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Polymorphism of Cattle Microsatellite Complexes

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Abstract

The article presents the results of genotyping dairy and beef cattle population by analyzing 15 microsatellite markers. For studying dairy animals, black-and-white Holstein heifers from the herd of educational and experimental farm Krasnodarskoye of the Kuban State Agrarian University (Krasnodar, Krasnodar Krai, Russia) were used. This selection was represented by 73 heads 11-12 months old. Beef animals in the selection were provided by the Stock Company Agrocomplex n.a. N. I. Tkachev (Vyselkovsky district, Krasnodar Krai, Russia). The material was obtained from 30 purebred Aberdeen-Angus heads, including 5 cows, 15 heifers and 10 bulls. The obtained biological samples were processed for obtaining the genomic DNA of the required quality. The molecular genetic identification of animals was performed based on microsatellite DNA markers (COrDIS Cattle kit manufactured by LLC Gordis, Russia). Certificates of genetic conformity were obtained in full for all dairy and beef animals. For all groups of animals, Fis values were within the confidence interval (95%), which confirmed the absence of both inbreeding and outbreeding, i.e. the studied population had stable genetic structure. The research made it possible to determine heterozygosity of the populations (0.67 to 0.95), to confirm the absence of close and closely-related inbreeding, to predict further directions of livestock breeding, to identify each animal individually, and to form and store a common database, which allowed to generate and issue passports for the animals that complied with the requirements of world standards.

Keywords: CATTLE BREEDING, GENETIC INFORMATION, MARKER, GENE, BREEDING, IDENTIFICATION, MOLECULAR-GENETIC METHODS, MICROSATELLITE MARKERS, PEAKS, ALLELES OF ORIGIN, INBREEDING, HETEROZYGOTES, HOMOZYGOTES, DNA REPEATS, PCR.

INTRODUCTION

Genotyping of animals is the scientific basis for defining thoroughbredness and modern breeding. Currently, methods of molecular genetic analysis are widely used for solving a wide range of tasks in animal breeding and husbandry. Their task is to identify polymorphic variants of genes (i.e. the presence of several gene alleles with the frequency of not less than 1% each in the population), which are the result of genetic variation of all traits of the organism. Genetic polymorphism refers to a specific locus that may be presented by at least two alleles. Genotyping is becoming a new form of animal assessment that is commonly used in addition to the phenotypic indicators of the animal. DNA analysis is in the basis of genetic diagnostics; therefore, phenotypic manifestations of traits are not important. Changes in the DNA structure are due to various types of mutations: point mutations, deletions and insertions (from one to more nucleotides) [1, 2, 3, 4]. The most common type of polymorphism is determined by the substitution of one nitrogenous base for another (SNP), which results in changing the DNA cleavage site by the restrictase in such a manner that one allele contains a section of nucleotide chain fragmentation, and the other does not have it. In this case, genome dimorphism is determined; heterozygosity does not exceed 50%. Therefore, the widely used method of determining the restriction fragment length polymorphism (RFLP) is less informative in the reference of origin or during analysis of the ancestry [5, 6, 7]. However, there is another reason that causes RFLP. The genome of agricultural animals is saturated (up to 30%) with the so-called short tandem (STR) or simple sequence (SSR) repeats. The repeating unit may be, for example, mononucleotide $[A]_n$ or dinucleotide $[AC]_n$ [8, 9]. However, the repeating unit may be also a trinucleotide, a tetranucleotide, etc. Very often, there are different numbers of repetitions on different chromosomes in the same locus. The number of alleles may be much higher due to changing the number of repeats "n". It should be noted that although each of these loci has different number of repeats in the alleles, the nucleotide sequences at the edges of these repeats are the same for the same loci. And if such repeat falls between two adjacent sites of a restrictase, the lengths of the obtained restriction fragments for different alleles will be different [10, 11, 12]. In families of tandem repeats as genetic markers, special attention of researchers was attracted to the minisatellites that consisted of repeating copies ("the motive") with the length of 9 or 10 up to hundreds of nucleotides each, and microsatellites, the repeating copies of which typically had the length of 1 to 4, and sometimes 6 nucleotides. A minisatellite locus may consist of two to several hundred repeats, a microsatellite locus – of 10 to 100 repeats. The highly polymorphous nature and the Mendeleev type of inheritance make microsatellites perfect DNA markers in farm animals [13, 14]. High veracity and specificity of DNA analysis can be achieved by using any types of biological material: blood, saliva, hair, semen, samples of soft tissues.

Currently, microsatellite DNA marker systems are used for solving various fundamental and applied problems in agricultural biology and biotechnology, such as genomic mapping, characterization of genetic structure of a population, and the degree of inbredness, assessing genetic distances between the lines, breeds and populations, phylogenetic studies, monitoring the origin [15, 16, 17]. Thus, the technology of DNA analysis of microsatellites is becoming an indispensable tool for solving problems in breeding and purposeful enhancement of the productive qualities of bred animals, and is the "gold" standard in the modern molecular genetic identification of animals, as recommended by the International Society for Animal Genetics (ISAG). The most common method of SSR analysis is PCR with the use of primers that are complementary to unique sequences (domains) that each microsatellite locus is flanked with. The use of PCR for analyzing microsatellite loci was first proposed in 1989 for analyzing $[CA]_n[GT]_n$, which was one of the most common motifs of repeats in human genomic DNA [18, 19]. Amplification products were separated by means of electrophoresis in polyacrylamide gel, which allowed to dramatically improve sensitivity and speed of analysis, compared to the methods based on hybridization of genomic blots. Currently, the standard method of analyzing DNA microsatellites with known flanking sequences is multiplex PCR followed by separation of amplification products by means of capillary electrophoresis and their simultaneous laser detection. It meets modern requirements for molecular genetic analysis: high informativeness of the analysis, minimum time of examination, ease of performing, and low cost of the examination.

MATHERIALS AND METHODS

In the practical part of this work, ready domestic modern solutions were used for all stages of genetic analysis. The Gordis company is today the only Russian manufacturer of products for molecular-genetic identification of animals based on microsatellite DNA markers (the COrDIS Cattle kit). ISAG recommended to use 11 loci as standard markers: ETH3, INRA023, TGLA227, TGLA126, TGLA122, SPS115, ETH225, TGLA53, BM2113, BM1824, ETH10. In addition, the kit from the Gordis company for determining the molecular-genetic characteristics of cattle with the purpose of analyzing kinship and DNA individualization of animals based on multiplex PCR analysis of the loci containing STR also contains three additional highly polymorphous microsatellite loci: CSSM66, ILSTS006, CSRM60 (Table 1).

Table 1. Description of microsatellite markers.

Locus name	Chromosomal localization	Nucleotide sequence of the repeat unit
Main		
ETH3	D19S2	$(GT)_n AC(GT)_6$
INRA023	D3S10	$(AC)_n$
TGLA227	D18S1	$(TG)_n$
TGLA126	D20S1	$(TG)_n$
TGLA122	D21S6	$(AC)_n(AT)_n$
SPS115	D15	$(CA)_n TA(CA)_6$
ETH225	D9S2	$(TG)_4CG(TG)(CA)_n$
TGLA53	D16S3	$(TG)_6CG(TG)_4(TA)_n$
BM2113	D2S26	$(CA)_n$
BM1824	D1S34	$(GT)_n$
ETH10	D5S3	$(AC)_n$
Additional		
CSSM66	D14S31	$(AC)_n$
ILSTS006	D7S8	$(GT)_n$
CSRM60	D10S5	(AC)n

The methods of sampling, DNA extraction, PCR and capillary phoreses

For the genetic analysis, blood samples were obtained by the members of the research team together with the specialists of the educational-experimental farm Krasnodarskoye (samples from animals of the black motley Holstein breed) and JSC Company Agrocomplex n.a. N. I. Tkachev (samples from animals of the Aberdeen-Angus breed). The samples were taken in a sterile manner in accordance with the manufacturer's instructions into special vacuum tubes SARSTEDT Monovette® (Sarshtedt, Germany) containing EDTA for preservative. From each animal, 3-5 ml of whole blood was taken. Tubes with the samples were stored and transported in cold (plus 4 °C), preventing freezing. The obtained blood samples were partially (150 to 200 µl) transferred to sterile cotton gauze in the conditions that excluded cross-contamination. Then they were dried in a sterile cabinet under laminar air flow and were packed into individual paper bags. The genomic DNA was extracted from the biological material with a special set of reactants in K-SORB-100 columns (Synthol, Russia). The protocol of extracting genomic DNA consisted of three steps (lysis, DNA precipitation, washing and DNA elution), and was applied to all blood samples used in this work. The DNA extracted this way was used in the work with the use of the COrDIS Cattle set (Gordis, Russia). The primers for PCR were chosen by the manufacturer, subject to amplification of 14 loci in a single tube. The size of all amplified PCR products was < 320 pairs of nucleotides (with regard to all known alleles). PCR results were analyzed by capillary electrophoresis with the use of automated genetic analyzers with laser-induced fluorescence detection. The COrDIS Cattle kit uses five fluorescent dyes characterized by different wavelengths of emission for simultaneous detection in different channels of fluorescence. The primers are marked with four fluorescent dyes detected in Blue, Green, Yellow, and Red channels. The S450 length standard is marked with the fifth fluorescent dye, and is detected in separate Orange channel simultaneously with PCR products.

RESULTS AND DISCUSSION

At present, genotyping of the microsatellite loci by alleles is the most widespread method in studying the structure of populations. Their location is important. The pesence of a large number of alleles allows accurate identification of any individual. Currently, origin assessment by the microsatellite markers is more precise than by blood groups [20, 21, 22]. Therefore, in the world practice of sales of breeding animals abroad, a proof of origin is required according to the analysis of microsatellite DNA. There is FAO recommendation about what microsatellites are to be used for genetic assessment of cattle. According to the database of INRA (French National Institute for Agricultural Research), 2,402 microsatellites were detected in cattle, out of which 2,244 had been mapped (microsatellites were present on all 30 pairs of chromosomes). For population-genetic analysis, packages of computer programs had been developed and improved - Arlequin, GDA, Gen-AlEx, GENEPOP, GeneClass, GeneticStudio, MicroSat, PowerMarker, which were widely used in agricultural genetics [23, 24]. The work was aimed at studying polymorphism of 15 microsatellite markers in two local populations of the black motley Holstein and Aberdeen-Angus cattle. The DNA extracted from blood samples of the following local breed populations were analyzed: Holstein breed at the educational-experimental farm Krasnodarskoye of the Kuban SAU – 73 heifers, and the Aberdeen Angus breed at AF IPC Vyselkovsky – 30 animals. 15 microsatellite markers were used: BM1824, BM2113, BM1818, CSRM60, CSSM66, ETH3, ETH10, ETH225, INRA023, ILSTS006, SPS115, TGLA227, TGLA126, TGLA122, TGLA53. For identifying alleles of the microsatellite loci, electrophoretogram, i.e. a section of the profile of short tandem repeats (alleles of microsatellite loci) obtained with the use of identification software, was used. As a result, graphs were obtained, by the location of the peaks of which the length of microsatellite was determined (h.p.). The frequency of heterozygotes' occurrence (P_{AA}) was calculated by the following formula:

$$P_{AA} = 1 / N \times n_{AA},$$

where n_{AA} is the number of homozygous, and N is the number of animals in the sample.

The Holstein breed

Analysis of the interrelation between the obtained results related to reliability of the used animals' genotype origin reveals practicability of studying the hereditary capacity in the stud bulls used to generate the livestock. Using animals of the Holstein breed from different populations (Dutch, German, North-American) had a positive effect on formation of the genealogical structure of the herd that was dominated by four genealogical lines. The breeding work performed at the present time has resulted in differentiation into two main lines: Vis Back Ideal 1013415, Reflexin Sovering 198988.

The Holstein breed is different from other breeds in the peculiarities of breeding and reproduction: it is well known that in the breed there is a limited number of lines and related groups, and massifs (populations) of Holstein cattle formed in the homeland under the intensive use of relatively small number of bulls. Thus, in the 4-5th generations of ancestors of the studied animals, there is at least one of the 20 founder stud bulls. That is, during formal outbreeding, it is actually hard to avoid selection of

pairs in the ancestry, none of which are founders or their descendants. On the one hand, such system of breeding with intensive selection contributes to breed consolidation; on the other hand, it increases the probability of transition into the homozygous condition of the complex of mutant genes that cause various disorders. For studying dairy animal, black motley Holstein heifers from the herd of the educational-experimental farm Krasnodarskoye of the Kuban State Agrarian University (Krasnodar, Krasnodar Krai, Russia) were used. This sample was represented by 73 animals at the age of 11-12 months. The weight of the animals at the time of blood collection ranged between 285 and 400 kg. During the analysis of 15 microsatellites of the Holstein breed, polymorphism for all loci was detected (Table 2).

Table 2. Characteristic of the Holstein breed microsatellite loci (n = 73).

Marker	Allele	Alleles' characteristic			The number of homozygotes	Observed heterozygosity
		the number of peaks	allele length, h.p.*			
			minimum	maximum		
BM2113	1	3	121	131	26	0.643
	2	5	121	135		
BM1824	1	4	176	186	18	0.753
	2	5	176	188		
BM1818	1	3	261	265	28	0.616
	2	3	261	267		
CSSM66	1	6	149	162	18	0.753
	2	6	152	162		
CSRM60	1	6	88	100	21	0.712
	2	5	90	100		
ETH3	1	5	113	125	6	0.917
	2	5	113	127		
ETH10	1	8	203	219	20	0.726
	2	6	207	219		
ETH225	1	6	136	148	12	0.836
	2	6	136	150		
INRA023	1	5	200	212	25	0.652
	2	5	204	212		
ILSTS006	1	6	287	297	14	0.808
	2	6	289	298		
SPS115	1	4	238	244	4	0.945
	2	7	252	240		
TGLA227	1	9	81	101	9	0.875
	2	9	87	103		
TGLA126	1	4	115	121	16	0.780
	2	5	115	123		
TGLA122	1	9	137	181	10	0.863
	2	10	139	181		
TGLA53	1	9	150	172	7	0.904
	2	8	150	182		

Note: * h.p. - the highest peaks

Different microsatellites had unequal maximum number of peaks in the alleles. It had been established that the average number of peaks per allele in the population was 5.4; fluctuations in the number of peaks were in the range between 3.00 (BM2113; BM1818; allele I) and 10 (TGLA122 allele II).

In analyzing 15 microsatellite loci, the total of 91 peaks were found. As an illustration, analysis of the structure of the microsatellites of three (BM2113, BM1824 and BM1818) of the fifteen studied loci was shown for allele I of the loci (Fig. 1, a-c).

The research had shown that the structure of microsatellites was dominated by peaks along allele I: 117 – 49% in TGLA126; 240 – 56.2% in SPS115; and 261 – 80.8% in BM1818. The smallest amount were the following: 206 – 5.4% in INRA023; 121 – 4.1% in ETH3; 157 – 1.37% in TGLA122; and 101, 93, 85 – 1.37% each in TGLA227. The structure of allele II in three (CSSM66, ETH225 and CSRM60) of the fifteen microsatellites is shown in Fig. 2 (a–c).

In terms of the microsatellite's ratio, the structure of allele II somewhat differed from that of allele I. The most frequent were peaks in: BM2113 – 131 – 52.06%; CSSM66 – 158 – 60.3%; ETH225 – 146 – 68.5%; and ILSTS006 – 295 - 69.9%. A few peaks were observed in microsatellites: INRA023 (206 and 201); TGLA227 (103 and 93); TGLA122 (171); SPS115 (242); ETH225 (144, 142, 136); and TGLA53 (174, 150) – 1.37% each. Variation of the length of microsatellites in both alleles was heterogeneous, for example, the largest one: CSSM66 – 149-162; ETH3 – 113-127; ETH225 – 136-150; TGLA122 – 137-181; and TGLA53 – 150-182. The smallest variation was observed in microsatellite BM1818 – 261-267. With equal number of microsatellite peaks, their lengths were different. Thus, lengths of BM2113 and BM1818 with equal number of peaks (three) differed: 121-131 – in the first case, and 261-265 in the second case. The observed heterozygosity was quite high in all microsatellites - from 0.616 (BM1818) to 0.945 (SPS115). Microsatellite BM1818 showed close inbreeding. The most heterozygous ones were the following: TGLA53 – 0.904; ETH3 – 0.917; and SPS115 – 0.945. For all groups, Fis values were within the confidence interval (95%), which confirmed the absence of both inbreeding and outbreeding, i.e. the studied population had stable genetic structure.

The Aberdeen Angus breed

Conscious control of the individual development requires studying the gene pool of the bred livestock. It should be noted that formation of the population of the Aberdeen-Angus breed in the Krasnodar region occurred due to import of heifers and stud bulls from Australia and Canada. As noted by the scientists of the Bryansk State Agricultural Academy (Russia) and the West Kazakhstan Agrarian Technical University n.a. Zhangir Khan (Kazakhstan), due to the peculiarities of breeding Aberdeen Angus cattle abroad, it is difficult to identify animals belonging to specific genealogical lines, and even more to factory lines – there are simply none of them. This fact is very important from the standpoint of rational use of the gene pool, preserving genetic variability among Aberdeen Angus cattle. As a result of studying the breeding certificates translated into Russian and registered at the Institute of Breeding (settl. Lesnyye Polyany, Moscow region), it has been found that stud stock and stud bulls belong to over 20 genealogical lines and kinship groups. The Australian database (access code: http://abri.une.edu.au/online/cgi-bin/i4.dll?1=22342A3D&2=2431&3=56&5=2B3C2B3) and the Canadian database (access code: http://abri.une.edu.au/online/cgi-bin) were used in the work. The most numerous groups and lines are shown in the Figures, where the lines are marked by the nicknames of the most famous stud bulls, and kinship groups are formed in a way to indicate the promising ones for further breeding. On the main ones among them, it is recommended to start factory lines, which may be extended in the future to increase the number. All progenitor bulls are entered into the pedigree register of Australia (HBR), which indicates the best breeding qualities of these purebred animals that are allowed for registration in foreign herd books. In addition, the results of bulls testing were analyzed for the presence of genetic abnormalities. In terms of the main ones – Arthrogryposis Multiplex (AM), Neuropathic Hydrocephalus (NH), Contractural Arachnodactyly (CA), Developmental Duplication (DD) – all bulls were free (F), as shown in the developed schemes. An exception was the successor in line New Trend 315, branch N. Desing 036 036 USA

– P. A. R. B. Design Plus 97 USA97, which was the DDC carrier. Therefore, it is recommended to exclude the offspring of this bull from breeding programs.

As a result of analyzing the number of the breeding and replacement stock of heifers, 23 lines and related groups were allocated, recommended for further formation in the region. Assessment of the origin of the Aberdeen Angus population that has been brought to the territory of the Krasnodar region from Australia and Canada shows that due to the spread of artificial insemination and intensive use of leader bulls, there is a tendency of increasing the size of lines and related groups. The formed complexes fully comply with the amount and the structure of the livestock used on the territory of Krasnodar Krai.

The beef animals for selection were provided by JSC Company Agrocomplex n.a. N. I. Tkachev (Vyselkovsky district, Krasnodar Krai, Russia). During the analysis of 15 microsatellites of the Aberdeen-Angus breed, polymorphism in all loci was also identified (Table 3).

Table 3. Characteristics of microsatellites of the Aberdeen-Angus breed (n = 30).

Marker	Allele	Characteristic			The number of homozygotes	Observed heterozygosity
		the number of peaks	allele length, h.p.*			
			minimum	maximum		
BM2113	1	4	121	131	7	0.767
	2	5	121	135		
BM1824	1	3	176	180	8	0.733
	2	4	176	188		
BM1818	1	4	259	265	7	0.767
	2	3	261	267		
CSSM66	1	6	150	166	1	0.967
	2	4	156	166		
CSRM60	1	6	90	120	7	0.767
	2	6	90	120		
ETH3	1	6	113	123	6	0.800
	2	8	113	127		
ETH10	1	4	209	215	11	0.633
	2	4	211	217		
ETH225	1	5	136	146	5	0.833
	2	5	140	148		
INRA023	1	3	200	206	10	0.667
	2	4	204	212		
ILSTS006	1	5	206	295	7	0.767
	2	2	212	301		
SPS115	1	5	204	246	3	0.900
	2	5	240	252		
TGLA227	1	4	81	89	3	0.900
	2	7	81	97		
TGLA126	1	4	113	119	4	0.867
	2	5	115	123		
TGLA122	1	6	139	151	10	0.667
	2	6	147	159		
TGLA53	1	4	156	166	7	0.767
	2	5	156	172		

Note: * h.p. - the highest peaks

It had been found that in the population the average number of peaks per allele was 4.7, fluctuations in the number of peaks were between 3.00 (BM1818; allele II) and 7-8 (TGLA227 allele II and ETH3 allele II). Analysis of 15 microsatellite loci revealed 69 peaks. Analysis of the microsatellites' structure on the example of I allele allowed to identify the differences (Figure 3, a-c). The illustration shows only three of the fifteen analyzed loci (BM2113, BM1824 and BM1818).

Analysis of the microsatellites' structure on the example of allele I allowed to identify the differences (Figure 4, a-c). The illustration shows only three of the fifteen analyzed loci (BM2113, BM1824 and BM1818).

The research had shown that the structure of microsatellites was dominated by peaks along allele I: in TGLA53 – 156 – 56.6%; and TGLA122 – 147 – 56.6%. The smallest amount for 3.33% in CSRM60 was 102, 120 and 90. The spread over the length of the microsatellites was unhomogeneous: in allele I – from ILSTS006 – 206-295; SPS115 – 204-246; CSRM60 – 90-120 to the minimum in BM1824 – 176-180; in allele II – from the maximum value in microsatellites ILSTS006 – 212-301; CSRM60 – 90-120 to the minimum in BM1818 – 261-267. With the equal number of microsatellite peaks, their lengths were different. Thus, lengths of ETH3 and ILSTS006 with equal number of peaks (8) differed: 113–127 – in the first case, and 212–301 - in the second case.

The observed heterozygosity was quite high in all microsatellites - from 0.633 (ETH10) to 0.967 (CSSM66). Close inbreeding had not been found in either studied microsatellites. The most heterozygous were the following: CSSM66 - 0.967; ILSTS006 and TGLA227 – 0.900 each, respectively. For all groups of animals, Fis values were within the confidence interval (95%), which confirmed the absence of both inbreeding and outbreeding, i.e. the studied population had stable genetic structure.

CONCLUSIONS

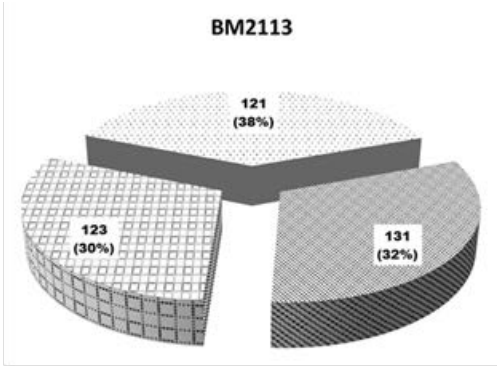
The livestock of the Holstein breed differentiated into two genealogical lines: Vis Back Ideal 1013415 and Reflexin Sovering 198988. The origin of father bulls has been registered (RHA-I and RHA-NA) in the international associations of the breed (USA, DEU, CAN, ITA, NLD) and the territory of the Krai (CRGP) at 99-100%. By the results of testing for carriage of inherited genetic diseases (BLAD, CVM), all father bulls have been free (TL, TV).

For the Aberdeen Angus breed, all ancestor bulls have been recorded in the pedigree register of Australia (HBR) and Canada, and tested for genetic anomalies: Arthrogryposis Multiplex (AM), Neuropathic Hydrocephalus (NH), Contractural Arachnodactyly (CA), Developmental Duplication (DD); all bulls have been free (F), as shown in the developed schemes. An exception has been the succ+essor in line New Trend 315, branch N. Desing 036 036 USA – P. A. R. B. Design Plus 97 USA97, which is a DDC carrier. Therefore, it is recommended to exclude the offspring of this bull from breeding programs.

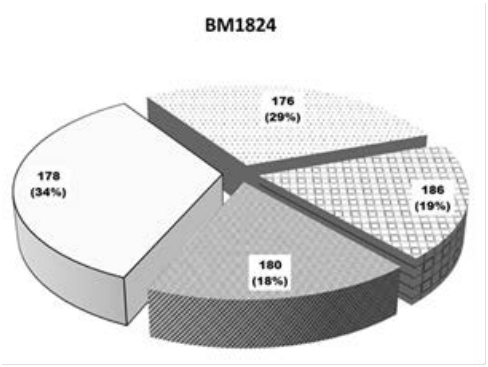
Genetic analysis was performed on blood samples from cattle of purebred populations: Holstein dairy breed – 73 heads (educational experimental farm Krasnodarskoye of the Kuban State Agrarian University), and the Aberdeen-Angus beef breed – 30 heads (JSC Company Agrocomplex n.a. N. I. Tkachev), with the use of 15 microsatellites (BM1824, BM2113, BM1818, CSRM60, CSSM66, ETH3, ETH10, ETH225, INRA023, ILSTS006, SPS115, TGLA227, TGLA126, TGLA122, TGLA53).

The research showed that in Holstein breed, there were 91 peaks, Aberdeen Angus – 69 peaks. The structure of the microsatellites of the Aberdeen-Angus breed was dominated by peaks for allele I: TGLA53 – 156 – 56.6%; TGLA122 -147 - 56.6%; for allele II: ILSTS006 – 212-301; CSRM60 – 90-120. In the Holstein breed, the structure of allele II was different from the structure of allele I. The most frequent were peaks in BM2113 – 131 – 52.06%; CSSM66 – 158 – 60.3%; ETH225 – 146 – 68.5%; ILSTS006 – 295 - 69.9%.

a



b



c

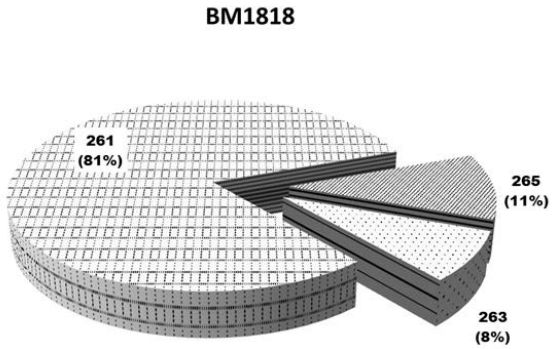
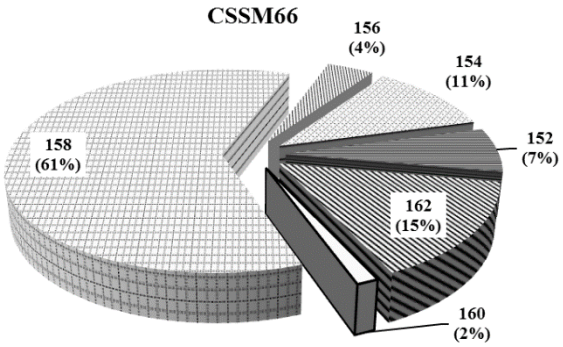


Figure 1. Structure of microsatellites along allele I (a-c).

a



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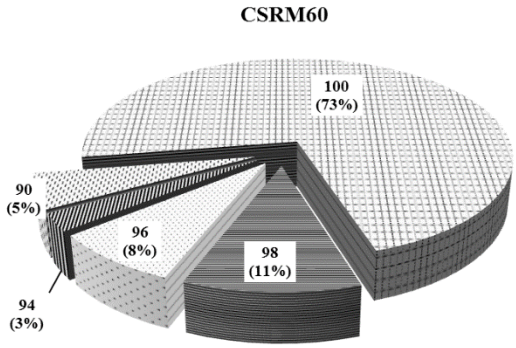

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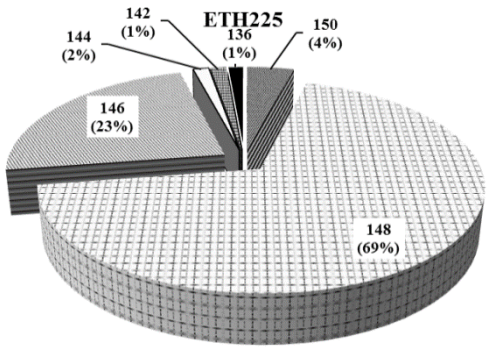

Figure 2. Structure of microsatellites along allele II (a-c).

a b

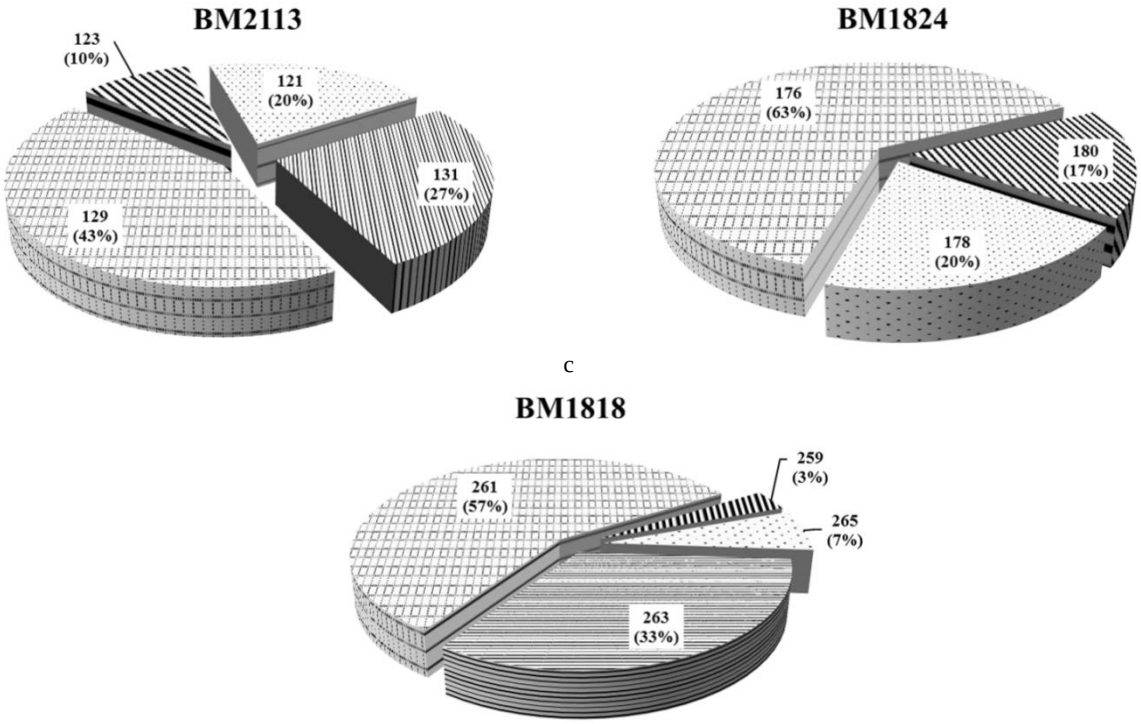

Figure 3. The structure of three of the fifteen microsatellite loci of allele I of the Aberdeen-Angus breed (a-c).

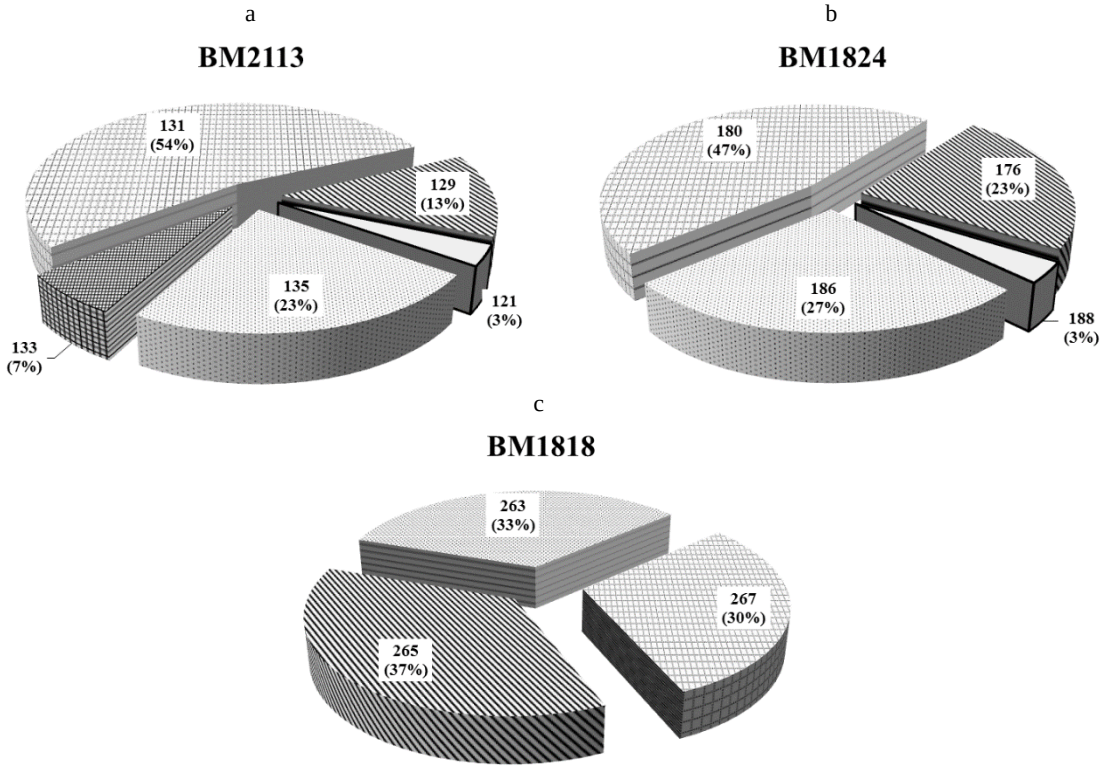

Figure 4. The structure of three of the fifteen microsatellite loci of allele II of the Aberdeen-Angus breed (a-c).

It had been found that with the equal number of microsatellite peaks, their lengths were different. Thus, in the Aberdeen Angus breed, in ETH3 and ILSTS006 with the equal number of peaks (8), the lengths differed: 113-127 in the first case, and 212-301 in the second case; in the Holstein breed, in BM2113 and BM1818 there were three peaks in each, the lengths of which differed: 121-131 in the first case, and 261-265 in the second case. The observed heterozygosity was high both in the Holstein population: 0.616 (BM1818) to 0.945 (SPS115), and in the Aberdeen Angus population: 0.633 (ETH10) to 0.967 (CSSM66). The most heterozygous in the Holstein breed were the following: TGLA53 – 0.904; ETH3 – 0.917; and SPS115 – 0,945; in the Aberdeen Angus breed: CSSM66 – 0.967; ILSTS006 and TGLA227– 0.900, respectively. For all groups of animals, Fis values were within the confidence interval (95%), which confirmed the absence of inbreeding, and the studied populations had stable genetic structure.

REFERENCES

1. Shcherbatov, V. I., Sidorenko, L. I., Koshchaev, A. G., Vorokov, V. K., Skvortsova, L. N., Chicken hatching syncronization for artificial incubation, *Journal of Pharmaceutical Sciences and Research*, 2018, *10(1)*, 148-151.
2. Zverzhanovskiy, M. I., Zabashta, S. N., Kataeva, T. S., Koshchaev, A. G., Nazarov, M. V., Epizootic trichinellosis situation and consortive links in jackals (Canis aureus L.) in North-western Region of Russia, *Indian Veterinary Journal*, 2017, *94(10)*, 29-32.
3. Grover, P., Sharma, S., Development and use of molecular markers: Past and present, *Critical Reviews in Biotechnology*, 2016. DOI 10.3109/07388551.2014.959891.
4. Radchenko, V. V., Ilnitskaya, E. V., Rodionova, A. S., Shuvaeva, T. M., Lysenko, Y. A., Plutakhin, G. A., Manolov, A. I., Donnik, I. M., Koshchaev, A. G., Identification of autochthonous strains as a basis for the development of the therapeutic and profylactic probiotics, *Russian Journal of Biopharmaceuticals*, 2016, *8(1)*, 3-12.
5. Koshchaev, A. G., Shchukina, I. V., Semenenko, M. P., Krivonogova, A. S., Vasilevich, K. V., Amino acid profile of meat of specialized beef breeds, *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2016, *7(5)*, 670-676.
6. Koba, I. S., Lysenko, A. A., Koshchaev, A. G., Rodin, I. A., Shantyz, A. U., Effective treatment of chronic endometritis in cows by florinazol preparation, *Indian Veterinary Journal*, 2017, *94(10)*, 15-18.
7. Serdyuchenko, I. V., Koshchaev, A. G., Guguchvili, N. N., Zholobova, I. S., Donnik, I. M., Smirnov, A. M., Usha, B. V., Microbiocenosis of the intestinal tract of honey bees and its correction, *OnLine Journal of Biological Sciences*, 2018, *18(1)*, 74-83.
8. Mason, A. S., SSR Genotyping, *Methods in Mol. Biol*, 2015, 1245. DOI 10.1007/978-1-4939-1966-6_6.
9. Donnik, I. M., Krivonogova, A. S., Isaeva, A. G., Koshchaev, A. G., Neverova, O. P., Bykova, O. A., Productivity and health markers for large cattle, *International Journal of Green Pharmacy*, 2017, *11(3)*, 620-625.
10. Starostina, N. G., Koshchaev, A. G., Ratner, E. N., Tsiomenko, A. B., Assessment of cell-surface hydrophobicity in methanotrophic bacteria by their adherence to hydrocarbons, *Microbiologiya*, 1997, *66(2)*, 151-156.
11. Starostina, N. G., Koshchaev, A. G., Ratner, E. N., Tsiomenko, A. B., Cell surface hydrophobicity in methanotrophic bacteria by their adherence to hydrocarbons, *Mikrobiologiya*, 1997, *66(2)*, 185-191.
12. Zane, L., Bargelloni, L., Patarnello, T., Strategies for microsatellite isolation: A review, *Mol. Ecol.*, 2002, *11*, 1-16.
13. Sobol, I. V., Donchenko, L. V., Rodionova, L. Y., Koshchaev, A. G., Stepovoy, A. V., Peculiarities of analytical characteristics of pectins extracted from sunflower hearts, *Asian Journal of Pharmaceutics*, 2017, *11(1)*, 97-100.
14. Onischuk, P. D., Semenenko, M. P., Kuzminova, E.,V., Koshchaev, A. G., Selective mechanisms of antiviral effect of triazole derivatives in a transplantable virus-producing cell culture of hamadryas baboon, *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *7(6)*, 1778-1782.
15. Ernst, L. K., Zinovieva, N. A., *Biologicheskie problemi zhivotnovodstva v XXI veke [Biological problems of livestock in the XXI century]*, RAAS, Moscow 2008.
16. Guichoux, E., Lagache, L., Wagner, S., Chaumell, P., Leger, P., Lepais, O., Lepoittevin, C., Malausa, T., Revardel, E., Salin, F., Petit, R. J., Current trends in microsatellite genotyping, *Molecular Ecology Resources*, 2011, *11*, 591-611.
17. Chasovshchikova, M. A., Sheveleva, O. M., Svjazhenina, M. A., Tatarkina, N. I., Satkeeva, A. B., Bakharev, A. A., Ponomareva, E. A., Koshchaev, A. G., Relationship between the genetic variants of kappa-casein and prolactin and the productive-biological characteristics of cows of the black-motley breed, *Journal of Pharmaceutical Sciences and Research*, 2017, *9(7)*, 1038-1044.
18. Zykova, S. S., Danchuk, M. S., Talismanov, V. S., Tokareva, N. G., Igidov, N. M., Rodin, I. A., Koshchaev, A. G., Gugushvili, N. N., Karmanova, O. G., Predictive and experimental determination of antioxidant activity in the series of substituted 4-(2,2-dimethylpropanoyl)-3-hydroxy-1,5-diphenyl-1,5-dihydro-2h-pyrrol-2-ones, *Journal of Pharmaceutical Sciences and Research*, 2018, *10(1)*, 164-166.
19. Weber, J. L., May, P. E., Abudant class of human DNA polymorphism which can be typed using the polymerase chain reaction, *American J. Human Genet.*, 1989, 44, 388-396.
20. Belyakova, A. N., Bogdanova, T. V., Boukarov, N. G., Bykova, A. N., Geneticheskie resursy i informatsionno-vichislitelnoe obespechenie – garantiya kachestvennogo vosproizvodstva stada [Genetic resources and hardware and software are the guarantee of successful herd reproduction], *Zootechny*, 2015, *5*, 2-4.
21. Plutakhin, G. A., Koshchaev, A. G., Donnik, I. M., Quality assessment of chicken meat by analysis-of-variance method, *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2016, *7(3)*, 2293-2299.
22. Koshchaev, A. G., Lysenko, Y. A., Lysenko, A. A., Luneva, A. V., Saleeva, I. P., Fisinin, V. I., Screening of microorganism symbiont strains as a base of probiotics for poultry industry, *Journal of Pharmaceutical Sciences and Research*, 2017, *9(8)*, 1373-1379.
23. Zinovieva, N. A., Gaplotipi fertilnosti golshtinskogo skota [Haplotypes of Holstein cattle fertility], *Agricultural biology*, 2016, *51(4)*, 423-435.
24. Hayes, B., The 1000 Bull Genomes project – toward genomic selection from whole genome sequence data in dairy and beef cattle, *Plant and Animal Genome XXI Conf.*, San Diego 2013.