970 cows) are extinctive (Guzev, 2012; Gladiy, 2014). Four Ukrainian breeds of pigs, two sheep breeds, and Hutsulskaya horse breed are completely lost or faced the extinction (Metlitskaya & Nor, 2013; Guzev et al., 2012).

Regional breeds have the advantage of adoption to certain climatic, forage, health conditions, they have better reactive qualities and diversity of genetic material, they also do not miss the advantage of "genetic type – environment" balance. Genetic pool of these breeds is cultural and historical heritage of our nation (Vishnevsky, 2017; Vishnevsky et al., 2017).

Aboriginal animals are highly valued in developed countries as a repository of unique genetic pools, providing formation of positive features which have been lost by modern commercial breeds, in particular: high fertility, long period of economic utilization, strong health security, ability to more effective use of natural pastures, etc. These animals do not need great resources and can be bred in conditions close to natural (FAO, 2016). But the next generations, being breed inter se, show genetic resolving and the process of heterosis fades. That is why knowledge-consumptive ideas from everyone involved in agriculture are in an urgent need; realization of them will provide genetic pool security in regional breeds and conditions for constant development of eco safe animal husbandry in different ecological and geographical conditions of Ukraine (Voitenko and Vishnevsky, 2014).

Lebedyn cattle breed has developed by the method of complex breeding of regional cows (mainly from Ukrainian grey cattle) with bulls of Schwyz breed, with proceeding inter se breeding. Cattle of the breed are early maturing, resistant to infectious and invasive diseases, with productive utilization for more than 4 lactations. Milk productivity on stud farms 4500-5500 kg, fat content – 3.93%. Calf yield from 100 cows is 87 heads. Food cost per kg of milk in studs is 1.12 feed units. Today Lebedyn cattle is bred on such stud farms as: Stud Farm "Mykhaylivka" (120 cows), Private Farming Enterprise "Komyshanske" (293 cows) in Sumy region, and LtD "Mriya" (300 cows) in Chernyghiv region.

Labedyn cattle have high adoptive rates and deserve preservation and intensive utilization, but it demands a substantial genetic basis. Genetic features of the cattle mentioned above are still important functional elements of production in the region. According to FAO recommendations concerning the rate of risks for genetic resources, Lebedyn cattle breed, from our point of view, can also be regarded as having a high risk of elimination. The mentioned status depends on effective number of animals, number of females and males, tendency and dynamics of pedigree activities (Rischkowsky and Pilling, 2007).

According to monitoring of local thin or eliminating breeds of different species according to their number and number of stud farms in Ukraine (2011-2017) provided by State Livestock Breeds Register there is a tendency of reduction in number of stud farms and pedigree stock in breeds. Number of Lebedyn cattle pedigree herds was reduced from 6 to 4 with the reduction of livestock population on 512 heads or 41,8%. It must be mentioned that the largest part of stud stock of Lebedyn cattle breed was taken from imported bulls or semen production of exterior selection Schwyz breed. The last, mentioned above, determines their definition as a new selected Ukrainian brown dairy breed. So, the real genetic pool of Lebedyn cattle breed is measured even by closer circle of pedigree cattle, it must be studied, by the specialists of scientific and educational institutions involved, in expeditionary way. According to the results of the analysis held, among the great number of thin farm breeds of Ukraine, we have chosen the most "vulnerable" populations which are extinctive, considering the number of females and number of stud farms involved in the process of their breeding.

Loss of breed diversity is characterized not only by the loss of the unique and genetically valuable diversity, but also by decay of genetic potential and makes a serious negative influence on selective work and breed developing process in future. In any case, in order to preserve the genetic diversity we need genetic monitoring system to study the structure of genetic pool objects demanding preservation by methods of modern genetics.

Material and Methods

The research has been held according to the data of livestock judgment (livestock management form – 7-Мол) of Lebedyn cattle breed on the farms of Sumy and Chernygiv regions: Stud Farm "Mykhaylivka" (114 heads), Private Farming Enterprise "Komyshanske" (275 heads) in Sumy region, and LtD "Mriya" (300 heads), AE "Victoriya" (96 heads) AE "Nadiya" (82 heads); Brown dairy breed: Scientific research enterprise under the control of NAAS of Ukraine (100 heads), AE "Victoriya" (96 heads), AE "Nadiya" (82 heads); Sumy interbreed type of Ukrainian black and white dairy breed: Scientific research enterprise under the control of NAAS of Ukraine (100 heads), AE "Vladana" (163 heads), AE "Iskra" (236 heads), AE "Pershe Travnya" (298 heads). The study of modern genealogy of population of cattle and study of milk productivity were done on the basis of the first, the third and the best lactation.

Fat and protein content in milk has been measured according to universal method in laboratory of former Sumy state selection centre having the Bentley facilities, in laboratory of Institute of livestock under the control of NAAS, in laboratory of Institute of breeding and genetics of animals having the same Bentley facilities.

One semen doze from five bulls of every breed (Holstein, Ukrainian black and white dairy, Schwyz and Lebedyn) has been taken for molecular-genetic researches. Four mql from

every semen doze were taken for DNA genomic extraction. For genetic characteristic of Brown dairy breed and Sumy interbreed type of Ukrainian black and white dairy breed, blood was taken from jugular in amount of 1 ml into pointed bottom tubes with 3.8% solution of sodium citrate.

For extraction of genomic DNA from the semen of bulls 20% ion exchanged resin "Chelex-100@" has been used according to Gregory and Rinderer (2004) along with laboratory modifications, involving ablution of example and its ultrasound treatment (the results are not presented, proceeding the stage of patent acquiring). DNA extraction from animal blood proceeded after receiving the lactation residue with the help of 5% "Chelex-100@" and residue of lactation mass (Walsh et al., 1991).

DNA amplification with ISSR primer has been held with the help of commercial kit "Fermentas" (Vilnius, Lithuania). Reactive mixture contains: reactive buffer – 2.5 mql; 100 pM of primer (0.5-1.0 mql); from 2 to 4 activity points Tag-polymerase (0.1-0.2 mql); DNA sample (1-3 mql); deionised water for mixture volume 25 mkl. Not more than 0.5-1 ng of DNA sample (DNA stock solution at the ratio 1:10) for every reaction has been used. Primer synthesis for ISSR analysis has been made at "Fermentas" (Vilnius, Lithuania) request.

Nucleonic structure and temperature regime of a primer: S1 3'-AGCAGCAGCAGCAG CAGCC-5', being the direct and reversed initiator of amplification in PCR. Amplification has been done in thermo cycler "Tercik" ("ДНК – технологія", Moscow, Russia). Program of amplification with primer S1: 1 cycle: 94°C – 4 min; 2-31 cycle: 57°C – 2 min; 72°C – 4 min, 94°C – 1 min; 32 cycle: 57°C – 3 min; 72°C – 7 min.

Electrophoresis division of amplified DNA areas with ISSR technology has been held in 2% agarose gel in single TBE electrophoresis buffer, according to recommendations (Maniatis, 1984). Gel coloring has been made with 0,5% solution of ethydium bromide during 10 min. Visualization of electrophoregramms has been made on transilluminator in ultraviolet specter with 340 nm wave length. Photos of gel have been done with orange color filter on "Canon" camera. Size of amplification products control in gel electrophoreogramm has been done with the help of molecular weight marker 1kb Ledderplus ("Fermentas", Vilnius, Lithuania). Only PCR products, being distinctly shown in gel in the range of molecular weight from 200 to 3000, under three times of amplification, have been taken for the analysis.

The procession of profiles has been made by typical software GenAlex6 (Peakall and Smouse, 2006). A dendrogram of relative genetic relatedness (Nei, 1972) was constructed using the UPGMA method (Sneath and Sokal, 1973) and software MEGA 4 (Tamura, 2004).

Results and Discussion

The research work showed that more than 75% of cows on stud farms breeding Lebedyn cattle in Sumy region are the ancestors of Schwyz bulls of American or West European selection (Fig. 1). The most numerous genetic structural items in livestock are the line of Distinction 159523 and Elegant 148551. Animals ancestors of bulls fecundators of Lebedyn cattle belong to 2 lines – Brave 1510 and Chutkiy 4281.

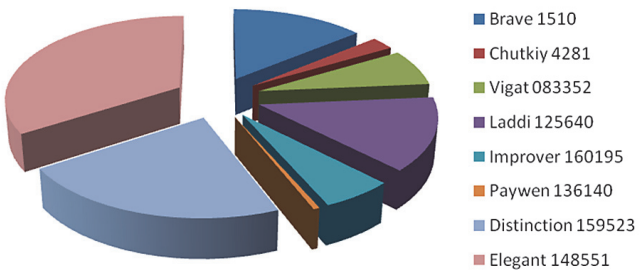


Fig. 1. Cows pedigree of Lebedyn cattle breed

So, the biggest part of pedigree cattle of Lebedyn cattle breed is from imported semen of bulls from Schwyz breed. That is why, there is a need in expeditionary study of all Lebedyn cattle breed animals on stud farms, by the specialists of scientific institutions and universities involved. Former stud farms of Chernigiv region can also be listed for expeditionary study.

Rate of milk productivity for Lebedyn cattle breed is in measures of 3000-7000 kg of milk (Fig. 2). It shoud be noted that according to breed standards it must be in measures: I lactation – 3600 kg of milk, II – 4000 kg, III – 5000 kg (Bashchenko et al., 2012). It must be mentioned that cows of stud farm "Mykhaylivka" and AE "Komyshanske" complaint with standards of breed in milk yields and exceed them in milk fat content. High rates of animal milk productivity in FE "Komyshanske" show a great potential of genetic diversity and ability to preserve it with the optimal conditions of husbandry.

Purpose-driven selection of Ukrainian brown dairy cattle is based on supplemented theory and practice of animal husbandry with the large scale and individual selection as a principally new system of genetic pool estimation of Lebedyn cattle and openness of population for better utilization of Brown cattle heredity (Ladyka et al., 2014).

Due to utilization of this breed in selection, with optimal rates of milk yields, and optimal milk contents and posture elements, the conditions appeared for creation of compatible, modern population of Brown cattle (Ladyka et al., 2012a, 2012b).

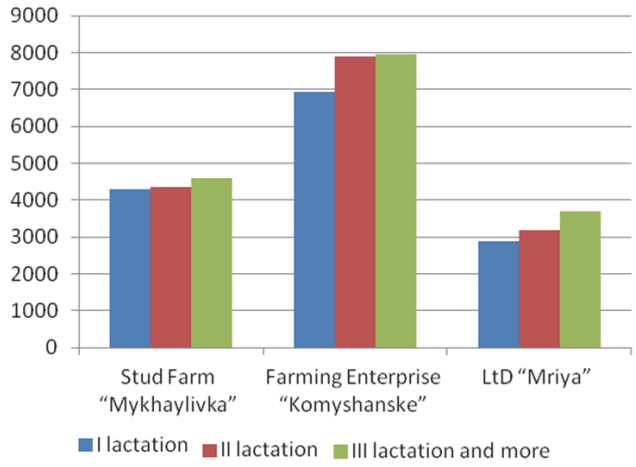


Fig. 2. Rate of milk productivity for Lebedyn cattle breed

Today in Sumy region, there are four stud farms breeding Ukrainian brown dairy cattle. Genealogic structure of Ukrainian brown dairy cattle has five lines, with more then 50% of livestock having the Distinction 159523 leading line (Fig. 3). The second position has the line Elegant 148551. Rate of milk productivity for the breed is in measures of 3000-7000 kg, being in normal measures in most of the enterprises (Fig. 4). The difference in milk productivity rates appears because of genotypic and paratypic factors. Most of livestock in AE "Victoriya" come from the lines Vigata 083352, Distinction 159523 and Elegant 148551, in Scientific research enterprise under the control of NAAS of Ukraine – from Distinction 159523 and Elegant 148551, in AE "Nadiya" – from Laylason 131528 and Stretch 143612. Variations of milk productivity are influenced by the system of feeding and milk production technology on a certain farm.

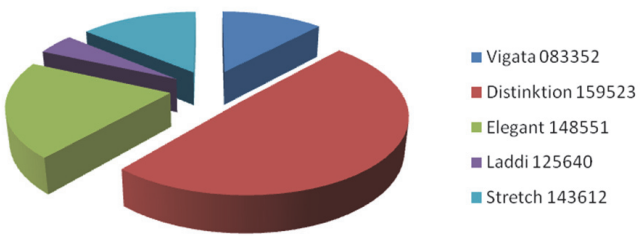


Fig. 3. Cows pedigree of Ukrainian brown dairy cattle

As a result of a long selection work, Ukrainian black and white dairy cattle, having five interbreed types, has been developed. Sumy region population of this breed has been formed by accumulation of black and white breed livestock

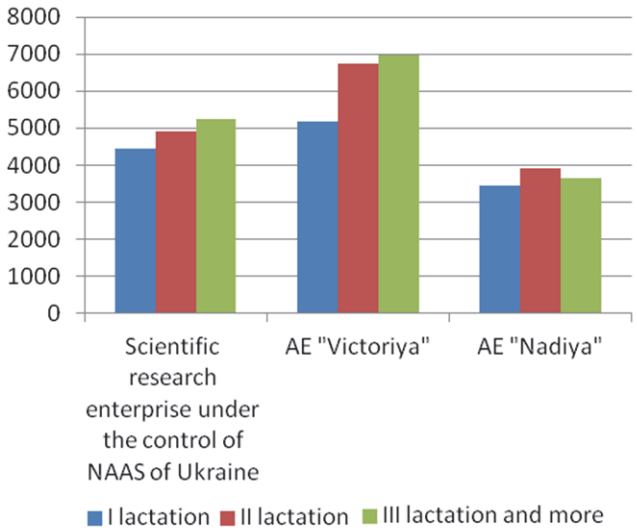


Fig. 4. Rate of milk productivity for Ukrainian brown dairy cattle

from different regions of Ukraine and republics of former USSR, and import of semen and bulls of Holstein breed. Sumy interbreed type of Ukrainian black and white cattle development was accompanied by utilization of several breeds of white and black color: Ukrainian black and white dairy cattle, Holstein breed and pedigree cattle of Lebedyn cattle breed.

Unfortunately this breed has no perspective for future utilization and it is the same with the common tendency for Ukrainian black and white dairy breed. SE "Sumy State Selection Centre" has no bulls of Ukrainian black and white dairy cattle, so it is not possible to use them with a planned pedigree after the Holstein breed of native selection, on stud farms.

Population of black and white dairy cattle is characterized by a high level of blood relationship (more than 87.5%) with Holstein breed. As a result some stud farms get the cer-

Fig. 5. Genealogic structure of cows from Sumy interbreed type of Ukrainian black and white dairy cattle (the most numerous lines)

tificates of Holstein breeders (LtD "Vladana"). Several-year situation analysis shows the decrease in livestock and number of stud farms.

Pedigree stock cows are the ancestors of 19 lines of bulls. The most numerous is the Chifa 1427381 line. It is close to Starbaka 352790 line, as for number of bulls and has the leading role. The important factor for domination of Holstein lines in genealogy of Ukrainian black and white dairy cattle is absence of native selection and system of bull estimation and utilization of semen from Holstein breed.

Rate of milk productivity of cows from Sumy interbreed type of Ukrainian black and white dairy cattle is the highest among breeds mentioned above, it is in measures of 5.0-8.0 thousand kg of milk.

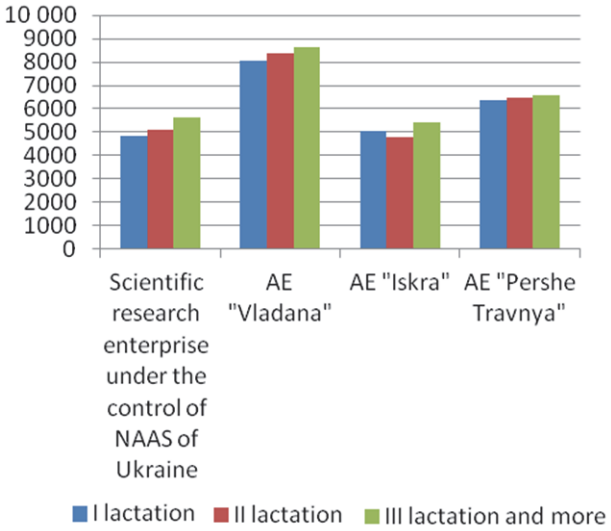


Fig. 6. Rate of milk productivity of cows from Sumy interbreed type of Ukrainian black and white dairy cattle

Stud stocks, breeding Sumy interbreed type of Ukrainian black and white dairy cattle, use different ways of bull selection and have different feeding and livestock management conditions. It determines, in our opinion, the difference in rates of milk productivity.

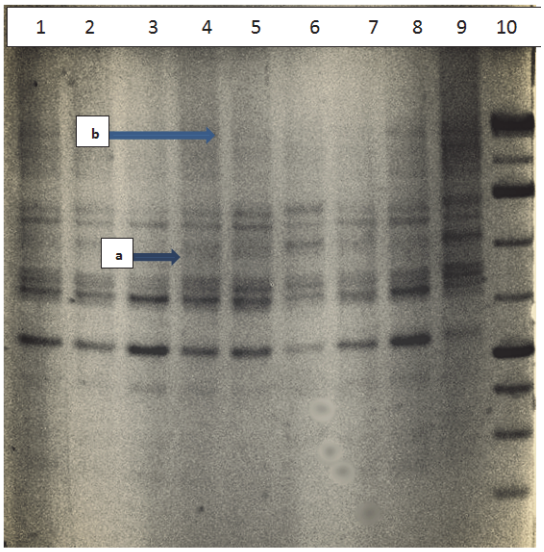
The results of the research show that Sumy region developed a layer of Ukrainian black and white dairy cattle, having its own selective and economic characteristics. Regional population has been developed with the help of introduction of black and white livestock with further holsteinization, together with development of Sumy interbreed type on the base of Lebedyn cattle breed.

Related origin of animals from the mentioned breeds, gave us the idea to study the genetic peculiarities of Lebedyn, Ukrainian black and white, and Sumy interbreed type of Ukrainian black and white dairy cattle.

The peculiarities of selection with some breeds, demands synthesis of data from different sources, namely the results of molecular-genetic researches analysis giving the objective criteria for estimation of interbreed diversity. The diversity of marker systems allows the researchers to find the optimal type of markers for real estimation of breed genetic pool. Common conception for DNA classification must have methods for a universal approach determining the peculiarities of genetic pool for any livestock, high polymorph and informational content of genetic markers, their high renewing ability and low cost of tests (Kopylova et al., 2013; Metlitskaya et al., 2016).

This question is especially important considering the genetic criteria of estimation for thin populations of cattle and characteristics of developed breeds in order to increase their consolidation, productiveness and breeding value.

Genetic and population analysis of animals from mentioned breeds with the technology of ISSR using the anchored microsatellite with trinucleotic AGC motive, allowed to have 25 products of amplification in measures from 250 to 1600 base pairs, and all gene locus were polymorph.



1-5 lines: DNA-profiles of bulls of SCHWYZ breed
6-9 lines: DNA profiles of Lebedyn cattle breed
9- Molecular weight marker DNA plus, size from the bottom upwords: 200, 300, 400, 500, 600, 800, 1000, 1200, 1500 base pairs
Arrows mark:
a – Fragment size 770 base pairs
b – Fragment size 1500 base pairs

Fig. 7. Product amplification electrophoregramm of animal DNA from Schwyz and Lebedyn breed with ISSR-S1 in 2% agarose gel primer

Table 1. Genetic and population analysis of six cattle breeds samples with poly locus system ISSR-S1

Breeds (N)	Genetic and population rates				
	Average number of amplicons	Rate of intergroup kindred	Heterozygosis	Number of genetic locus	Part of polymorph locus
Holstein (5)	13,000*** ±0,894	0.822	0,156***	11,244	0,200
Ukrainian black and white dairy cattle (5)	15,200 ±1,241	0,846	0,162***	13,081	0,236
Schwyz (5)	12,600*** ±0,400	0,730***	0,283**	9,818	0,390
Lebedyn (5)	12,600*** ±0,872	0.779**	0,194***	10,557	0,242
Ukrainian brown dairy cattle (20)	12,450*** ±0,531	0.778***	0,360[a]	9,151	0,782
Sumy interbreed type of Ukrainian black and white dairy cattle (20)	15,050[a] ±0266	0.914[a]	0,100***	13,678	0,196

Note: N – number of animals in a group; the difference in rates in measures of one column of a table is possible, in comparison with the number marked with Fisher criteria: ** – $p < 0.01$; *** – $p < 0.001$

Analysis of separate DNA fragments division, gave the opportunity to determine some peculiarities in genome of some breeds. For example, we have found the PCR amplification product having size of 770 base pairs (Fig. 7), occurring with a frequency of 0.60 in animals of Schwyz breed, and with frequency of 0.20 and 0.30 in animals of Lebedyn and Brown cattle breeds, respectively. Considering the fact that bulls of Schwyz breed were the part of development and further improvement of Lebedyn breed and the Lebedyn breed had the same role in schemes of development for Ukrainian brown dairy breed, we consider the allele found to be the mark for Schwyz breed. The animals studied have shown the absence of DNA fragment of 560 base pairs, which was different by its frequency from 0.30 in cows to 0.60 in bulls of Ukrainian black and white dairy cattle. That is why such silent allele can be determined as one of the markers characteristic to Schwyz breed. The biggest number of allele markers for Ukrainian brown dairy cattle, characterized by presence of amplicon of 350 base pairs size, and absence of (silent allele) of DNA fragments of 1350 and 1500 base pairs size. We must admit that the last of mentioned amplicons was found with the frequency 1.00 in bulls of Schwyz breed and 0.80 in animals of Lebedyn cattle breed and only in 5% of cases with Sumy interbreed type of Ukrainian black and white dairy cattle.

Genetic-population analysis of animals from different breeds, according to the results of DNA tests haves shown substantial differences between them in some rates. The fragment used as a primer, allowed to mark from 15.2 genome fragments in bulls of Ukrainian black and white dairy cattle to 12.6 in animals of Schwyz and Lebedyn cattle. The highest rate of intergroup kinship (genetic likeness of animals in measures of a group studied) shown by animals of Sumy interbreed type of Ukrainian black and white dairy cattle (0.914), it exceeded the rates shown by Schwyz, Lebedyn and Ukrainian black and white dairy cattle – 0.730; 0.779 and 0.778, respectively ($p \leq 0{,}001$). The rate of estimated heterozygosis was minimal and was 0.100, with the lowest rate of polymorph locus in compared breeds – 0.196. These results are the consequence of "chokepoints" effect and the effect of new interbreed group developer, with possible influence by the genetic homogeneity of bulls, close group of newly developed population and further processes of breed development under a great influence of artificial selection.

On the contrary, the rate of genetic heterogeneity has been found in cows of Ukrainian brown cattle. This fact can be explained by the results of animal selection according to the lines, influencing the differentiation of cows from pedigree stock according to genetic complexes of the best bulls, connected with productivity of their ancestors. It is well known that, so called blood relationships, taken into consideration in schemes of breed hybridization or genealogic structures, from genetic point of view are mostly factitious. It is influenced by lots of biological (imbalance in linkage, domination and hereditary capacity, epistazis, prezygotic selection, etc.) and paratypic factors, especially artificial selection and environmental components.

One of the tasks for our research was to model the scheme of genetic relations between the breeds of cattle, connected together by historic relations and to estimate if the results of DNA tests show the adequate scheme of breed differentiation. It is very important instrument of planning and realization for the activities devoted to preservation of thin livestock breeds, first of all for Lebedyn cattle breed, which is no close to extinction.

Table 2. Rates of genetic kinship between the micro populations of cattle, being estimated

Breeds	Holstein	Ukrainian black and white dairy	Schwyz	Lebedyn	Ukrainian brown cattle	Sumy interbreed type of Ukrainian black and white dairy cattle
Holstein	0.000	**0.771**	0.654	**0.594**	**0.531**	0.652
Ukrainian black and white dairy cattle		0.000	0.709	0.704	0.684	**0.765**
Schwyz			0.000	**0.743**	0.622	0.721
Lebedyn				0.000	0.648	0.741
Ukrainian brown dairy cattle					0.000	**0.768**
Sumy interbreed type of Ukrainian black and white dairy cattle						0.000

Note: The method of genetic kinship estimation for livestock has been used (Nei, 1972)

The results of estimation for genetic relations in cattle micro populations of different breeds are shown in Table 2. The biggest rate of genetic kinship, according to the data of microsatellite analysis with primer S1, is determined for Ukrainian brown cattle and Sumy interbreed type of Ukrainian black and white dairy cattle (0.768). The explanation for the results is in history of breed development, because the basis for the first was the genetic basin of Schwyz and Lebedyn breeds, and the genetic structure of the second was influenced by animals of Lebedyn breed, it formed common sub-cluster by representatives of these two populations (Fig. 8). If the first one has been developed from two breeds Lebedyn and Schwyz, the second has been influenced by four breeds. The development process of Sumy interbreed type of Ukrainian black and white dairy cattle had females of Lebedyn cattle breed, and crossbred cows of Lebedyn and Schwyz breed with different rates of blood relationships. To manage the process, bulls of Holstein and Ukrainian black and white dairy breed has been used. Crossbreeding was planned to be present till gaining the part of Holstein breed not more than 87.5%. Genetic kinship of a new breed and type, on our opinion, is determined by the same Lebedyn mother breed, it gives the opportunity to registered mating with stud bulls of these breeds as a result.

We cannot say that the marker system ISSR-S1 used for this research is completely appropriate for estimation the genetic breed differentiation, because of the appearing DNA fragments connected with sex. For example, we have found an amplicon sized 470 base pairs, appearing only in cows of Ukrainian brown breed and Sumy interbreed type of Ukrainian black and white dairy cattle. On the contrary amplification fragment sized 1120 base pairs, has been found only in bulls with the frequency of 0.75. Our previous research have found that DNA fragments found among invert oriented fragments of microsatellites, are the unique, very often coding genome sequences, being found in sex chromosomes as well, are very often connected with features of animal productivity, influencing the cladogram configuration under the cluster analysis (Metlitskaya, 2012). Despite the fact that ISSR technology is thought to be old-fashioned, it allows to find new genes, quantity features and create more exact SNP markers on the base of amplicons found, it has a great informational potential being used while researching population of livestock with minimal costs, it is essential for studying, especially with understudied biological objects (Askari et al., 2011; Metlitskaya, 2012; Nesteruk et al., 2016; Costa et al., 2016).

It should be noted that the breeds of the common cluster are closely connected by history. Schwyz and Lebedyn cattle

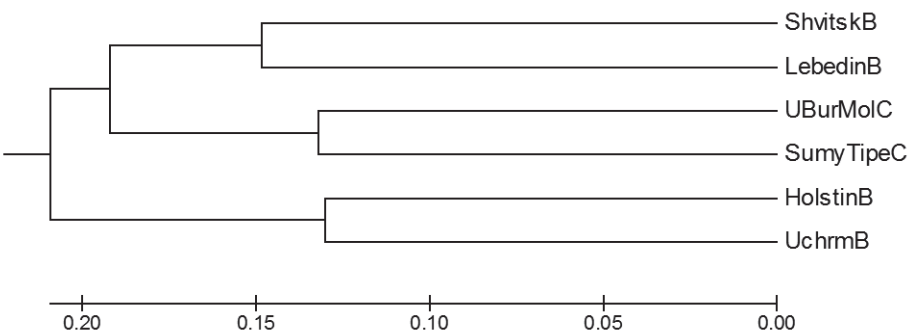


Fig. 8. Dendrogram of ISSR-typing of 5 cattle breeds and one interbreed type, constructed using the UPGMA method, software MEGA 4

breeds are united into one sub cluster together with early mentioned breeds Ukrainian brown breed and Sumy interbreed type of Ukrainian black and white dairy cattle. Genetic kinship of Schwyz and Lebedyn cattle can be explained by the fact that the first started its development on the beginning of the previous century by means of Schwyz bulls utilization on regional livestock, containing mainly Ukrainian grey cattle breed, with the proceeding development inter se. Genetic kinship of Lebedyn and Schwyz cattle gives the possibility to save the mentioned breed from extinction, with the help of existing modern selection activities.

It is absolutely normal to give the separate sub cluster line for two breeds: Holstein and Ukrainian black and white dairy cattle breed as the estimated index of genetic kinship between them (77.1%) practically coincides with the absolute number of estimated blood relationship for regional brown dairy cows which are in fact the subgroup of Holstein cattle, adopted to Ukrainian conditions.

So, six micro populations of Ukrainian breeds researched have shown at the end lack of genetic diversity with existing distinctions between them. Low rates of genetic polymorph ability and heterozygosity, especially in animals of Sumy interbreed type of Ukrainian black and white dairy cattle, Ukrainian black and white dairy cattle breed and Lebedyn cattle, potentially create the possibility to lose genetic diversity, loss in unique alleles and increase in inbreeding of next generations. Our researc, has strengthen the importance of development special breeding programs for thin and extinctive breeds, using the genetic information needed for planned mating. One more important fact is the importance of highly informative usage of markers for estimation of breed's genetic specificity using the standard panels of microsatellites, confirmed by international organizations ISAG and ICAR, with monitoring research of every existing population of every cattle breed (Adamov et al., 2011; Beja-Pereira et al. 2003; Uffo et al., 2017).

Special attention must be paid to DNA specification of aboriginal animal breeds and identification of unique adaptive genetic complexes and alleles associated with important economic features (Hu et al., 2013; Kopylov et al., 2014; Kopylov et al., 2016), the above mentioned is determined as the main point for further research and development of effective strategy for preservation of genetic pool in Lebedyn cattle breed.

Conclusions

In Sumy region of Ukraine the layer of brown and black and white dairy cattle has been developed, it has its own selection and economic characteristics. Micro populations researched have shown at the end lack of genetic diversity with existing distinctions between them. Low rates of genetic polymorph ability and heterozygosity, especially in animals of Sumy interbreed type of Ukrainian black and white dairy cattle, Ukrainian black and white dairy cattle breed and Lebedyn cattle, potentially create the possibility to lose genetic diversity, loss in unique alleles and increase in inbreeding of next generations. The biggest rate of genetic kinship, according to the data of microsatellite analysis with primer S1, was determined for Ukrainian brown cattle and Sumy interbreed type of Ukrainian black and white dairy cattle (0.768). The explanation for the results is in history of breed development, because the basis for the first was the genetic basin of Schwyz and Lebedyn breeds, and the genetic structure of the second was influenced by animals of Lebedyn breed. It formed common sub-cluster by representatives of these two populations. Schwyz breed which have taken art in development of Lebedyn breed can be also attracted to preservation of Lebedyn breed.

References

Adamov, N., Mickov, L., Petkov, V., & Adamov, M. (2011). Microsatellite markers for pedigree verification in cattle. *Macedonian Journal of Animal Science*, *1*(1), 9-15.

Askari, N., Mohammad, A. M., & Baghizadeh, A. (2011). ISSR markers for assessing DNA polymorphism and genetic characterization of cattle, goat and sheep populations. *Iranian Journal of Biotechnology*, **9**(3): 222-229.

Belew, A. K., Tesfaye, K., Belay, G., & Assefa, G. (2016). The state of conservation of animal genetic resources in developing countries: a review. *International Journal of Pharma Medicine and Biological Sciences*, *5*(1), 58-66.

Bashchenko, M., Bratusha, R., Boyko, Yu., Ladyka, V., Sklyarenko, Y., Ivanniko, V., Herman, D., & Golod, L. (2012) Regional program for preserving the Lebedyn breed gene pool, Sumy, "Mriya" (Ru).

Beja-Pereira, A., Alexandrino, P., Bessa, I., Carretero, Y., Dunner, S., Ferrand, N., Jordana, J., Laloë, D., Moazamigoudarzi, K., Sanchez, A. & Canon, J. (2003). Genetic characterization of southwestern European bovine breeds: a historical and biogeographical reassessment with a set of 16 microsatellites. *Journal of Heredity*, *94*(3), 243-250.

Boettcher, P. J., & Akin, O. (2010). Current arrangements for national and regional conservation of animal genetic resources. *Animal Genetic Resources*, *47*, 73-83.

Costa, R., Pereira, G., Garrido, I., Tavares-de-Sousa, M. M., & Espinosa, F. (2016). Comparison of RAPD, ISSR, and AFLP molecular markers to reveal and classify orchardgrass (Dactylis glomerata L.) germplasm variations. *PloS one*, *11*(4), e0152972.

FAO (2013). In vivo conservation of animal genetic resources. FAO Animal Production and Health Guidelines, no. 14, Rome, 244 p.

FAO (2015). Coping with climate change – the roles of genetic resources for food and agriculture, Rome.

FAO (2016). What is agro biodiversity? http://www.fao.org/docrep/007/y5609e/y5609e01.htm (accessed 14th January 2016).

Gladiy, M., Sharan, P., & Kruglyak, O. (2014). State support for preservation of the gene pool of local small breeds in livestock. *Economy of Autonomous Republic of Crimea*, 11, 15-22.

Gregory, P. G., & Rinderer, T. E. (2004). Non-destructive sources of DNA used to genotype honey bee (Apis mellifera) queens. *Entomologia Experimentalis et Applicata*, *111*(3), 173-177.

Guzev, I. (2012). Results of modern identification of current resources of the risk of disappearance of genetic resources of meat cattle breeding of Ukraine. *Visnyk of Sumy National Agrarian University, Series: Animal Husbandry*, 12, 36-42 (Ru).

Guzev, I., Similar, B., & Reznikov, N. (2012). Some topical issues of preservation of the gene pool of animals in the modern context. *Breeding and Genetics of Animals*, *46*, 69-73 (Ru).

Guzeev, Yu. (2014). Genesis of the gene pool of aboriginal breeds of cattle of Ukraine. *Scientific and Technical Bulletin of the Institute of Animal Biology and the State Scientific-Research Control, Institute of Veterinary Preparations and Feed Additives*, *15*(4), 245-251 (Ru).

Hu, Z. L., Park, C. A., Wu, X. L., & Reecy, J. M. (2012). Animal QTLdb: an improved database tool for livestock animal QTL/association data dissemination in the post-genome era. *Nucleic acids research*, *41*(D1), D871-D879.

Kopylova, K., Shelov, A., Berezovsky, O., Kopylov, K., & Rossokha, V. (2013). Genetic structure of different breeds of cattle for molecular-genetic markers. *Scientific and Technical Bulletin of National Academy of Sciences of Ukraine, 110*, 76-83 (Ru).

Kopylov, K., Kopylova, K., Balatsky, V., & Metlitskaya, O. (2014). Methodology for assessing the genotype of animals by molecular genetic markers in livestock production in Ukraine. By the ed. of acad. M. I. Gladiy, Kyiv: Agrarian Science (Ru).

Kopylov, K., Metlitskaya, O., Mokhnacheva, N., & Suprovich, T. (2016). Molecular-genetic monitoring in the system of genetic resources preservation of animals. *Bulletin of Agrarian Science*, *6*, 43-47 (Ru).

Ladyka, V., Bratusha, R., & Boyko, Yu. (2012). Prospects for the selection of brown breeds of dairy cattle. *Breeding and Genetics of Animals*, *46*, 13-15.

Ladyka, V., Khmelnychy, L., & Salogub, A. (2014). Organization and main directions of selection and breeding work in dairy cattle breeding of the Sumy region. *Visnyk of Sumy National Agrarian University, Series: Animal Husbandry*, *24*, 3-10.

Ladyka, V., Khmelnychy, L., Salogub, A., & Shevchenko, A. (2012). Perspectives of selection and breeding work in dairy cattle breeding of Sumy region. *Breeding and Genetics of Animals, 46*, 34-37.

Maniatis, T., Fric, E., & Sambruck, D. (1984). Molecular cloning. Transl. from English by the ed. of A. A. Baeva, Moscow: Mir (Ru).

Metlitskaya, O., Kopylov, K., Berezovsky, O. (2016). Modern molecular genetic approaches for increasing the efficiency of breeding process in livestock breeding in Ukraine. *Breeding and Genetics of Animals*, *51*, 193-200.

Metlitskaya, O., & Nor, V. (2013). Population-genetic survey as a justification of ways to preserve the gene pool of pigs of the Mirgorod pig breed. *Breeding and Genetics of Animals*, *47*, 61-73.

Metlitskaya, O. (2012). The methodology of DNA-passporting of genetic funds of agricultural animals for the genus hypervariable locus. Doctoral Thesis.

Nei, M. (1972). Genetic distance between populations. *The American Naturalist*, *106*(949), 283-292.

Nesteruk, L. V., Makarova, N. N., Evsyukov, A. N., Svishcheva, G. R., Lhasaranov, B. B., & Stolpovsky, Y. A. (2016). Comparative Estimate of the sheep breed gene pools using ISSR-analysis. *Russian Journal of Genetics*, *52*(3), 304-313.

Peakall, R. O. D., & Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, *6*(1), 288-295.

Rischkowsky, B., & Pilling, D. (2007). *The state of the world's animal genetic resources for food and agriculture*. FAO.

Sneath, P. H., & Sokal, R. R. (1973). *Numerical taxonomy. The principles and practice of numerical classification*. San Francisco, W. H. Freeman and Co.

Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, *24*(8), 1596-1599.

Uffo, O., Martínez, N., Acosta, A., Sanz, A., Martín-Burriel, I., Osta, R., ... & Zaragoza, P. (2017). Analysis of microsatellite markers in a Cuban water buffalo breed. *Journal of Dairy Research*, *84*(3), 289-292.

Voytenko, S., & Vishnevsky, L. (2014). Risks of loss of domestic breeds of pigs and cattle. *Scientific Bulletin of the National University of Bioresources and Natural Resources of Ukraine*, 202, 186-192.

Vishnevsky, L. (2017). Information system in cattle breeding as a component of the strategy of biodiversity conservation. *Breeding and Genetics of Animals*, 53, 15-21.

Vishnevsky, L., Porkhun, M., Sidorenko, O., & Dzhus, P. (2017). Bank of genetic resources of animals of IABG nd. a. M.V. Zubets of NAASU in the system of biodiversity conservation of cattle of Ukraine. *Breeding and Genetics of Animals*, 53, 21-27.

Walsh, P. S., Metzger, D. A., & Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*, *10*(4), 506-509.

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